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Annual Report

Institute of Medical Science
University of Tokyo

2024

Preface

We are pleased to present the FY2024 Annual Report of the Institute of Medical Science at the University of Tokyo (IMSUT). The predecessor of IMSUT, the Institute of Infectious Diseases (IID), was founded in 1892 by Dr. Shibasaburo Kitasato and incorporated into the University of Tokyo in 1916. At that time, infectious diseases were the greatest threat to public health, and IID was a key center of infectious disease research in Asia and a leading international research center. As this report shows, IMSUT, as the successor to IID, has continued to dedicate itself to tackling infectious diseases such as COVID-19, but has also expanded its mission cancer and other intractable diseases.

In 1967, IMSUT was reorganized as a research institute covering a wider range of medical fields to meet the demands of modern medicine arising after World War II, and adopted its present name. IMSUT's mission has evolved to contribute to the development and welfare of human society through advanced medical research and the practice of cutting-edge medicine, based on the history and traditions of the more than 130 years since its founding. To achieve this mission, IMSUT promotes interdisciplinary research and conducts a wide range of practical research projects, from the establishment of artificial intelligence (AI) and support for state-of-the-art AI medicine, to the development of pharmaceuticals, including gene, virus and vaccine therapies, to cell and organ transplantation using stem cells and iPS cells, to new developments in genomic medicine.

For more than a century, IMSUT has followed the three guiding principles established by Dr. Kitasato: "practical learning" for the benefit of society, "diverse and comprehensive research," and "disease prevention." These three principles are the foundation of medical practice and rapidly developing research at IMSUT, and we are further opening up new intellectual horizons based on them by harnessing vast amounts of information with AI. We are accelerating the efficient use of new technologies by promoting the establishment of medical AI research with our dedicated supercomputer "Shirokane" and have already realized AI-guided medicine for hematopoietic tumors and other diseases at IMSUT hospital.

Importantly, IMSUT was accredited by the Minister of Education, Culture, Sports, Science and Technology in 2018 as the only International Joint Usage/Research Center in Japan in the field of life sciences. In recognition of its activities and achievements, IMSUT was reaccredited in 2021 and is continuing its center project for six years. Through this platform, IMSUT is supporting 34 international collaborative research projects in FY2024. Furthermore, the interim evaluation of the center projects was conducted last year, and our center received the highest rating of S. As a world-leading medical research institute, we endeavor to further contribute to the development of the basic, translational and clinical research undertaken by the global research community.

This annual report summarizes the scientific achievements of IMSUT in 2024. It is our sincere hope that these results will inspire further research, foster collaboration with scientists around the world, and ultimately contribute to the improvement of global healthcare.

January 2025

Makoto Nakanishi, M.D., Ph.D.
Dean
The Institute of Medical Science
The University of Tokyo

Organization and Faculty Members

機構および職員

〈as of December, 2024〉

Department of Microbiology and Immunology　感染・免疫部門

Division of Infectious Genetics …… 18

感染遺伝学分野

Professor	Kensuke Miyake, M.D., Ph.D.	教　授	医学博士	三　宅　健　介
Project Associate Professor	Ryutaro Fukui, Ph.D.	特任准教授	博士(医学)	福　井　竜太郎
Assistant Professor	Ryota Sato, Ph.D.	助　教	博士(医学)	佐　藤　亮　太

Division of Molecular Virology …… 21

ウイルス病態制御分野

Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	教　授	博士(獣医学)	川　口　　寧
Associate Professor	Akihisa Kato, Ph.D.	准教授	博士(医学)	加　藤　哲　久
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教	博士(生命科学)	小　柳　直　人
Assistant Professor	Yuhei Maruzuru, Ph.D.	助　教	博士(生命科学)	丸　鶴　雄　平

Division of Vaccine Science …… 23

ワクチン科学分野

Professor	Ken Ishii, M.D., Ph.D.	教　授	博士(医学)	石　井　　健
Associate Professor	Kouji Kobiyama, Ph.D.	准教授	博士(医学)	小檜山　康　司
Assistant Professor	Burcu Temizoz, Ph.D.	助　教	博士(医学)	テミズオズ　ブルジュ
Assistant Professor	Tomoya Hayashi, Ph.D.	助　教	博士(医学)	林　　智　哉

Division of Malaria Immunology …… 26

マラリア免疫学分野

Professor	Cevayir Coban, M.D., Ph.D. (Clinical Microbiology)	教　授	博士(医学) (臨床微生物学位)	チョバン　ジェヴァイア
Associate Professor	Niloufar Kavian-Tessler, Pharm.D., Ph. D.	准教授	博士(医学)	カビアン・テスラー ニルファー
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Project Assistant Professor	Michelle S.J. Lee, Ph.D.	特任助教	博士(医学)	リー　ミシェル

Division of Systems Virology …… 30

システムウイルス学分野

Professor	Kei Sato, Ph.D.	教　授	博士(医学)	佐　藤　　佳
Associate Professor	Jumpei Ito, Ph.D., D.V.M.	准教授	博士(理学)	伊　東　潤　平
Project Assistant Professor	Yu Kaku, Ph.D., M.D.	特任助教	博士(医学)	郭　　悠

Department of Cancer Biology　癌・細胞増殖部門

Division of Genetics …… 34

腫瘍抑制分野

Professor	Yuji Yamanashi, Ph.D.	教　授	理学博士　山　梨　裕　司
Associate Professor	Akane Inoue-Yamauchi, Ph.D.	准教授	博士(医学)　山内（井上）茜
Assistant Professor	Wu Ji, Ph.D.	助　教	博士(科学)　吴　　际

Division of Cancer Cell Biology …… 39

癌防御シグナル分野

Professor	Makoto Nakanishi, M.D., Ph.D.	教　授	医学博士　中　西　　真
Associate Professor	Atsuya Nishiyama, Ph.D.	准教授	博士(理学)　西　山　敦　哉
Assistant Professor	Satoshi Kawakami, Ph.D.	助　教	博士(理学)　川　上　聖　司
Project Assistant Professor	Yoshimi Imawari, M.D., Ph.D.	特任助教	博士(医学)　井　廻　良　美

Division of Aging and Regeneration …… 42

老化再生生物学分野

Professor	Emi K. Nishimura, M.D., Ph.D.	教　授	博士(医学)　西　村　栄　美
Associate Professor	Takuma Shibata, Ph.D.	准教授	博士(医学)　柴　田　琢　磨
Assistant Professor	Yasuaki Mohri, Ph.D.	助　教	博士(農学)　毛　利　泰　彰
Assistant Professor	Kyosuke Asakawa, Ph.D.	助　教	博士(工学)　浅　川　杏　祐

Department of Basic Medical Sciences　基礎医科学部門

Division of Cell Signaling and Molecular Medicine …… 44

分子シグナル制御分野

Professor	Mutsuhiro Takekawa, M.D., Ph.D.	教　授	博士(医学)　武　川　睦　寛
Senior Assistant Professor	Yuji Kubota, Ph.D.	講　師	博士(理学)　久保田　裕　二
Assistant Professor	Hisashi Moriizumi, Ph.D.	助　教	博士(医科学)　森　泉　寿　士
Assistant Professor	Ryosuke Hiranuma, Ph.D.	助　教	博士(医科学)　平　沼　亮　祐

Division of RNA and Gene Regulation …… 48

RNA 制御学分野

Professor	Toshifumi Inada, Ph.D.	教　授	博士(理学)　稲　田　利　文
Associate Professor	Yoshitaka Matsuo, Ph.D.	准教授	博士(理学)　松　尾　芳　隆
Assistant Professor	Toru Suzuki, Ph.D.	助　教	博士(理学)　鈴　木　　亨
Assistant Professor	Sihan Li, Ph.D.	助　教	博士(薬科学)　李　　思　涵

Division of Protein Metabolism …… 52

タンパク質代謝制御分野

Professor	Yasushi Saeki, Ph.D.	教　授	博士(薬学)　佐　伯　　泰
Associate Professor	Taeko Kobayashi, Ph.D.	准教授	博士(理学)　小　林　妙　子
Assistant Professor	Takuya Tomita, Ph.D.	助　教	博士(薬科学)　冨　田　拓　哉

Human Genome Center　ヒトゲノム解析センター

Laboratory of Molecular Medicine …… 55
ゲノム医科学分野

Professor	Tatsuhiro Shibata, M.D., Ph.D.	教　授	医学博士　柴　田　龍　弘
Senior Assistant Professor	Atsushi Niida, Ph.D.	講　師	博士(理学)　新井田　厚　司
Assistant Professor	Kazuki Takahashi, Ph.D.	助　教	博士(農学)　高　橋　数　冴

Laboratory of Genome Technology …… 58
シークエンス技術開発分野

Project Professor	Koichi Matsuda, M.D., Ph.D.	特任教授	博士(医学)　松　田　浩　一

Laboratory of Functional Analysis *In Silico* …… 65
機能解析イン・シリコ分野

Professor	Kenta Nakai, Ph.D.	教　授	博士(理学)　中　井　謙　太
Associate Professor	Sung-Joon Park, Ph.D.	准教授	博士(工学)　朴　　聖　俊
Assistant Professor	Martin Loza, Ph.D.	助　教	博士(生命機能学)　ロサ　マルティン

Laboratory of Genome Database
ゲノムデータベース分野

Professor	Kenta Nakai, Ph.D.	教　授	博士(理学)　中　井　謙　太

Department of Public Policy …… 72
公共政策研究分野

Professor	Kaori Muto, Ph.D.	教　授	博士(保健学)　武　藤　香　織
Associate Professor	Izen Ri, Ph.D.	准教授	博士(学際情報学)　李　　怡　然

Division of Medical Data Informatics …… 75
医療データ情報学分野

Professor	Tetsuo Shibuya, Ph.D.	教　授	博士(理学)　渋　谷　哲　朗
Assistant Professor	Robert Daniel Barish, Ph.D.	助　教	博士(学術)　ロバート ダニエル バリッシュ

Division of Health Medical Intelligence …… 81
健康医療インテリジェンス分野

Professor	Seiya Imoto, Ph.D.	教　授	博士(数理学)　井　元　清　哉
Project Associate Professor	Yao-zhong Zhang, Ph.D.	准教授	博士(情報理工学)　張　　耀　中
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Project Assistant Professor	Satoshi Ito	特任助教	伊　東　　聰
Project Assistant Professor	Yusri Dwi Heryanto, Ph.D.	特任助教	博士(医学)　ユスリ ドウィ ヘリヤント

Laboratory of Sequence Analysis
シークエンスデータ情報処理分野

Professor	Seiya Imoto, Ph.D.	教　授	博士(数理学)　井　元　清　哉
Associate Professor	Kotoe Katayama, Ph.D.	准教授	博士(情報学)　片　山　琴　絵

Division of Metagenome Medicine …… 89
メタゲノム医学分野

Project Professor	Satoshi Uematsu, M.D., Ph.D.	特任教授	博士(医学)　植　松　　智
Project Associate Professor	Kosuke Fujimoto, M.D., Ph.D.	特任准教授	博士(医学)　藤　本　康　介

Division of Digital Genomics …… 92
デジタル・ゲノミクス分野

Professor	Natsuhiko Kumasaka, Ph.D.	教　授	博士(理学) 熊　坂　夏　彦

Center for Experimental Medicine and Systems Biology　システム疾患モデル研究センター

Laboratory of Innate Immunity …… 95
自然免疫研究分野

Professor	Kensuke Miyake, M.D., Ph.D.	教　授	医学博士　三　宅　健　介

Laboratory of Reproductive Systems Biology …… 98
生殖システム研究分野

Project Professor	Masahito Ikawa, Ph.D.	特任教授	博士(薬学) 伊　川　正　人
Associate Professor	Manabu Ozawa, Ph.D.	准教授	博士(農学) 小　沢　　学

Division of Genome Engineering …… 101
ゲノム編集研究分野

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学) 真　下　知　士
Associate Professor	Kazuto Yoshimi, Ph.D.	准教授	博士(医科学) 吉　見　一　人

Division of Cell Regulation …… 104
細胞制御研究分野

Professor	Satoshi Yamazaki, Ph.D.	教　授	博士(生命科学) 山　﨑　　聡
Associate Professor	Yosuke Tanaka, Ph.D.	准教授	博士(医学) 田　中　洋　介
Assistant Professor	Hans Jiro Becker, M.D., Ph.D.	助　教	博士(医学) ベッカーハンス次郎
Project Assistant Professor	Hyojung Jeon, Ph.D.	特任助教	博士(医学) 全　　孝　静

Core Laboratory for Developing Advanced Animal Models …… 106
先進モデル動物作製コア

Professor	Satoshi Yamazaki, Ph.D.	教　授	博士(生命科学) 山　﨑　　聡
Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学) 真　下　知　士
Visiting Professor	Kimi Araki, Ph.D.	客員教授	博士(理学) 荒　木　喜　美
Associate Professor	Manabu Ozawa, Ph.D.	准教授	博士(農学) 小　沢　　学
Project Assistant Professor	Jumpei Taguchi, Ph.D.	特任助教	博士(医科学) 田　口　純　平

Advanced Clinical Research Center　先端医療研究センター

Division of Infectious Diseases …… 108
感染症分野

Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学) 四　柳　　宏
Senior Assistant Professor	Michiko Koga, M.D., D.M.Sc.	講　師	博士(医学) 古　賀　道　子
Assistant Professor	Makoto Saito, M.D., D.Phil.	助　教	博士(医学) 齋　藤　　真
Assistant Professor	Aya Ishizaka, Ph.D.	助　教	博士(理学) 石　坂　　彩
Assistant Professor	Yoshiaki Kanno, M.D., D.M.Sc.	助　教	博士(医学) 菅　野　芳　明

Division of Clinical Genome Research …… 115
臨床ゲノム腫瘍学分野

Professor	Yoichi Furukawa, M.D., Ph.D.	教　授	博士(医学) 古　川　洋　一
Associate Professor	Kiyoshi Yamaguchi, Ph.D.	准教授	博士(薬学) 山　口　貴世志
Assistant Professor	Kiyoko Takane, M.D., Ph.D.	助　教	博士(医学) 高　根　希世子
Assistant Professor	Saya Nakagawa, Ph.D.	助　教	博士(医科学) 中　川　沙　弥

Division of Innovative Cancer Therapy …… 119
先端がん治療分野

Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士(医学)	藤　堂　具　紀
Project Professor	Minoru Tanaka, M.D., Ph.D.	特任教授	博士(医学)	田　中　　実
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Assistant Professor	Yoshinori Sakata, M.D., Ph.D.	助　教	博士(医学)	坂　田　義　詞
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Assistant Professor	Seisaku Kanayama, M.D.	助　教		金　山　政　作

Division of Advanced Medicine Promotion …… 121
先端医療開発推進分野

Professor	Fumitaka Nagamura, M.D., D.M.Sc.	教　授	博士(医学)	長　村　文　孝
Associate Professor	Masanori Nojima, M.D., Ph.D., M.P.H.	准教授	博士(医学)	野　島　正　寛

Division of Advanced Genome Medicine …… 123
先端ゲノム医学分野

Associate Professor	Yoshihiro Hirata, M.D., Ph.D.	准教授	博士(医学)	平　田　喜　裕

Division of Frontier Surgery …… 125
フロンティア外科学分野

Professor	Dai Shida, M.D., Ph.D.	教　授	博士(医学)	志　田　　大
Associate Professor	Susumu Aikou, M.D., Ph.D.	准教授	博士(医学)	愛　甲　　丞
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Assistant Professor	Naoki Sakuyama, M.D., Ph.D.	助　教	博士(医学)	柵　山　尚　紀
Assistant Professor	Satoko Monma, M.D.	助　教		門　間　聡　子
Assistant Professor	Junko Mukohyama, M.D., Ph.D.	助　教	博士(医学)	向　山　順　子

Division of Hematopoietic Disease Control …… 127
造血病態制御学分野

Professor	Yasuhito Nannya, M.D., Ph.D.	教　授	博士(医学)	南　谷　泰　仁
Associate Professor	Takaaki Konuma, M.D., Ph.D.	准教授	博士(医学)	小　沼　貴　晶
Assisstant Professor	Koji Jimbo, M.D., Ph.D.	助　教	博士(医学)	神　保　光　児

Division of Advanced Gastroenterology and Endoscopy …… 132
先端消化器内視鏡学分野

Professor	Hiroaki Ikematsu, M.D., Ph.D.	教　授	博士(医学)	池　松　弘　朗
Assistant Professor	Tatsunori Minamide, M.D.	助　教		南　出　竜　典

Division of Anesthesia and Surgical Homeostasis …… 134
侵襲防御医学分野

Professor	Masahiko Bougaki, M.D., Ph.D.	教　授	博士(医学)	坊　垣　昌　彦

Division of Hematology and Tumor Biology …… 135
血液・腫瘍生物学分野

Associate Professor	Ayana Kon, M.D., Ph.D.	准教授	博士(医学)	昆　　彩　奈

Division of Bioethics and Medical Law …… 137
生命倫理・医事法研究分野

Associate Professor	Waki Toya, Ph.D.	准教授	博士(医学)	遠　矢　和　希

Center for Stem Cell Biology and Regenerative Medicine　幹細胞治療研究センター

Division of Regenerative Medicine …… 139
再生医学分野

Professor	Hideki Taniguchi, M.D., Ph.D.	教　授	博士(医学) 谷　口　英　樹
Associate Professor	Naoki Tanimizu, Ph.D.	准教授	博士(農学) 谷　水　直　樹
Assistant Professor	Yun-Zhong Nie, Ph.D.	助　教	博士(医学) 聶　　運　中
Project Assistant Professor	Yasuharu Ueno, Ph.D.	特任助教	博士(医学) 上　野　康　晴
Project Assistant Professor	Takayoshi Oba, M.D., Ph.D.	特任助教	博士(医学) 大　場　敬　義

Division of Stem Cell and Molecular Medicine …… 144
幹細胞分子医学分野

Professor	Atsushi Iwama, M.D., Ph.D.	教　授	博士(医学) 岩　間　厚　志
Senior Assistant Professor	Motohiko Oshima, Ph.D.	講　師	博士(医学) 大　島　基　彦
Assistant Professor	Yaeko Nakajima, Ph.D.	助　教	博士(医学) 中　島　やえ子
Assistant Professor	Masayuki Yamashita, M.D., Ph.D.	助　教	博士(医学) 山　下　真　幸
Project Assistant Professor	Takako Yokomizo, Ph.D.	特任助教	博士(医学) 横　溝　貴　子
Project Assistant Professor	Shuhei Koide, Ph.D.	特任助教	博士(医学) 小　出　周　平
Project Assistant Professor	Ola Rizq, M.D., Ph.D.	特任助教	博士(医学) オ ラ　リ ズ ク

Division of Stem Cell Transplantation …… 147
幹細胞移植分野

Professor	Yasuhito Nannya, M.D., D.M.Sc.	教　授	博士(医学) 南　谷　泰　仁
Project Professor	Satoshi Takahashi, M.D., D.M.Sc.	特任教授	博士(医学) 高　橋　　聡

Division of Stem Cell Processing …… 151
幹細胞プロセシング分野

Professor	Hideki Taniguchi, M.D., Ph.D.	教　授	博士(医学) 谷　口　英　樹

Division of Mammalian Embryology …… 152
再生発生学分野

Project Associate Professor	Toshihiro Kobayashi, Ph.D.	特任准教授	博士(生命科学) 小　林　俊　寛

Division of Stem Cell Aging Medicine …… 154
幹細胞加齢医学分野

Professor	Emi K. Nishimura, M.D., Ph.D.	教　授	博士(医学) 西　村　栄　美

Division of Somatic Stem Cell Research …… 156
体性幹細胞研究分野

Associate Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.	准教授	博士(医学) 長　村　登紀子
Project Assistant Professor	Kazuhiro Sudo, Ph.D.	特任助教	博士(医学) 須　藤　和　寛

Division of Cell Engineering …… 157
幹細胞基盤技術研究分野

Professor	Satoshi Yamazaki, Ph.D.	教　授	博士(生命科学) 山　﨑　　聡

Division of Stem Cell and Genome Biology …… 159
幹細胞ゲノム生物学分野

Associate Professor	Ayana Kon, M.D., Ph.D.	准教授	博士(医学) 昆　　彩　奈

FACS Core Laboratory …… 161
FACS コアラボラトリー
Professor　Atsushi Iwama, M.D., Ph.D.　教　授　博士(医学) 岩　間　厚　志

International Research Center for Infectious Diseases　感染症国際研究センター

Department of Special Pathogens …… 162
高病原性感染症系
Professor　Kei Sato, Ph.D.　教　授　博士(医学) 佐　藤　　佳
Visiting Professor　Masaki Imai, D.V.M., Ph.D.　客員教授　博士(獣医学) 今　井　正　樹
Visiting Professor　Seiya Yamayoshi, D.V.M., Ph.D.　客員教授　博士(医学) 山　吉　誠　也
Associate Professor　Takeshi Ichinohe, Ph.D.　准教授　博士(工学) 一　戸　猛　志
Associate Professor　Jumpei Ito, Ph.D., D.V.M.　准教授　博士(理学) 伊　東　潤　平

Department of Infectious Disease Control …… 165
感染制御系
Professor　Yasushi Kawaguchi, D.V.M., Ph.D.　教　授　博士(獣医学) 川　口　　寧
Associate Professor　Akihisa Kato, Ph.D.　准教授　博士(医学) 加　藤　哲　久
Assistant Professor　Naoto Koyanagi, Ph.D.　助　教　博士(生命科学) 小　柳　直　人
Assistant Professor　Yuhei Maruzuru, Ph.D.　助　教　博士(生命科学) 丸　鶴　雄　平

Department of Infectious Disease Control Division of Viral Infection …… 167
感染制御系・ウイルス学分野
Associate Professor　Takeshi Ichinohe, Ph.D.　准教授　博士(工学) 一　戸　猛　志

International Vaccine Design Center　国際ワクチンデザインセンター

Division of Systems Immunology (Human Immune-Profiling Team) …… 169
ヒト免疫プロファイリング系・数理免疫学分野
Professor　Kei Sato, Ph.D.　教　授　博士(医学) 佐　藤　　佳

Division of Human Immunology (Human Immune-Profiling Team) …… 172
ヒト免疫プロファイリング系・ヒト免疫学分野
Professor　Ken Ishii, M.D., Ph.D.　教　授　博士(医学) 石　井　　健

Division of Infection Immunology (Human Immune-Profiling Team) …… 174
ヒト免疫プロファイリング系・感染免疫学分野
Professor　Cevayir Coban, M.D., Ph.D. (Clinical Microbiology)　教　授　博士(医学) チョバン　ジェヴァイア (臨床微生物学位)
Associate Professor　Niloufar Kavian-Tessler, Pharm.D., Ph.D.　准教授　博士(医学) ｶﾋﾞｱﾝ･ﾃｽﾗｰ　ﾆﾙﾌｧｰ
Visiting Professor　Anavaj Sakuntabhai, M.D., Ph.D.　客員教授　博士(医学) ｻｸﾝﾀﾊﾞｲ　ｱﾅｳﾞｧｼﾞ
Assistant Professor　Jalal Alshaweesh, Ph.D.　助　教　博士(医学) ｱﾙｼｬｳｨｼｭ　ｼﾞｬﾗﾙ
Assistant Professor　Michelle S.J. Lee, Ph.D.　特任助教　博士(医学) リー　ミシェル

Division of Vaccine Engineering (New Dimensional Vaccine Design Team) …… 177
新次元ワクチンデザイン系・ワクチン工学分野
Project Professor　Kouhei Tsumoto, Ph.D.　特任教授　博士(工学) 津　本　浩　平

Division of Adjuvant Innovation (New Dimensional Vaccine Design Team) …… 186
新次元ワクチンデザイン系・アジュバント開発分野

Professor	Ken Ishii, M.D., Ph.D.	教　授	博士(医学) 石　井　　健
Associate Professor	Kouji Kobiyama, Ph.D.	准教授	博士(医学) 小檜山　康　司
Visiting Professor	Jun Kunisawa, Ph.D.	客員教授	博士(薬学) 國　澤　　純

Division of Mucosal Vaccines (New Dimensional Vaccine Design Team) …… 189
新次元ワクチンデザイン系・粘膜ワクチン分野

Project Professor	Kohtaro Fujihashi, D.D.S., Ph.D.	特任教授	博士(歯学) 藤　橋　浩太郎
Visiting Professor	Koji Hase, Ph.D.	客員教授	博士(薬学) 長　谷　耕　二
Visiting Professor	Tomonori Nochi, Ph.D.	客員教授	博士(農学) 野　地　智　法

Division of Immunology and Genomics (New Dimensional Vaccine Design Team) …… 192
新次元ワクチンデザイン系・ゲノム免疫学分野

Professor	Ken Ishii, M.D., Ph.D.	教　授	博士(医学) 石　井　　健
Visiting professor	Anavaj Sakuntabhai, M.D., Ph.D.	客員教授	博士(医学) ｻｸﾝﾀﾊﾞｲ　ｱﾅｳﾞｧｼﾞ

Center for Gene & Cell Therapy　遺伝子・細胞治療センター

Division of Molecular and Medical Genetics …… 194
分子遺伝医学分野

Professor	Takashi Okada, M.D., Ph.D.	教　授	博士(医学) 岡　田　尚　巳
Project Associate Professor	Yasushi Soda, M.D., Ph.D.	特任准教授	博士(医学) 曽　田　　泰
Project Senior Assistant Professor	Yasunari Matsuzaka, Ph.D.	特任講師	博士(医学) 松　坂　恭　成
Project Senior Assistant Professor	Yuko Nitahara-Kasahara, Ph.D.	特任講師	博士(工学) 笠　原　優　子
Assistant Professor	Yuji Tsunekawa, Ph.D.	助　教	博士(医学) 恒　川　雄　二
Project Assistant Professor	Hiromi Hayashita-Kinoh, Ph.D.	特任助教	博士(医学) 喜　納　裕　美
Project Assistant Professor	Ken Sugo, Ph.D.	特任助教	博士(工学) 菅　生　　健

Center for Gene & Cell Therapy …… 197
遺伝子・細胞治療センター

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Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士(医学) 藤　堂　具　紀
Professor	Fumitaka Nagamura, M.D., D.M.Sc.	教　授	博士(医学) 長　村　文　孝
Invited Professor	Koji Tamada, M.D., Ph.D.	教授(委嘱)	博士(医学) 玉　田　耕　治
Project Professor	Satoshi Takahashi, M.D., D.M.Sc.	特任教授	博士(医学) 高　橋　　聡
Associate Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.	准教授	博士(医学) 長　村　登紀子

Laboratory Animal Research Center　実験動物施設

Division of Animal Genetics …… 204
先進動物ゲノム研究分野

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学) 真　下　知　士
Associate Professor	Kazuto Yoshimi, Ph.D.	准教授	博士(医科学) 吉　見　一　人
Assistant Professor	Saeko Ishida, D.V.M., Ph.D.	助　教	博士(医学) 石　田　紗恵子

Animal Center ····· 208

動物センター

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　士
Associate Professor	Kazuto Yoshimi, Ph.D.	准教授	博士(医科学)	吉　見　一　人
Assistant Professor	Saeko Ishida, D.V.M., Ph.D.	助　教	博士(医学)	石　田　紗恵子

Amami Laboratory of Medical Science ····· 209

奄美医科学研究施設

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　士
Visiting Associate Professor	Takeshi Annoura, Ph.D.	客員准教授	医学博士	案　浦　　健
Assistant Professor	Shin-Ichi Yokota, D.V.M., Ph.D.	助　教	博士(人間科学)	横　田　伸　一

Medical Proteomics Laboratory ····· 210

疾患プロテオミクスラボラトリー

Professor	Mutsuhiro Takekawa, M.D., Ph.D.	教　授	博士(医学)	武　川　睦　寛
Project Professor	Kouhei Tsumoto, Ph.D.	特任教授	博士(工学)	津　本　浩　平
Associate Professor	Masaaki Oyama, Ph.D.	准教授	博士(医学)	尾　山　大　明
Associate Professor	Satoru Nagatoishi, Ph.D.	准教授	博士(生命科学)	長門石　　曉
			(大学院工学系研究科)	
Senior Assistant Professor	Makoto Nakakido, Ph.D.	講　師	博士(生命科学)	中木戸　　誠
			(大学院工学系研究科)	
Assistant Professor	Ryo Matsunaga, Ph.D.	助　教	(生命科学)	松　長　　遼
			(大学院工学系研究科)	
Project Assistant Professor	Hiroshi Sagara, Ph.D.	特任助教	博士(医学)	相　良　　洋

Research Center for Asian Infectious Diseases ····· 225

アジア感染症研究拠点

Director/Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	拠点長／教授	博士(獣医学)	川　口　　寧
Project Professor	Xuan Xuenan, D.V.M., Ph.D.	特任教授	農学博士	玄　　学　南
Project Professor	Mitsue Hayashi, Ph.D.	特任教授	法学博士	林　　光　江
Visiting Professor	Masaki Imai, D.V.M., Ph.D.	客員教授	博士(獣医学)	今　井　正　樹
Visiting Professor	Seiya Yamayoshi, D.V.M., Ph.D.	客員教授	博士(医学)	山　吉　誠　也
Associate Professor	Akihisa Kato, Ph.D.	准教授	博士(医学)	加　藤　哲　久
Project Associate Professor	Jin Gohda, Ph.D.	特任准教授	博士(薬学)	合　田　　仁
Project Senior Assistant Professor	Mizuki Yamamoto, Ph.D.	特任講師	博士(医学)	山　本　瑞　生
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教	博士(生命科学)	小　柳　直　人
Assistant Professor	Yuhei Maruzuru, Ph.D.	助　教	博士(生命科学)	丸　鶴　雄　平

Laboratory of Molecular Genetics (Frontier Research Unit) ····· 230

遺伝子解析施設（フロンティア研究領域）

Professor	Makoto Nakanishi, M.D., Ph.D.	教　授	医学博士	中　西　　真
Associate Professor	Kazuo Tatebayashi, Ph.D.	准教授	博士(薬学)	舘　林　和　夫

IMSUT Hospital　附属病院

Department of Hematology/Oncology ····· 232

血液腫瘍内科

Professor	Yasuhito Nannya, M.D., Ph.D.	教　授	博士(医学) 南　谷　泰　仁
Project Professor	Satoshi Takahashi, M.D., Ph.D.	特任教授	博士(医学) 高　橋　　聡
Associate Professor	Tokiko Nagamura-Inoue M.D., Ph.D.	准教授	博士(医学) 長　村　登紀子
Associate Professor	Takaaki Konuma, M.D., Ph.D.	准教授	博士(医学) 小　沼　貴　晶
Associate Professor	Kazuaki Yokoyama, M.D., Ph.D.	准教授	博士(医学) 横　山　和　明
Assistant Professor	Seiko Kato, M.D., Ph.D.	助　教	博士(医学) 加　藤　せい子
Assistant Professor	Aki Sato, M.D., Ph.D.	助　教	博士(医学) 佐　藤　亜　紀
Assistant Professor	Koji Jimbo, M.D., Ph.D.	助　教	博士(医学) 神　保　光　児

Department of Infectious Diseases and Applied Immunology ····· 241

感染免疫内科

Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学) 四　柳　　宏
Senior Assistant Professor	Eisuke Adachi, M.D., D.M.Sc.	講　師	博士(医学) 安　達　英　輔
Senior Assistant Professor	Michiko Koga, M.D., D.M.Sc.	講　師	博士(医学) 古　賀　道　子
Assistant Professor	Yoshiaki Kannno, M.D., D.M.Sc.	助　教	博士(医学) 菅　野　芳　明
Assistant Professor	Makoto Saito, M.D., D.Phil.	助　教	博士(医学) 齋　藤　　真

Department of Rheumatology and Allergy ····· 245

アレルギー免疫科

Associate Professor	Motohisa Yamamoto, M.D., D.M.Sc.	准教授	博士(医学) 山　本　元　久
Assistant Professor	Masaaki Uehara, M.D., D.M.Sc.	助　教	博士(医学) 上　原　昌　晃

Department of Oncology and General Medicine ····· 248

腫瘍・総合内科

Head, Professor	Narikazu Boku, M.D., D.M.Sc.	教　授	博士(医学) 朴　　成　和
Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学) 四　柳　　宏
Project Senior Assistant Professor	Koichi Kimura, M.D., D.M.Sc.	特任講師	博士(医学) 木　村　公　一
Assistant Professor	Keisuke Baba, M.D., D.M.Sc.	助　教	博士(医学) 馬　場　啓　介

Department of Applied Genomics ····· 253

ゲノム診療科

Department of Clinical Genomics

ゲノム診療部

Professor	Yoichi Furukawa, M.D., Ph.D.	教　授	博士(医学) 古　川　洋　一

Department of Radiology ····· 255

放射線科

Associate Professor	Hiroyuki Akai, M.D., D.M.Sc.	准教授	博士(医学) 赤　井　宏　行
Senior Assistant Professor	Toshihiro Furuta, M.D., D.M.Sc.	講　師	博士(医学) 古　田　寿　宏
Assistant Professor	Shimpei Kato, M.D., D.M.Sc.	助　教	博士(医学) 加　藤　伸　平
Project Assistant Professor	Naomasa Okimoto, M.D., D.M.Sc.	特任助教	博士(医学) 沖　元　斉　正

Department of Radiological Technology

放射線部

Associate Professor	Hiroyuki Akai, M.D., D.M.Sc.	准教授	博士(医学) 赤　井　宏　行
Head Radiologic Technologist	Kenji Ino, RT	放射線技師長	井　野　賢　二

Department of Palliative Medicine and Advanced Clinical Oncology …… 258
先端緩和医療科
Project Senior Assistant Professor Tetsuya Ito, M.D., Ph.D. 特任講師 博士(医学) 伊 藤 哲 也

Department of Diagnostic Pathology …… 260
病理診断科

Department of Pathology
病理部
Associate Professor Yasunori Ota, M.D., Ph.D. 准教授 博士(医学) 大 田 泰 徳
Project Assistant Professor Tamami Denda, Ph.D. 特任助教 博士(保健学) 傳 田 珠 美

Department of Gastroenterology …… 263
消化器内科
Professor Hiroaki Ikematsu, M.D., Ph.D. 教 授 博士(医学) 池 松 弘 朗
Associate Professor Yoshihiro Hirata, M.D., D.M.Sc. 准教授 博士(医学) 平 田 喜 裕
Assistant Professor Tatsunori Minamide, M.D. 助 教 南 出 竜 典

Department of Surgery …… 267
外科
Professor Dai Shida, M.D., Ph.D. 教 授 博士(医学) 志 田 大
Associate Professor Susumu Aikou, M.D., Ph.D. 准教授 博士(医学) 愛 甲 丞
Assistant Professor Naoki Sakuyama, M.D., Ph.D. 助 教 博士(医学) 柵 山 尚 紀
Assistant Professor Satoko Monma, M.D. 助 教 門 間 聡 子
Assistant Professor Junko Mukohyama, M.D., Ph.D. 助 教 博士(医学) 向 山 順 子
Assistant Professor Ai Sadatomo, M.D., Ph.D. 助 教 博士(医学) 佐田友 藍
Assistant Professor Yuka Ahiko, M.D. 助 教 阿 彦 友 佳
Assistant Professor Haruna Onoyama, M.D., Ph.D. 助 教 博士(医学) 小野山 温 那

Department of Anesthesia …… 270
麻酔科
2024.4.1~
Professor Masahiko Bougaki, M.D., Ph.D. 教 授 博士(医学) 坊 垣 昌 彦
Assistant Professor Fumiko Seto, M.D., Ph.D. 助 教 博士(医学) 瀬 戸 富美子
~2024.3.31
Associate Professor Ryo Orii, M.D., Ph.D. 准教授 博士(医学) 折 井 亮
Assistant Professor Miho Asahara, M.D., Ph.D. 助 教 博士(医学) 浅 原 美 保

Department of Joint Surgery …… 272
関節外科
Project Professor Minoru Tanaka, M.D., Ph.D. 特任教授 博士(医学) 田 中 実

Department of Surgical Neuro-Oncology …… 273
脳腫瘍外科
Professor Tomoki Todo, M.D., Ph.D. 教 授 博士(医学) 藤 堂 具 紀
Project Professor Minoru Tanaka, M.D., Ph.D. 特任教授 博士(医学) 田 中 実
Assistant Professor Hirotaka Ito, M.D., Ph.D. 助 教 博士(医学) 伊 藤 博 崇
Assistant Professor Seisaku Kanayama, M.D. 助 教 金 山 政 作
Assistant Professor (Thoracic surgeon) Yoshinori Sakata, M.D., Ph.D. 助 教 博士(医学)(呼吸器外科医) 坂 田 義 詞

Department of Urology ····· 276

泌尿器科

Professor	Haruki Kume, M.D., Ph.D.	教　授	博士(医学)	久　米　春　喜
Project Associate Professor	Sayuri Takahashi, M.D., Ph.D.	特任准教授	博士(医学)	髙　橋　さゆり
Project Assistant Professor	Yuji Hakozaki, M.D., Ph.D.	特任助教	博士(医学)	箱　崎　勇　治
Project Assistant Professor	Jun Takahashi, M.D.	特任助教		髙　橋　　潤

Department of Medical Informatics ····· 278

医療情報部

Associate Professor	Hiroyuki Akai, M.D., D.M.Sc.	准教授	博士(医学)	赤　井　宏　行
Senior Assistant Professor	Toshihiro Furuta, M.D., D.M.Sc.	講　師	博士(医学)	古　田　寿　宏

Department of Cell Processing and Transfusion ····· 279

セルプロセッシング・輸血部

Clinical Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.	病院教授	博士(医学)	長　村　登紀子
Associate Professor	Kazuaki Yokoyama, M.D., Ph.D.	准教授	博士(医学)	横　山　和　明
Project Assistant Professor	Kazuhiro Sudo, Ph.D.	特任助教	博士(医学)	須　藤　和　寛

Surgical Center ····· 281

手術部

Project Professor	Minoru Tanaka, M.D., Ph.D.	特任教授	博士(医学)	田　中　　実

Department of Laboratory Medicine ····· 283

検査部

Clinical Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.	部長／病院教授	博士(医学)	長　村　登紀子
Assistant Professor	Tomohiro Ishigaki, M.D., Ph.D.	副部長／助教	博士(医学)	石　垣　知　寛
Project Senior Assistant Professor	Koichi Kimura, M.D., Ph.D.	特任講師	博士(医学)	木　村　公　一
Chief Technologist	Hironori Shimosaka	技師長	臨床検査技師	下　坂　浩　則

Center for Clinical Safety and Infection Control ····· 286

医療安全・感染制御センター

Head, Professor	Yasuhito Nannya, M.D., D.M.Sc.	教　授	博士(医学)	南　谷　泰　仁

Department of Medical Safety Management

医療安全管理部

Head, Associate Professor	Susumu Aikou, M.D., D.M.Sc.	准教授	博士(医学)	愛　甲　　丞
Associate Professor	Motohisa Yamamoto, M.D., D.M.Sc.	准教授	博士(医学)	山　本　元　久
Nurse Manager	Nozomi Linzbichler	看護師長		リンツビヒラ希
Director of Pharmacy	Seiichiro Kuroda	薬剤部長		黒　田　誠一郎

Department of Infection Prevention and Control

感染制御部

Head, Senior Assistant Professor	Eisuke Adachi, M.D., D.M.Sc.	講　師	博士(医学)	安　達　英　輔
Nurse Manager	Fumie Kameda	看護師長		亀　田　史　絵
Head of Nursing Department	Mika Kogayu	看護部長		小　粥　美　香
Pharmacist	Naoki Furukawa	薬剤師		古　川　直　樹
Pharmacist	Mika Yamamura	薬剤師		山　村　美　佳
Clinical laboratory technician	Takashi Momoda	臨床検査技師		百　田　尭　史
Clinical laboratory technician	Hiroko Shibata	臨床検査技師		柴　田　浩　子

Center for Translational Research ····· 288
トランスレーショナルリサーチ・治験センター

Professor	Fumitaka Nagamura, M.D., D.M.Sc.	教　授	博士(医学)	長　村　文　孝
Associate Professor	Masanori Nojima, M.D., Ph.D., M.P.H.	准教授	博士(医学)	野　島　正　寛

Therapeutic Vector Development Center ····· 290
治療ベクター開発センター

Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士(医学)	藤　堂　具　紀
Project Professor	Minoru Tanaka, M.D., Ph.D.	特任教授	博士(医学)	田　中　　実

IMSUT CORD ····· 291
臍帯血・臍帯バンク

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Department of Pharmacy ····· 295
薬剤部

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Pharmacist	Mai Yokota	薬剤師	横　田　　舞

Department of AIDS Vaccine Development ····· 297
エイズワクチン開発担当

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IMSUT Distinguished Professor Unit　東京大学特任教授部門

Division of Virology ····· 300
ウイルス感染部門

Project Professor	Yoshihiro Kawaoka, D.V.M., Ph.D.	特任教授	獣医学博士	河　岡　義　裕

Social Cooperation Research Programs　社会連携研究部門

Project Division of RNA Medical Science …… 304
RNA 医科学社会連携研究部門
Project Associate Professor　Kaku Goto, Ph.D.　特任准教授 博士(医学) 後　藤　　覚

Project Division of Advanced Biopharmaceutical Science …… 306
先進的バイオ医薬品学社会連携研究部門
Project Professor　Kouhei Tsumoto, Ph.D.　特任教授　博士(工学) 津　本　浩　平
Project Associate Professor　Susana de Vega, Ph.D.　特任准教授 博士(生物学) スサーナ　デ　ベガ

Project Division of Genomic Medicine and Disease Prevention …… 316
ゲノム予防医学社会連携研究部門
Project Professor　Toru Suzuki, M.D., Ph.D.　特任教授　博士(医学) 鈴　木　　亨

Project Division of Clinical Precision Research Platform …… 318
臨床精密研究基盤社会連携研究部門
Project Professor　Satoshi Takahashi, M.D., D.M.Sc.　特任教授　博士(医学) 高　橋　　聡
Project Assistant Professor　Kimihito Kawabata, M.D., D.M.Sc.　特任助教　博士(医学) 川　畑　公　人

Project Division of Generative AI Utilization Aging Cells …… 323
生成 AI 活用加齢医学社会連携研究部門
Project Associate Professor　Teh-Wei Wang, Ph.D.　特任准教授 博士(理学) 王　　徳　瑋

Project Division of International Healthcare Innovation Research …… 325
国際健康医療推進社会連携研究部門
Project Associate Professor　Koichiro Yuji, M.D., Ph.D.　特任准教授 博士(医学) 湯　地　晃一郎

Corporate Sponsored Research Program　寄付研究部門

Project Division of Oncolytic Virus Development …… 327
ウイルス療法開発寄付研究部門
Project Professor　Minoru Tanaka, M.D, Ph.D.　特任教授　博士(医学) 田　中　　実

Consortium　コンソーシアム

Consortium for Gene Therapy and Regenerative Medicine …… 329
遺伝子治療・再生医療コンソーシアム
Professor　Atsushi Iwama, M.D., Ph.D.　教　授　博士(医学) 岩　間　厚　志
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Dean's Office　所長オフィス

Project Coordination Office ····· 330
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Research Platform Office ····· 332
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BioBank Japan ····· 334
バイオバンク・ジャパン

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RESEARCH ACTIVITIES

Department of Microbiology and Immunology

Division of Infectious Genetics
感染遺伝学分野

Professor	Kensuke Miyake, M.D., Ph.D.	教　授	医学博士	三　宅　健　介
Project Associate Professor	Ryutaro Fukui, Ph.D.	特任准教授	博士(医学)	福　井　竜太郎
Assistant Professor	Ryota Sato, Ph.D.	助　教	博士(医学)	佐　藤　亮　太

Immune cells express multiple Toll-like receptors (TLRs) that are simultaneously activated by various pathogen-derived products from microorganisms and viruses. Recent reports have demonstrated that imbalances in TLR responses can result in the development of autoimmune diseases. Nucleic acid(NA) -sensing TLRs detect not only bacterial and viral NAs, but also host-derived NAs. To prevent excessive immune responses to host-derived NA, there may exist regulatory mechanisms that control TLR expression, localization, and function. Based on this hypothesis, it is believed that TLRs are involved not only in autoimmune diseases, but also in the pathogenesis of a variety ofother diseases. Our research endeavors to uncover the regulatory mechanisms that control TLR-mediated recognition of pathogenic ligands, as well as the identification of endogenous ligands. Our research goal is to clarify the pathogenic mechanisms of histiocytosis and autoimmune diseases that are thought to be mediated by TLRs.

1. Nucleosides drive histiocytosis in SLC29A3 disorders by activating TLR7

Takuma Shibata[1], Ryota Sato[1], Masato Taoka[2], Shin-Ichiroh Saitoh[1], Mayumi Komine[3], Kiyoshi Yamaguchi[4], Susumu Goyama[5], Yuji Motoi[1], Jiro Kitaura[6], Kumi Izawa[6], Yoshio Yamauchi[2], Yumiko Tsukamoto[7], Takeshi Ichinohe[8], Etsuko Fujita[3], Ryosuke Hiranuma[1], Ryutaro Fukui[1], Yoichi Furukawa[4], Toshio Kitamura[9], Toshiyuki Takai[10], Arinobu Tojo[11], Mamitaro Ohtsuki[3], Umeharu Ohto[12], Toshiyuki Shimizu[12], Manabu Ozawa[13], Nobuaki Yoshida[13], Toshiaki Isobe[2], Eicke Latz[14], Kojiro Mukai[15], Tomohiko Taguchi[15], Kensuke Miyake[1]

[1] Division of Innate Immunity, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [2]Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University; Tokyo 192-0397, Japan. [3] Department of Dermatology, Jichi Medical University; Tochigi 329-0498, Japan. [4] Division of Clinical Genome Research, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [5] Division of Molecular Oncology, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo; Tokyo 108-8639, Japan. [6] Atopy Research Center, Juntendo University Graduate School of Medicine; Tokyo 113-8421, Japan. [7] Department of Mycobacteriology, Leprosy Research Center, National Institute of Infectious Diseases; Tokyo 189-0002, Japan. [8] Division of Viral Infection, Department of Infectious Disease Control, International Research Center for Infectious Diseases, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [9] Division of Cellular Therapy, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [10] Department of Experimental Immunology, Institute of Development, Aging and Cancer, Tohoku University; Sendai 980-8575, Japan. [11] Department of Hematology and Oncology, Research Hospital, The In-

stitute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [12] Graduate School of Pharmaceutical Sciences, The University of Tokyo; Tokyo 113-0033, Japan. [13] Laboratory of Developmental Genetics, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo; Tokyo 108-8639, Japan. [14] Institute of Innate Immunity, University Hospital Bonn, University of Bonn; 53127 Bonn, Germany. [15] Laboratory of Organelle Pathophysiology, Department of Integrative Life Sciences, Graduate School of Life Sciences, Tohoku University; Sendai 980-8577, Japan.

Loss-of-function mutations in the lysosomal nucleoside transporter SLC29A3 cause lysosomal nucleoside storage and histiocytosis: phagocyte accumulation in multiple organs. However, little is known about the mechanism by which lysosomal nucleoside storage drives histiocytosis. Herein, histiocytosis in *Slc29a3*$^{-/-}$ mice was shown to depend on Toll-like receptor 7 (TLR7), which senses a combination of nucleosides and oligoribonucleotides (ORNs). TLR7 increased phagocyte numbers by driving the proliferation of Ly6C^{hi} immature monocytes and their maturation into Ly6C^{low} phagocytes in *Slc29a3*$^{-/-}$ mice. Downstream of TLR7, FcRγ and DAP10 were required for monocyte proliferation. Histiocytosis is accompanied by inflammation in SLC29A3 disorders. However, TLR7 in nucleoside-laden splenic macrophages failed to activate inflammatory responses. Enhanced production of pro-inflammatory cytokines was observed only after stimulation with ssRNAs, which would increase lysosomal ORNs. Patient-derived monocytes harboring the G208R *SLC29A3* mutation showed enhanced survival and proliferation in a TLR8 antagonist-sensitive manner. These results demonstrated that TLR7/8 responses to lysosomal nucleoside stress drive SLC29A3 disorders.

2. TLR7 responses in glomerular macrophages accelerate the progression of glomerulonephritis in NZBWF1 mice

Reika Tanaka[1, 8], Yusuke Murakami[1,2, 8], Dorothy Ellis[3], Jun Seita[3], Wu Yinga[4,5], Shigeru Kakuta[4,6,7], Keiki Kumano[2], Ryutaro Fukui[1], Kensuke Miyake[11] Division of Innate Immunity, The Institute of Medical Science, TheUniversity of Tokyo; Minato-ku, Tokyo 108-8639, Japan.[2]Faculty of Pharmacy, Department of Pharmaceutical Sciences & Research Institute of Pharmaceutical Sciences, Musashino University,Nishitokyo-shi, Tokyo, 202-8585, Japan[3] Laboratory for Integrative Genomics, RIKEN Center for Integrative Medical Sciences, Yokohama, 230-0045, Japan[4]Laboratory of Biomedical Science, Department of Veterinary Medical Science, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan [5]Department of Animal Resource Sciences, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan[6]Research Center for Food Safety, Graduate School of Agricultural andLife Sciences, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan[7]Collaborative Research Institute for Innovative Microbiology, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo, 113-8657, Japan[8]These authors contributed equally to this work.

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by the production of autoantibodies and damage to multiple organs. Glomerulonephritis, a manifestation involving glomerular deposition of immune complexes and complement components, significantly contributes to disease morbidity. Although the endosomal single-stranded RNA sensor TLR7 is known to drive glomerulonephritis by promoting autoantibody production in B cells, the contribution of macrophage TLR7 responses to glomerulonephritis remains poorly understood. Here, we have examined *Tlr7*$^{-/-}$ NZB-WF1 mice and found that TLR7-deficiency ameliorates lupus nephritis by abolishing autoantibody production against RNA-associated antigens, C3 deposition, and macrophage accumulation in glomeruli. Furthermore, TLR7 signaling increased CD31 expression on glomerular endothelial cells and Ly6C^{low} macrophages but not on T and B cells, suggesting that CD31 mediates TLR7-dependent migration of monocyte into glomeruli. Compared to their splenic counterparts, glomerular macrophages produced IL-1β in a TLR7-dependent manner. In addition, single cell RNA sequencing (scRNA-seq) of glomerular macrophages revealed that TLR7 signaling induced expression of lupus associated genes including those encoding Chitinase 3 like 1, ferritin heavy chain 1, IKKε, and complement factor B (CfB). Although serum CfB did not increase in NZBWF1 mice, TLR7-dependent CfB protein expression was detected in glomerular macrophages. In addition, TLR7 signaling promoted C3 cleavage and deposition predominantly on mesangial cells. These findings suggest that TLR7 responses in glomerular macrophages accelerates the progression of glomerulonephritis in NZBWF1 mice.

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Department of Microbiology and Immunology

Division of Molecular Virology
ウイルス病態制御分野

Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	教　授	博士(獣医学)　川　口　　寧
Associate Professor	Akihisa Kato, Ph.D.	准教授	博士(医学)　加　藤　哲　久
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教	博士(生命科学)　小　柳　直　人
Assistant Professor	Yuhei Maruzuru, Ph.D.	助　教	博士(生命科学)　丸　鶴　雄　平

In our laboratory, we are promoting strategic fundamental research aimed at developing a novel method of viral infection control by elucidating the mechanism underlying viral proliferation/pathology. Moreover, using viruses as a biological probe, we are also challenging next-generation virology to reconsider viruses as a homeostasis factor and explore their significance, in addition to unraveling cells and physiological control mechanisms that cannot be elucidated by research on normal human hosts.

1. Identification of a novel neurovirulence factor encoded by the cryptic orphan gene UL31.6 of herpes simplex virus 1

Akihisa Kato, Ryoji Iwasaki, Kousuke Takeshima, Yuhei Maruzuru, Naoto Koyanagi, Tohru Natsume[1], Hideo Kusano[1,2], Shungo Adachi[1,2], Shuichi Kawano[3], and Yasushi Kawaguchi. [1]Molecular Profiling Research Center for Drug Discovery (molprof), National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, [2]Department of Proteomics, National Cancer Center Research institute, Tokyo, [3]Faculty of Mathematics, Kyushu University, Fukuoka

Although the herpes simplex virus type 1 (HSV-1) genome was thought to contain approximately 80 different protein coding sequences (CDSs), recent multi-omics analyses reported HSV-1 encodes more than 200 potential CDSs. However, few of the newly identified CDSs were confirmed to be expressed at the peptide or protein level in HSV-1-infected cells. Furthermore, the impact of the proteins they encode on HSV-1 infection is largely unknown. This study focused on a newly identified CDS, UL31.6. Re-analyzation of our previous chemical proteomics data verified that UL31.6 was expressed at the peptide level in HSV-1-infected cells. Antisera raised against a viral protein encoded by UL31.6 (pUL31.6) reacted with a protein with an approximate molecular mass of 37 kDa in lysates of Vero cells infected with each of three HSV-1 strains. pUL31.6 was efficiently dissociated from virions in high salt solution. A UL31.6-null mutation had a minimal effect on HSV-1 gene expression, replication, cell-to-cell spread, and morphogenesis in Vero cells; in contrast, it significantly reduced HSV-1 cell-to-cell spread in three neural cells but not in four non-neural cells including Vero cells. The UL31.6-null mutation also significantly reduced the mortality and viral replication in the brains of mice after intracranial infection, but had minimal effects on pathogenic manifestations in and around the eyes, and viral replication detected in the tear films of mice after ocular infection. These results indicated that pUL31.6 was a tegument protein and specifically acted as a neurovirulence factor by potentially promoting viral transmission between neuronal cells in the central nervous system.

2. Impact of the Interaction between Herpes Simplex Virus 1 ICP22 and FACT on Viral Gene Expression and Pathogenesis.

Shaocong Liu, Yuhei Maruzuru, Kosuke Takeshima, Naoto Koyanagi, Akihisa Kato, and Yasushi Kawaguchi

Facilitates chromatin transcription (FACT) interacts with nucleosomes to promote gene transcription by regulating the dissociation and reassembly of nucleosomes downstream and upstream of RNA polymerase II (Pol II). A previous study reported that herpes simplex virus 1 (HSV-1) regulatory protein ICP22 interacted with FACT and was required for its recruitment to the viral DNA genome in HSV-1 infected cells. However, the biological importance of interactions between ICP22 and FACT in relation to HSV-1 infection is unclear. Here, we mapped the minimal domain of ICP22 required for its efficient interaction with FACT to a cluster of five basic amino acids in ICP22. A recombinant virus harboring alanine substitutions in this identified cluster led to the decreased accumulation of viral mRNAs from UL54, UL38, and UL44 genes, reduced Pol II occupancy of these genes in MRC-5 cells, and impaired HSV-1 virulence in mice following ocular or intracranial infection. Furthermore, the treatment of mice infected with wild-type HSV-1 with CBL0137, a FACT inhibitor currently being investigated in clinical trials, significantly improved the survival rate of mice. These results suggested that the interaction between ICP22 and FACT was required for efficient HSV-1 gene expression and pathogenicity. Therefore, FACT might be a potential therapeutic target for HSV-1 infection.

3. MYBBP1A is required for efficient replication and gene expression of herpes simplex virus 1

Moeka Nobe, Yuhei Maruzuru, Kosuke Takeshima, Naoto Koyanagi, Akihisa Kato and Yasushi Kawaguchi

More than 100 different herpes simplex virus 1 (HSV-1) genes belong to three major classes, and their expression is coordinately regulated and sequentially ordered in a cascade. This complex HSV-1 gene expression is thought to be regulated by various viral and host cellular proteins. A host cellular protein, Myb-binding protein 1A (MYBBP1A), has been reported to be associated with HSV-1 viral genomes in conjunction with viral and cellular proteins critical for DNA replication, repair, and transcription within infected cells. However, the role(s) of MYBBP1A in HSV-1 infections remains unclear. In this study, we examined the effects of MYBBP1A depletion on HSV-1 infection and found that MYBBP1A depletion significantly reduced HSV-1 replication, as well as the accumulation of several viral proteins. These results suggest that MYBBP1A is an important host cellular factor that contributes to HSV-1 replication, plausibly by promoting viral gene expression.

Publications

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Department of Microbiology and Immunology

Division of Vaccine Science
ワクチン科学分野

Professor	Ken Ishii, M.D., Ph.D.	教　授　博士(医学)	石　井　　健
Associate Professor	Kouji Kobiyama, Ph.D.	准教授　博士(医学)	小檜山　康　司
Assistant Professor	Burcu Temizoz, Ph.D.	助　教　博士(医学)	テミズオズ　ブルジュ
Assistant Professor	Tomoya Hayashi, Ph.D.	助　教　博士(医学)	林　　智　哉

***Primary goal** of our laboratory is to understand the immunological mechanisms of the intra- and inter-cellular signaling pathways that mediate the immunogenicity of successful vaccines, as well as biological responses to adjuvants. Such knowledge will enable us to develop novel concepts, modalities and next generation immuno-preventive and/or therapeutic agents against infectious diseases, cancer and allergy as well as other non-communicable diseases.*

The development of vaccines for COVID-19 (LNP-mRNA, Protein+adjuvant)

Supported by AMED, we contributed to the development of the firs LNP-mRNA vaccine in Japan by Daiichi Sankyo (DS-5670) and the first adjuvanted recombinant protein based vaccine by Shionogi Pharma (S-268019) against SARS-CoV2 virus approved in 2023 and 2024 respectively. doi: 10.1038/s41598-024-57308-3 doi: 10.1126/science.adh0968,

Innovative vaccine evaluation system for 100 days mission

Supported by AMED SCARDA, 2022-, our lab leads the project of the tile above with our colleagues in IMSUT, National Institute of Infectious Diseases (NIID) and National Institute of Biomedical Innovation, Health and Nutrition (NIBIOHN). The purpose of this research is to establish an innovative vaccine evaluation system to provide vaccines to the world in 100 days in the next outbreak, epidemic, or pandemic of infectious diseases. The pandemic of the new coronavirus showed the aspect of a simultaneous worldwide disaster and revealed the importance of global health coverage through international collaboration as well as the importance and urgency of research on vaccine development in one's own country. The G7 countries have set a goal to provide vaccines within the next 100 days. In Japan, the proposal for this research on infectious diseases, immunological research, and animal studies calls for the participation of Japan's top research institutes and their collaboration with CROs in order to ignite an innovation in non-clinical studies for vaccine R&D in Japan. With the ultimate goal of providing vaccines in 100 days, researchers in the four essential areas of pathogen research, infection immunology research, vaccine design research, and animal model research will work together in an organic manner at all times to achieve the ultimate goal of providing vaccines in 100 days. The team of researchers in the four essential areas of pathogen research, infection immunity research, vaccine design research, and animal model research will be organically integrated at all times during normal times, and an implementation system will be established to enable seamless, rapid, accurate, and high-level preclinical drug efficacy testing (humoral and cellular immunity) in the event of an emergency. https://www.amed.go.jp/content/000136233.pdf

5,6-dimethylxanthenone-4-acetic acid (DMXAA), a partial STING agonist, competes for human STING activation

5,6-dimethylxanthenone-4-acetic acid (DMXAA) is a mouse-selective stimulator of interferon gene (STING) agonist exerting STING-dependent anti-tumor activity. Although DMXAA cannot fully activate human STING, DMXAA reached phase III in lung cancer clinical trials. How DMXAA is effective against human lung cancer is completely unknown. Here, we show that DMXAA is a partial STING agonist interfering with agonistic STING activation, which may explain its partial anti-tumor effect observed in humans, as STING was reported to be pro-tumorigenic for lung cancer cells with low antigenicity. Furthermore, we developed a DMXAA derivative-3-hydroxy-5-(4-hydroxybenzyl)-4-methyl-9H-xanthen-9-one (HHMX)-that can potently antagonize STING-mediated immune responses both in humans and mice. Notably, HHMX suppressed aberrant responses induced by STING gain-of-function mutations causing STING-associated vasculopathy with onset in infancy (SAVI) in in vitro experiments. Furthermore, HHMX treatment suppressed aberrant STING pathway activity in peripheral blood mononuclear cells from SAVI patients. Lastly, HHMX showed a potent therapeutic effect in SAVI mouse model by mitigating disease progression. Thus, HHMX offers therapeutic potential for STING-associated autoinflammatory diseases. doi: 10.3389/fimmu.2024.1353336.

Tridecylcyclohexane in incomplete Freund's adjuvant (IFA) is a critical component in inducing experimental autoimmune diseases.

Incomplete Freund's adjuvant (IFA) has been used for many years to induce autoimmune diseases in animal models, including experimental autoimmune encephalitis and collagen-induced arthritis. However, it remains unclear why it is necessary to emulsify autoantigen and heat-killed Mycobacterium tuberculosis (HKMtb) with IFA to induce experimental autoimmune diseases. Here, we found that immunization with self-antigen and HKMtb was insufficient to induce autoimmune diseases in mice. Furthermore, IFA or one of its components, mineral oil, but not mannide monooleate, was required for the development of experimental autoimmune disease. Immunization with autoantigen and HKMtb emulsified in mineral oil facilitated innate immune activation and promoted the differentiation of pathogenic $CD4^+$ T cells, followed by their accumulation in neuronal tissues. Several water-soluble hydrocarbon compounds were identified in mineral oil. Of these, immunization with HKMtb and autoantigen emulsified with the same amount of hexadecane or tridecylcyclohexane as mineral oil induced the development of experimental autoimmune encephalitis. In contrast, immunization with HKMtb and autoantigen emulsified with tridecylcyclohexane, but not hexadecane, at doses equivalent to those found in mineral oil, resulted in neuronal dysfunction. These data indicate that tridecylcyclohexane in mineral oil is a critical component in the induction of experimental autoimmune disease. doi: 10.1002/eji.202350957

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Department of Microbiology and Immunology

Division of Malaria Immunology
マラリア免疫学分野

Professor	Cevayir Coban, M.D., Ph.D. (Clinical Microbiology)		教　授	博士(医学)(臨床微生物学位)	チョバン　ジェヴァイア
Associate Professor	Niloufar Kavian-Tessler, Pharm.D., Ph. D.		准教授	博士(医学)	カビアン・テスラー ニルファー
Assistant Professor	Jalal Alshaweesh, Ph.D.		助　教	博士(医学)	アルシャウィシュ ジャラル
Project Assistant Professor	Michelle S.J. Lee, Ph.D.		特任助教	博士(医学)	リー ミシェル

Summary of Activity (Less than 70 words)

Our laboratory investigates how pathogens interact with the host immune system. Initially specializing in malaria immunology, we have expanded our research to include respiratory viral diseases and neglected parasitic infections like leishmaniasis. Our goal is to deepen understanding of immune responses against these pathogens to enhance vaccine and drug development, with potential applications beyond infectious diseases.

1. Elucidation of host-pathogen interactions

MyD88 in osteoclast and osteoblast lineages differentially controls bone remodeling in homeostasis and malaria. Chronic bone loss is an under-recognized complication of malaria, the underlying mechanism of which remains incompletely understood. We have previously shown that persistent accumulation of *Plasmodium* products in the bone marrow leads to chronic inflammation in osteoblast (OB) and osteoclast (OC) precursors causing bone loss through MyD88, an adaptor molecule for diverse inflammatory signals (*Lee et al., Science Immunology, 2017*). However, the specific contribution of MyD88 signaling in OB or OC precursors in malaria-induced bone loss remains elusive. To assess the direct cell-intrinsic role of MyD88 signaling in adult bone metabolism under physiological and infection conditions, we used the Lox-Cre system to specifically deplete MyD88 in the OB or OC lineages. Mice lacking MyD88 primarily in the maturing OBs showed a comparable decrease in trabecular bone density by microcomputed tomography to that of controls after *Plasmodium yoelii* non-lethal infection. In contrast, mice lacking MyD88 in OC precursors showed significantly less trabecular bone loss than controls, suggesting that malaria-mediated inflammatory mediators are primarily controlled by MyD88 in the OC lineage. Surprisingly, however, depletion of MyD88 in OB, but not in OC, precursors resulted in reduced bone mass with decreased bone formation rates in the trabecular areas of femurs under physiological conditions. Notably, insulin-like growth factor-1, a key molecule for OB differentiation, was significantly lower locally and systemically when MyD88 was depleted in OBs. Our data demonstrated an indispensable intrinsic role for MyD88 signaling in OB differentiation and bone formation, while MyD88 signaling in OC lineages played a partial role in controlling malaria-induced inflammatory mediators and following bone pathology. These findings may lead to the identification of novel targets for specific intervention of bone pathologies, particularly in malaria-endemic regions ***(Alshaweesh et al., International Immunology, 2024. This study was chosen as Featured Article)***.

Acute malaria suppresses the B lymphocytic niche in the bone marrow through the alteration of CXCL12-abundant reticular cells. Bone marrow is a dynamic organ composed of stem cells that constantly receive signals from stromal cells and other hematopoietic cells in the niches of the bone marrow to maintain hematopoiesis and generate immune cells.

Perturbation of the bone marrow microenvironment by infection and inflammation affects hematopoiesis and may affect immune cell development. Little is known about the effect of malaria on the bone marrow stromal cells that govern the hematopoietic stem cell (HSC) niche. In this study, we demonstrated that the mesenchymal stromal CXCL12-abundant reticular (CAR) cell population is reduced during acute malaria infection. The reduction of CXCL12 and interleukin-7 signals in the bone marrow impairs the lymphopoietic niche, leading to the depletion of common lymphoid progenitors, B cell progenitors, and mature B cells, including plasma cells in the bone marrow. We found that interferon-γ (IFNγ) is responsible for the upregulation of Sca1 on CAR cells, yet the decline in CAR cell and B cell populations in the bone marrow is IFNγ-independent. In contrast to the decline in B cell populations, HSCs and multipotent progenitors increased with the expansion of myelopoiesis and erythropoiesis, indicating a bias in the differentiation of multipotent progenitors during malaria infection. Our findings suggest that malaria may affect host immunity by modulating the bone marrow niche ***(Lee et al., International Immunology, 2024. This study was chosen as Featured Article).***

Diagnostic challenges in cutaneous leishmaniasis due to atypical Leishmania infantum. Leishmaniasis, a parasitic infection affecting both humans and animals, is increasingly spreading across Mediterranean and European regions, largely driven by human migration and environmental changes. In countries like Türkiye and across Europe, which have seen large influxes of migrants, the incidence of cutaneous leishmaniasis (CL) is rising, with cases now appearing in cities where the disease was previously undocumented. In these previously non-endemic areas, physicians unfamiliar with the characteristic lesions may misdiagnose CL, particularly in cases with only cutaneous manifestations. This study aimed to evaluate the impact of re-emerging CL on the routine diagnostic practices of pathologists in Türkiye, by retrospectively reviewing cases. We conducted a retrospective analysis of CL cases diagnosed between 2013 and 2022 at a single pathology center in Türkiye, covering multiple provinces. Twelve cases of CL were identified and analyzed based on clinical presentation, pre-diagnosis, histopathological findings, and molecular diagnostics. DNA extraction and PCR were performed on paraffin-embedded tissue samples to identify the *Leishmania* species involved. Out of the twelve CL cases reviewed, seven exhibited morphological findings strongly suggestive of CL (MFSS of CL), warranting further microbiological evaluation. All patients presented with non-healing skin lesions characterized by central ulceration, crater-like formations, or papulonodular lesions. Notably, CL was included in the clinical pre-diagnosis in only 58.3% of cases, while it was not considered in the remaining 41.7% of cases. Clinicians initially pre-diagnosed skin tumors in six cases (50%), four of which led to wide surgical excision. Histopathological examination in all cases revealed chronic or mixed (acute/chronic) inflammation, predominantly rich in histiocytes. To further investigate the role of *Leishmania* species in the pre-diagnosis, DNA extraction and PCR were performed on paraffin-embedded tissue samples, identifying *L. infantum* as the causative agent in 10 cases and *L. major* in two cases. Notably, *L. infantum* was the causative agent in all five cases initially misdiagnosed as skin tumors, which were also associated with a granulomatous type of chronic inflammation ***(Ekemen et al., Frontiers in Medicine, 2024).***

Histone H3.3 variant plays a critical role on zygote-to-oocyst development in malaria parasites. The *Plasmodium* life cycle involves differentiation into multiple morphologically distinct forms, a process regulated by developmental stage-specific gene expression. Histone proteins are involved in epigenetic regulation in eukaryotes, and the histone variant H3.3 plays a key role in the regulation of gene expression and maintenance of genomic integrity during embryonic development in mice. However, the function of H3.3 through multiple developmental stages in *Plasmodium* remains unknown. To examine the function of H3.3, h3.3-deficient mutants (Δh3.3) were generated in *P. berghei*. The deletion of h3.3 was not lethal in blood stage parasites, although it had a minor effect of the growth rate in blood stage; however, the in vitro ookinete conversion rate was significantly reduced, and the production of the degenerated form was increased. Regarding the mosquito stage development of Δh3.3, oocysts number was significantly reduced, and no sporozoite production was observed. The h3.3 gene complemented mutant have normal development in mosquito stage producing mature oocysts and salivary glands contained sporozoites, and interestingly, the majority of H3.3 protein was detected in female gametocytes. However, Δh3.3 male and female gametocyte production levels were comparable to the wild-type levels. Transcriptome analysis of Δh3.3 male and female gametocytes revealed the upregulation of several male-specific genes in female gametocytes, suggesting that H3.3 functions as a transcription repressor of male-specific genes to maintain sexual identity in female gametocytes. This study provides new insights into the molecular biology of histone variants H3.3 which plays a critical role on zygote-to-oocyst development in primitive unicellular eukaryotes ***(Tateishi et al., Parasitology International, 2024).***

2. Adjuvant discovery and development platform

5,6-dimethylxanthenone-4-acetic acid (DMXAA), a partial STING agonist, competes for human STING activation. Stimulator of interferon genes (STING) is one of the key molecules at the intersection of various cytosolic nucleic acid-sensing pathways,

including cyclic GMP-AMP synthase (cGAS), DEAD-box helicase family, and interferon gamma inducible protein. DMXAA is a mouse-selective stimulator of interferon gene (STING) agonist exerting STING-dependent anti-tumor activity. Although DMXAA cannot fully activate human STING, DMXAA reached phase III in lung cancer clinical trials. How DMXAA is effective against human lung cancer is completely unknown. Here, we show that DMXAA is a partial STING agonist interfering with agonistic STING activation, which may explain its partial anti-tumor effect observed in humans, as STING was reported to be pro-tumorigenic for lung cancer cells with low antigenicity. Furthermore, we developed a DMXAA derivative—3-hydroxy-5-(4-hydroxybenzyl)-4-methyl-9H-xanthen-9-one (HHMX)—that can potently antagonize STING-mediated immune responses both in humans and mice. Notably, HHMX suppressed aberrant responses induced by STING gain-of-function mutations causing STING-associated vasculopathy with onset in infancy (SAVI) in in vitro experiments. Furthermore, HHMX treatment suppressed aberrant STING pathway activity in peripheral blood mononuclear cells from SAVI patients. Lastly, HHMX showed a potent therapeutic effect in SAVI mouse model by mitigating disease progression. Thus, HHMX offers therapeutic potential for STING-associated autoinflammatory diseases ***(Temizoz et al., Frontiers Immunology, 2024).***

3. Infection and beyond

Our previous research demonstrated that Lipocalin 2 (LCN2), also known as siderocalin or neutrophil gelatinase-associated lipocalin (NGAL), enhances innate and adaptive immune responses in malaria by modulating iron metabolism (*Zhao et al., Cell Host Microbe, 2012*). Interestingly, LCN2 expression is also elevated in cancer, highlighting its broader role beyond infection. In tumorigenesis, alongside somatic mutations, stroma-associated immunity significantly influences tumor progression. Tumor cells create a supportive microenvironment by releasing mediators, attracting monocytes and leukocytes, and disrupting iron balance through excessive consumption, potentially upregulating LCN2 as an intracellular iron transporter. Recently, we investigated the expression of LCN2 and the immune checkpoint molecule programmed cell death ligand-1 (PD-L1) in breast cancers across molecular subtypes. This retrospective analysis of 89 primary breast cancer cases revealed that LCN2 expression correlates with poor prognostic factors, including high histological grade, elevated Ki-67 proliferation index, and ER/PR negativity. Elevated LCN2 and PD-L1 expressions were significantly associated with triple-negative and HER2-positive breast cancers. These findings demonstrate the prognostic potential of LCN2 and its relevance in immune modulation within the tumor microenvironment. Furthermore, this research suggests the potential for immunotherapeutic applications of LCN2, advancing breast cancer management ***(Ekemen et al., Breast Cancer: Targets and Therapy, 2024).*** By bridging infection and cancer research, our work demonstrates the versatile roles of LCN2 in regulating immunity and iron metabolism, offering insights into its therapeutic potential in diverse pathological contexts.

Publications

Ekemen S, Nalcaci M, Toz S, Sanjoba C, Demirkesen C, Cetin ED, Tecimer T, Yildiz P, Gursel M, Ince U, Ozbel Y, Coban C. Diagnostic challenges in cutaneous leishmaniasis due to atypical *Leishmania infantum*: pathologists' insights from re-emergence zones. Front Med (Lausanne). 2024 Sep 12;11:1453211. doi: 10.3389/fmed.2024.1453211. eCollection 2024.

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Temizoz B, Shibahara T, Hioki K, Hayashi T, Kobiyama K, Lee MSJ, Surucu N, Sag E, Kumanogoh A, Yamamoto M, Gursel M, Ozen S, Kuroda E, Coban C, Ishii KJ. 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a partial STING agonist, competes for human STING activation. Front Immunol. 2024 Mar 12;15:1353336. doi: 10.3389/fimmu.2024.1353336. eCollection 2024.

Lee MSJ, Matsuo Dapaah J, Del Rosario Zorrilla C, Omatsu Y, Nagasawa T, Uemura S, Iwama A, Ishii KJ, Coban C. Acute malaria suppresses the B lymphocytic niche in the bone marrow through the alteration of CXCL12-abundant reticular cells. International Immunology, 2024; dxae012. https://doi.org/10.1093/intimm/dxae012. **Editor's Choice**

Tateishi YS, Araki T, Kawai S, Koide S, Umeki Y, Imai T, Saito-Nakano Y, Kikuchi M, Iwama A, Hisaeda H, Coban C, Annoura T. Histone H3.3 variant plays a critical role on zygote-to-oocyst development in malaria parasites. Parasitol Int. 2024 Jan 9;100:102856. doi: 10.1016/j.parint.2024.102856.

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Department of Microbiology and Immunology

Division of Systems Virology
システムウイルス学分野

Professor	Kei Sato, Ph.D.	教　授	博士(医学)	佐　藤　　佳
Associate Professor	Jumpei Ito, Ph.D., D.V.M.	准教授	博士(理学)	伊　東　潤　平
Project Assistant Professor	Yu Kaku, Ph.D., M.D.	特任助教	博士(医学)	郭　　悠

The aim of our laboratory is to expand the knowledge and methodology on virology, which were unable to shed light on by conventional experimental approach alone. To investigate the co-evolutionary relationship between viruses and hosts, we perform bioinformatic and molecular phylogenetic analyses as well as experimental virology. The interdisciplinary investigations based on experimental virology and other scientific fields/methods will pioneer a new science for deeply understanding infectious diseases.

1. Understanding the evolution of SARS-CoV-2

Yu Kaku, Keiya Uriu, Kaho Okumura, Luo Chen, Jarel Elgin Tolentino, Maximilian Stanley Yo, Yusuke Kosugi, Arnon Plianchaisuk, Shusuke Kawakubo, Ziyi Guo, Alfredo Amolong Hinay, Jr., Kaoru Usui, Wilaiporn Saikruang, Luca Nishimura, Spyridon Lytras, Shigeru Fujita, Lin Pan, Wenye Li, Yukun Zhu, Ruojin Tian, Yueying Zhang, Naoko Misawa, Mai Suganami, Adam Patrick Strange, Naomi Ohsumi, Shiho Tanaka, Eiko Ogawa, Mika Chiba, Kyoko Yasuda, Keiko Iida, Tsuki Fukuda, Tamaki Yoshihara, Keiko Koizumi, Hiroaki Unno, Jumpei Ito, Kei Sato.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a causative agent of coronavirus disease 2019 (COVID-19). SARS-CoV-2 emerged at the end of 2019 and has spread all over the world. Since then, more than 770 million people have been infected with this virus and more than 7 million people have died of COVID-19, meaning that COVID-19 is ongoing pandemic and a most urgent and crucial problem in the current human society. To proceed and accelerate COVID-19-releated researches in Japan, we launched a consortium, called "The Genotype to Phenotype Japan (G2P-Japan) Consortium" in January 2021. As of December 2024, more than 10 principal investigators in Japan and Czech Republic join this consortium and proceed fruitful collaboration. We aim to elucidate the virological characteristics of the SARS-CoV-2 variants continuously emerging in the world.

2. Predicting viral fitness and evolution

Spyridon Lytras, Adam Patrick Strange, Shusuke Kawakubo, Arnon Plianchaisuk, Luca Nishimura, Kaho Okumura, Mai Suganami, Hiroaki Unno, Jumpei Ito, Kei Sato.

One of the reasons controlling viral infections is challenging is that viruses evolve and change their characteristics over time. Leveraging the vast viral genome information obtained through genomic epidemiological studies, phenotypic data measured by high-throughput experiments, and state-of-the-art machine learning techniques such as protein language models, it is expected to become possible to predict viral phenotypes directly from their genotypes. This year, we developed a protein model called CoVFit, which predicts the fitness (transmissibility) of SARS-CoV-2 variants based on their spike proteins. CoVFit enables the prediction of the fitness of previ-

ously unseen variants that had not emerged at the time the model was trained. Furthermore, using CoV-Fit for evolutionary simulations, we demonstrated the ability to predict which mutations a virus is likely to acquire next, effectively forecasting viral evolution.

Publications

Yu Kaku, Kaho Okumura, Miguel Padilla-Blanco, Yusuke Kosugi, Keiya Uriu, Alfredo A. Hinay, Luo Chen, Arnon Plianchaisuk, Kouji Kobiyama, Ken J. Ishii, Genotype to Phenotype Japan (G2P-Japan) Consortium, Jiri Zahradnik, Jumpei Ito, Kei Sato. Virological Characteristics of the SARS-CoV-2 JN.1 Variant. **Lancet Infectious Diseases** 24(2):e82 (2024).

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Department of Cancer Biology

Division of Genetics
腫瘍抑制分野

Professor	Yuji Yamanashi, Ph.D.		教　授	理学博士	山　梨　裕　司
Associate Professor	Akane Inoue-Yamauchi, Ph.D.		准教授	博士(医学)	山内（井上）茜
Assistant Professor	Wu Ji, Ph.D.		助　教	博士(科学)	吴　　际

The major interest of this division is in molecular signals that regulate a variety of cellular activities. Our aim is to address and elucidate how dysregulated cellular signals give rise to neoplastic, immune, neural, metabolic, or developmental disorders. Our goal is to understand the molecular bases of tumorigenesis and the development of other intractable diseases as a path toward uncovering therapeutic targets. Currently, we are investigating regulatory mechanisms in protein-tyrosine kinase (PTK)-mediated signaling pathways, their pathophysiological roles and the potential for therapeutic intervention.

1. Activation of the receptor tyrosine kinase MuSK by the cytoplasmic protein Dok-7 in neuromuscular synaptogenesis.

Inoue-Yamauchi, A., Eguchi, T.[1], Tokuoka, T., Zhong, Z., Yoda, M., Hwang, J., Ueta, R., Tezuka, T.[2], Weatherbee, SD.[3], Watanabe, Y.[4], Sagara, H.[4], Nagatoishi, S.[4], Tsumoto, K.[4], and Yamanashi, Y.: [1,2]Present affiliation: [1]Brain-Skeletal Muscle Connection in Aging Project Team, Geroscience Research Center, National Center for Geriatrics and Gerontology and [2]Center for the Promotion of Interdisciplinary Education and Research, Kyoto University. [3]Department of Genetics, Yale University. [4]Medical Proteomics Laboratory, IMSUT.

Protein-tyrosine kinases (PTKs) play crucial roles in a variety of signaling pathways that regulate proliferation, differentiation, motility, and other activities of cells. Therefore, dysregulated PTK signals give rise to a wide range of diseases such as neoplastic disorders. To understand the molecular bases of PTK-mediated signaling pathways, we identified Dok-1 as a common substrate of many PTKs in 1997. Since then, the Dok-family has been expanded to seven members, Dok-1 to Dok-7, which share structural similarities characterized by N-terminal pleckstrin homology (PH) and phosphotyrosine binding (PTB) domains, followed by Src homology 2 (SH2) target motifs in the C-terminal moiety, suggesting an adaptor function. Indeed, as described below, Dok-1 and Dok-2 recruit p120 rasGAP upon tyrosine phosphorylation to suppress Ras-Erk signaling. However, we found that Dok-7 acts as an essential cytoplasmic activator of the muscle-specific receptor tyrosine kinase (RTK) MuSK in the formation and maintenance of the neuromuscular junction (NMJ), providing a new insight into RTK-mediated signaling. It seems possible that local levels of cytoplasmic activators, like Dok-7, control the activity of RTKs in concert with their extracellular ligands.

The NMJ is a synapse between a motor neuron and skeletal muscle, where the motor nerve terminal is apposed to the endplate (the region of synaptic specialization on the muscle). The contraction of skeletal muscle is controlled by the neurotransmitter acetylcholine (ACh), which is released from the presynaptic motor nerve terminal. To achieve efficient neuromuscular transmission, acetylcholine receptors (AChRs) must be densely clustered on the postsynaptic muscle membrane of the NMJ. Failure of AChR clustering is associated with disorders of neuromuscular transmis-

sion such as congenital myasthenic syndromes (CMS) and myasthenia gravis (MG), which are characterized by fatigable muscle weakness. The formation of NMJs is orchestrated by MuSK and by neural agrin, an extracellular activator of MuSK. However, experimentally when motor nerves are ablated, AChRs form clusters in the correct, central region of muscle during embryogenesis in a MuSK-dependent process known as prepatterning of the receptors. In addition, in vivo overexpression of MuSK causes neuromuscular synapse formation in the absence of agrin, suggesting that muscle-intrinsic, cell-autonomous activation of MuSK may be adequate to trigger presynaptic and postsynaptic differentiation in vivo. However, the mechanisms by which MuSK is activated independently of nerve and agrin had long been unclear.

Because both MuSK and the adaptor-like cytoplasmic protein Dok-7 are localized to the postsynaptic region of NMJs, we examined their interaction and found that Dok-7 is an essential cytoplasmic activator of MuSK. In addition, we found that Dok-7 directly interacts with the cytoplasmic portion of MuSK and activates the RTK, and that neural agrin requires Dok-7 in order to activate MuSK. Indeed, in vivo overexpression of Dok-7 increased MuSK activation and promoted NMJ formation. Conversely, mice lacking Dok-7 formed neither NMJs nor AChR clusters. In addition, we found that postnatal knockdown of *dok-7* gene expression in mice causes structural defects in NMJs and myasthenic pathology, suggesting an essential role for Dok-7 not only in the embryonic formation but also in the postnatal maintenance of NMJs. Furthermore, we found that forced expression of Dok-7 lacking the C-terminal region rescued Dok-7 knockout mice from neonatal lethality caused by the lack of NMJs, indicating restored MuSK activation and NMJ formation. However, these mice showed only marginal activation of MuSK and died by 3 weeks of age apparently due to an abnormally small number and size of NMJs. Therefore, Dok-7's C-terminal region plays a key, but not fully essential, role in MuSK activation and NMJ formation.

Interestingly, mice lacking Lrp4, which forms a complex with MuSK and acts as an essential agrin-binding module, do not show MuSK-dependent AChR prepatterning or NMJ formation. This suggests that Lrp4 is required for MuSK activation under physiological conditions, in contrast to our observation that Dok-7 can activate MuSK in the absence of Lrp4 or its ligand agrin, at least in vitro. Thus, we examined the effects of forced expression of Dok-7 in skeletal muscle on NMJ formation in the absence of Lrp4 and found that it indeed induces MuSK activation in mice lacking Lrp4. However, the activation level of MuSK was significantly lower in the absence of Lrp4. Together, these data indicate that Lrp4 is required for efficient activation of MuSK by Dok-7 in the muscle. Since Lrp4 is also essential for presynaptic differentiation of motor nerve terminals in the embryonic NMJ formation (*Nature* 489:438-442, 2012), this apparent cooperation between Lrp4 and Dok-7 in MuSK activation may be complicated.

The NMJs are cholinergic synapses characterized by ultrastructural specializations, including the presynaptic active zones, the acetylcholine (ACh) release sites of the motor nerve terminal, and the postsynaptic junctional folds of muscle membrane, where ACh receptors (AChRs) cluster in the nearby areas of active zones for efficient neuromuscular transmission. Interestingly, overexpression of Dok-7 in skeletal muscle abnormally activates MuSK, leading to the formation of abnormally large NMJs in mice. However, these mice with abnormally large NMJs show no obvious motor dysfunction. Recently, we have found that Dok-7 overexpression enhances NMJ transmission less markedly than NMJ size. Consistent with this, ultrastructural analyses revealed that the densities of active zones and synaptic vesicles in the presynaptic motor nerve terminals were reduced. In addition, the density and size of postsynaptic junctional folds in the muscle membrane were also reduced. Moreover, terminal Schwann cells (tSCs) exhibits significantly greater penetration of their processes into the synaptic clefts, which connect the pre- and post-synaptic specializations. Together, our findings demonstrate that forced expression of Dok-7 in muscle enhances neuromuscular transmission with significant enlargement and ultrastructural alterations of NMJs, implying increased robustness of neuromuscular transmission. We are investigating Dok-7/MuSK-mediated signaling, including downstream effectors, in regulating formation, maintenance and function of NMJs to develop mechanism-based therapies for NMJ disorders. Recently, we have identified novel downstream genes/proteins critical for NMJ formation and maintenance, including calcium-binding protein 7 (Cabp7) (see below).

2. Cabp7 negatively regulates age-related degeneration of NMJs.

Inoue-Yamauchi, A., Eguchi, T., Tezuka, T., Watanabe, Y., Sagara, S., Ozawa, M.[1], and Yamanashi Y.: [1]Core Laboratory for Developing Advanced Animal Models, IMSUT.

As mentioned above, formation and maintenance of NMJs in the central region of the skeletal muscle are governed by MuSK. Interestingly, the transcripts and protein products of AChR subunit and other NMJ-related genes are expressed and accumulated also in the central, synaptic region of myotube in a manner dependent on MuSK. Indeed, we previously reported that midmuscle expression of AChR subunit gene Chrna1 and MuSK transcripts are lost or enhanced in mouse embryos lacking the essential MuSK activator Dok-7 or overexpressing it specifically in skeletal muscle, respectively. Thus, to identify

NMJ-related genes required for the formation and/or maintenance of NMJs, we performed RNA sequencing analysis of the synaptic and extrasynaptic regions of diaphragm muscles in WT mice (3 months old) and found that Cabp7 gene is the most upregulated among significantly upregulated genes in the synaptic region in comparison with the extrasynaptic region. Furthermore, we found that the expression level of the Cabp7 gene is significantly higher in the synaptic region in Dok-7 transgenic (Tg) mice, which overexpress Dok-7 specifically in skeletal muscle, than in wild-type (WT) mice (3 months old). Also, whole-mount in situ hybridization analysis on embryos confirmed that Cabp7 transcripts are specifically expressed in the central region of the diaphragm muscle in Dok-7 Tg and wild-type mice and that the synaptic expression is significantly enhanced in Dok-7 Tg mice compared with that in WT mice. These findings together indicates that Dok-7-MuSK axis regulates expression of Cabp7 gene in skeletal muscle.

To explore the role for Cabp7 in muscle, we generated Cabp7 cKO mice, which lacks Cabp7 protein specifically in skeletal muscle, and found that Cabp7 cKO mice showed a significant reduction in motor function and muscle strength at 12 and 24, but not 3 or 6, months of age in comparison with the controls, as determined by rotarod, forelimb grip, and hindlimb twitch/tetanic force tests. Furthermore, Cabp7 cKO mice showed acceleration of age-related degeneration of NMJs as early as 6 to 12 months of age; namely, axonal swelling, nerve sprouting, denervation and size-reduction of NMJs. Because impaired NMJ function may lead to muscle atrophy and weakness as observed in patients with myasthenia, we investigated if muscle-specific deletion of Cabp7 in mice affects muscle homeostasis and found that tibialis anterior and gastrocnemius muscle masses were significantly reduced in Cabp7 cKO mice at 12 and 24, but not 3 or 6, months of age in comparison with the controls. In addition, the myofiber cross-section area (CSA) of gastrocnemius muscles was significantly reduced in Cabp7 cKO mice at 12 and 24, but not 3, months of age and showed significant shifts in CSA distribution with a higher frequency of small fibers in comparison with the controls. Taken together, these findings indicates that Cabp7 plays a protective role against age-related NMJ degeneration, muscle weakness and atrophy, and motor dysfunction. Indeed, Cabp7 cKO mice showed a lifespan about 8 weeks shorter than the control mice.

In addition, we found that Cabp7 cKO mice showed increased protein expression of p25, a potent activator of Cdk5, in the tibialis anterior muscle at 3 and 12 months of age in comparison with the control mice. Because Cdk5 negatively regulates NMJ formation and maintenance, we generated Adeno-associated virus-based vector (AAV-p25), which expressed p25 in myotubes under the control of the muscle-specific CK8 promoter, and found that AAV-p25 administration induces NMJ degeneration. Moreover, we also generated AAV-CIP, which expressed Cdk5 Inhibitory Peptide (CIP), and found that AAV-CIP administration restores NMJ integrity and muscle strength, and heals muscle atrophy in Cabp7 cKO mice. We are currently investigating how Dok-7-MuSK-Cabp7-p25-Cdk5 axis contributes to NMJ homeostasis and how CIP expression counteracts NMJ degeneration, muscle weakness and atrophy caused by the loss of Cabp7.

3. Agrin's role aside from MuSK activation in the postnatal maintenance of NMJs.

Inoue-Yamauchi, A., Eguchi, T., Tezuka, T., Mao, Y., Fan, W., Ochiai, C., Ma, WT., Burgess, RW.[1], Ueta, R., and Yamanashi, Y.: [1]The Jackson Laboratory.

Although NMJ formation requires agrin under physiological conditions, it is dispensable for NMJ formation experimentally in the absence of the neurotransmitter acetylcholine, which inhibits postsynaptic specialization. Thus, it was hypothesized that MuSK needs agrin together with Lrp4 and Dok-7 to achieve sufficient activation to surmount inhibition by acetylcholine. To test this hypothesis, we examined the effects of forced expression of Dok-7 in skeletal muscle on NMJ formation in the absence of agrin and found that it indeed restores NMJ formation in agrin-deficient embryos. However, these NMJs rapidly disappeared after birth, whereas exogenous Dok-7-mediated MuSK activation was maintained. These findings indicate that the MuSK activator agrin plays another role essential for the postnatal maintenance, but not for embryonic formation, of NMJs. Because pathogenic mutations of agrin in patients with congenital myasthenic syndromes (see below) did not show impaired ability to activate MuSK at least in vitro (*Am. J. Hum. Genet.*, 85:155-167, 2009; *JCI Insight*, 5:e132023, 2020), the novel role of agrin may be relevant to pathogenicity of the mutations. We are investigating molecular mechanisms underlying the agrin-mediated postnatal maintenance of NMJs by utilizing mice expressing various forms of agrin mutants, including those related to congenital myasthenic syndromes (see below).

4. Pathophysiological mechanisms underlying *DOK7* myasthenia.

Inoue-Yamauchi, A., Eguchi, T., Tezuka, T., Ueta, R., Fukudome, T.[1], Watanabe, Y., Sagara, H., Motomura, M.[2], Beeson, DMW.[3], and Yamanashi, Y.: [1]Department of Neurology, Nagasaki Kawatana Medical Center. [2]Department of Engineering, Faculty of Engineering, Nagasaki Institute of Applied Science. [3]Weatherall Institute of Molecular Medicine, University of Oxford.

As mentioned above, impaired clustering of AChRs could underlie NMJ disorders, be they autoimmune (myasthenia gravis) or genetic (congenital myasthenic syndromes (CMSs)) in origin. Therefore, our findings that Dok-7 activates MuSK to cluster AChRs and to form NMJs suggested *DOK7* as a candidate gene for mutations associated with CMS. Indeed, we demonstrated that biallelic mutations in *DOK7* underlie a major subgroup of CMS with predominantly proximal muscle weakness that did not show tubular aggregates on muscle biopsy but were found to have normal AChR function despite abnormally small and simplified NMJs. We further demonstrated that several mutations, including one associated with the majority of patients with the disease, impaired Dok-7's ability to activate MuSK. This new disease entity is termed "*DOK7* myasthenia."

To investigate pathophysiological mechanisms underlying *DOK7* myasthenia, we established knock-in mice (Dok-7 KI mice) that have a mutation associated with the majority of patients with *DOK7* myasthenia. Dok-7 KI mice showed characteristic features of severe muscle weakness and died by postnatal day 21. Furthermore, they showed abnormally small NMJs lacking postsynaptic folding, a pathological feature seen in patients with *DOK7* myasthenia. Consistent with this, Dok-7 KI mice exhibited decreased MuSK activity in skeletal muscle, indicating that the Dok-7 KI mice develop defects similar to those found in patients with *DOK7* myasthenia, although the mice exhibit a more severe phenotype. In collaboration with Prof. David Beeson's group, we examined NMJ formation, maintenance and functions in the Dok-7 KI mice in the absence or presence of salbutamol, a β2-adrenergic agonist, which is an effective treatment for *DOK7* myasthenia. This study revealed that salbutamol can increase NMJ number and enhance its function together with lifespan in Dok-7 KI mice, suggesting a similar mode of action in patients. We are investigating molecular pathways underlying NMJ defects and muscle weakness in Dok-7 KI mice to develop mechanism-based therapeutic approaches against *DOK7* myasthenia.

5. *DOK7* gene therapy that enlarges and regenerates NMJs.

Inoue-Yamauchi, A., Wu J., Eguchi, T., Ueta, R., Lin, S., Sugita, S.[1], Minegishi, Y.[1], Motomura, M., Beeson, DMW., Shimotoyodome, A.[1], Ota, N.[1], Ogiso, N.[2], Takeda, S.[3], Okada, T.[4], and Yamanashi, Y.: [1]Biological Science Research, Kao Corporation. [2]Laboratory of Experimental Animals, National Center for Geriatrics and Gerontology. [3]Department of Molecular Therapy, National Institute of Neuroscience. [4]Division of Molecular and Medical Genetics, Center for Gene and Cell Therapy, IMSUT

As mentioned above, *DOK7* myasthenia is associated with impaired NMJ formation due to decreased ability of Dok-7 to activate MuSK in myotubes at least in part. Interestingly, in vivo overexpression of Dok-7 increased MuSK activation and promoted NMJ formation in the correct, central region of the skeletal muscle. Because these genetically manipulated mice did not show obvious defects in motor activity, overexpression of Dok-7 in the skeletal muscle of patients with *DOK7* myasthenia might ameliorate NMJ formation and muscle weakness. To test this possibility, we generated an Adeno-associated virus-based vector (AAV-D7), which strongly expressed human Dok-7 in myotubes and enhanced MuSK activation and AChR cluster formation. Indeed, therapeutic administration of AAV-D7 to Dok-7 KI mice described above resulted in enlargement of NMJs and substantial increases in muscle strength and life span. Furthermore, when applied to model mice of another neuromuscular disorder, autosomal dominant Emery-Dreifuss muscular dystrophy, therapeutic administration of AAV-D7 (*DOK7* gene therapy) likewise resulted in enlargement of NMJs as well as positive effects on motor activity and life span. Interestingly, *DOK7* gene therapy suppressed denervation (nerve detachment) at NMJs, and enhanced motor activity and life span in a mouse model of familial amyotrophic lateral sclerosis (ALS), a progressive motor neurodegenerative disease with severe muscle atrophy. These results suggest potential for *DOK7* gene therapy in age-related decline in motor function, where NMJ denervation appears to play a crucial role similar to that observed in ALS model mice. Indeed, we found that *DOK7* gene therapy significantly enhances motor function and muscle strength together with NMJ reinnervation in aged mice. We are further investigating the effects, including ultrastructural and electrophysiological ones, of AAV-D7 administration in multiple types of muscle weakness.

6. Roles of Dok-1 to Dok-6.

Inoue-Yamauchi, A., Wu, W., Sato, T., Jozawa, H., Kanno, T., Arimura, S.[1], Katayama, K.[2], Imoto, S.[2], Nunès J. A.[3], Andre A.[4], and Yamanashi, Y.: [1]Present affiliation: Department of Medicine Section of Gastroenterology and Hepatology, Baylor College of Medicine. [2]Laboratorye of Sequence Analysis, Human Genome Center, IMSUT. [3]Centre de Recherche en Cancérologie de Marseille, CRCM, Immunity and Cancer Team, Institut Paoli-Calmettes, Inserm, CNRS, Aix Marseille Univ, Marseille. [4]The Laboratory of Molecular Oncology, Institut de recherches cliniques de Montréal.

Dok-family proteins can be classified into three subgroups based on their structural similarities and expression patterns; namely, 1) Dok-1, -2, and -3, which are preferentially expressed in hematopoietic

cells, 2) Dok-4, -5, and -6, which are preferentially expressed in non-hematopoietic cells, and 3) Dok-7, which is preferentially expressed in muscle cells. As mentioned above, Dok-1 and its closest paralog, Dok-2, recruit p120 rasGAP upon tyrosine phosphorylation to suppress Ras-Erk signaling. Although Dok-3 does not bind with p120 rasGAP, it also inhibits Ras-Erk signaling. Consistently, we demonstrated that Dok-1, Dok-2 and Dok-3 are key negative regulators of hematopoietic growth and survival signaling. For example, Dok-1, Dok-2, and Dok-3 cooperatively inhibit macrophage proliferation and *Dok-1*$^{-/-}$*Dok-2*$^{-/-}$ *Dok-3*$^{-/-}$ mice develop histiocytic sarcoma, an aggressive malignancy of macrophages. Also, we found that Dok-1 and Dok-2 negatively regulate intestinal inflammation in the dextran sulfate sodium-induced colitis model, apparently through the induction of IL-17A and IL-22 expression. However, we found that Dok-1/-2 and Dok-3 play distinctive roles in cell fusion and proliferation during osteoclastogenesis and cooperatively protect mice from osteopenia. Additionally, we recently demonstrated that Dok-1/-2 and Dok-3 play distinctive roles in intestinal tumor growth and malignant progression. Interestingly, Dok-3 deficiency in non-tumor cells induces malignant conversion of benign tumors without intensifying mutagenesis in tumors, providing a new insight into the regulation of tumor malignant progression. We are currently investigating molecular mechanisms underlying the Dok-3-mediated suppression of malignant progression of intestinal tumors, which may lead to developing new therapeutic approaches against non-tumor cell-driven malignant progression. Also, we are investigating physiological and pathological roles of Dok-1 to Dok-6, including those in T cell memory, macrophage function, tumor malignancy, inflammatory disorders and tissue injury.

7. Omic analyses.

Inoue-Yamauchi, A., Eguchi, T., Jozawa, H., Fan, W., Tokuoka, Y., Yoda, M., Wu, W., Ueta, R., Iemura, S.[1], Natsume, T.[2], Kozuka-Hata, H.[3], Oyama, M.[3], Katayama, K., Imoto, S., and Yamanashi, Y.: [1]Translational Research Center, Fukushima Medical University. [2]National Institute of Advanced Science and Technology, Molecular Profiling Research Center for Drug Discovery. [3]Medical Proteomics Laboratory, IMSUT.

To gain insights into signaling mechanisms underlying a variety of physiological and pathophysiological events, including NMJ formation, muscle atrophy, neurodegeneration, inflammation, tumorigenesis, and tumor metastasis, we have performed proteomic and transcriptomic analyses. We are investigating the roles of candidate proteins and genes that appear to be involved in each of these biological events. For instance, we are conducting transcriptomic and phospho-proteomic analyses related to muscle weakness due to defects of cytoplasmic protein kinases. In addition, we have prepared experimental settings for other omic approaches such as metabolomic analysis.

8. Screening of chemical compound and siRNA libraries.

Inoue-Yamauchi, A., Hwang, J., Eguchi, T., Tsoumpra, M., Ueta, R., Nagatoishi, S., Tsumoto, K., and Yamanashi, Y.

In addition to the omic analyses described above, we performed high throughput screenings of chemical compound and siRNA libraries, aiming to intervene in pathogenic signals or to gain insights into signaling mechanisms underlying NMJ formation. We are investigating in vivo and in vitro effects, including therapeutic ones in mouse models of human diseases, of hit compounds or down- or up-regulation of candidate genes, and continue the ongoing screenings to further collect appropriate hit compounds and candidate genes that may be involved in the regulation of NMJ formation. We are also investigating target proteins, including those in humans, for the hit compounds or protein products of the candidate genes to understand their modes of actions.

Publication

Eguchi T, Tezuka T, Watanabe Y, Inoue-Yamauchi A, Sagara H, Ozawa M, and Yamanashi Y. Calcium-binding protein 7 expressed in muscle negatively regulates age-related degeneration of neuromuscular junctions in mice. ***iScience*** 27:108997, 2024

Laletin V, Bernard P.-L., Monstersino C, Yamanashi Y, Olive D, Castellarno R, Guittard G, Nunès J. A. DOK1 and DOK2 regulate CD8 T cell signaling and memory formation without affecting tumor cell killing. ***Sci. Rep.*** 14:15053, 2024

Department of Cancer Biology

Division of Cancer Cell Biology
癌防御シグナル分野

Professor	Makoto Nakanishi, M.D., Ph.D.	教　授	医学博士	中　西　　真
Associate Professor	Atsuya Nishiyama, Ph.D.	准教授	博士(理学)	西　山　敦　哉
Assistant Professor	Satoshi Kawakami, Ph.D.	助　教	博士(理学)	川　上　聖　司
Project Assistant Professor	Yoshimi Imawari, M.D., Ph.D.	特任助教	博士(医学)	井　廻　良　美

There is some evidence that senescent cells play an important role in aging and healthy lifespan. However, little is known about the molecular basis of aging-related pathologies. Our research is focused on understanding the common pathologies underlying a variety of aging-related diseases. Currently, we are interested in the role of p16-positive senescent cells in the age-dependent decline of various organ functions and the mechanism of senescent cell accumulation with aging. In addition, we are focusing on the mechanism underlying the accumulation of abnormal proteins as a cause of aging. By understanding the degradation mechanisms of misfolded proteins, we are promoting research on abnormal cellular functions caused by the accumulation of protein aggregates, especially in the pathogenesis of neurodegenerative diseases. We are also investigating the molecular link between DNA methylation and the maintenance of genome stability.

1. Pre-existed senescent fibroblasts in aged bladder create tumor-permissive niche via CXCL12 expression

Satoru Meguro, Yoshikazu Johmura[1], Teh-Wei Wang, Satoshi Kawakami, Shota Tanimoto, Satotaka Omori, Yuki T. Okamura, Seiji Hoshi[2], Emina Kayama[2], Kiyoshi Yamaguchi[3], Seira Hatakeyama[3], Satoshi Yamazaki[4], Eigo, Shimizu[5], Seiya Imoto[5], Yoichi Furukawa[3], Yoshiyuki Kojima2, and Makoto Nakanishi:

[1]Division of Cancer and Senescence Biology, Cancer Research Institute, Kanazawa University, [2]Department of Urology, Fukushima Medical University School of Medicine

[3]Division of Clinical Genome Research, [4]Division of Stem Cell Biology, Center for Stem Cell Biology and Regenerative Medicine, 5Division of Health Medical Intelligence, Human Genome Center, IMSUT, University of Tokyo.

Aging is a major risk factor for cancer. The incidence of most cancers increases abruptly after the sixth decade of life. Therefore, cancer is considered an age-related disease, although the molecular and mechanistic links between aging and cancer remain largely unknown. All cancers acquire, to a greater or lesser extent, gene mutations in either or both proto-oncogenes and tumor suppressor genes that drive malignant transformation and cancer progression. The critical role of the accumulation of driver gene mutations during carcinogenesis may explain the reason why cancer incidence increases with age. Recently, several lines of evidence have suggested that the age-related increase in cancer incidence is not simply the result of the accumulation of gene mutations but is also regulated by biological processes.

Here, using genetically modified mouse models, we show that $p16^{high}$ senescent fibroblasts ($p16^{h}$-sn fibroblasts) accumulate with age, constitute inflammatory cancer-associated fibroblasts (iCAFs), and promote tumor growth in bladder cancer models. Single-cell RNA sequencing of fibroblasts in aged mice revealed higher expression of *Cxcl12* in $p16^{h}$-sn

fibroblasts than in p16^{low} fibroblasts. Elimination of p16^{h}-sn cells or inhibition of CXCL12 signaling significantly suppressed bladder tumor growth *in vivo*. Bladder cancer is one of the most challenging cancers with a poor prognosis. As systemic therapies for metastatic urothelial carcinoma, in addition to typical cisplatin-based combination regiments or immune checkpoint inhibitors, the identification of the practicality of novel agents, such as FGFR tyrosine kinase inhibitors and enfortumab vedotin, has been integrated. However, 5-year survival rates for patients with muscle-invasive bladder cancer are still unsatisfactory. We identified the high expression of *SMOC2*, *GUCY1A1* (*GUCY1A3*), *CXCL12*, *CRISPLD2*, *GAS1,* and *LUM* as a p16^{high} senescent CAFs signature in mice and humans, which was associated with age and poor prognosis of advanced and non-advanced bladder cancer patients. Our results suggest that p16^{h}-sn fibroblasts in the aged bladder serve as a cancer-permissive niche and promote tumor growth by secreting CXCL12.

2. Signaling networks in cancer stromal senescent cells establish malignant microenvironment

Yue Zhang, Teh-Wei Wang, Maho Tamatani, Xinyi Zeng, Lindo Nakamura, Satotaka Omori, Kiyoshi Yamaguchi[1], Seira Hatakeyama[1], Eigo Shimizu[2], Satoshi Yamazaki[3], Yoichi Furukawa[1], Seiya Imoto[2], Yoshikazu Johmura[4], Makoto Nakanishi

[1]Division of Clinical Genome Research, [2]Division of Health Medical Intelligence, [3]Division of Stem Cell Biology, IMSUT, The University of Tokyo, [4]Division of Cancer and Senescence Biology, Cancer Research Institute, Kanazawa University

The tumor microenvironment (TME) encompasses various cell types, blood and lymphatic vessels, and non-cellular constituents like extracellular matrix and cytokines. These intricate interactions between cellular and non-cellular components contribute to the development of a malignant TME, such as immunosuppressive, desmoplastic, angiogenic conditions and the formation of a niche for cancer stem cells, but there is limited understanding of the specific subtypes of stromal cells involved in this process.

Cellular senescence is a double-edged sword, exerting opposing effects in tumorigenesis. This phenomenon has generally been regarded as a tumor-suppressive process by preventing the proliferation of cells carrying transforming mutations. However, the accumulation of senescent cells during natural aging leads to chronic inflammation, emerging as a risk factor for overall tumor incidence. Furthermore, chemotherapy, radiotherapy, or other cell cycle inhibitors have been shown to induce cellular senescence in cancer cells. These intratumoral senescent cells may recruit immune cells through the secretion of pro-inflammatory factors, thereby enhancing blood vessel permeability and immune surveillance against cancer. On the other hand, the cytokines secreted by senescent cells promote angiogenesis, metastasis, and extracellular matrix (ECM) remodeling. While stromal cells lacking transforming mutations are prone to senescence induction, the characteristics and identification of senescent stromal cells are not as well-understood as those of senescent cancer cells.

Here, we utilized p16-Cre^{ERT2}-tdTomato mouse models to investigate the signaling networks established by senescent cancer stromal cells, contributing to the development of a malignant TME. In pancreatic ductal adenocarcinoma (PDAC) allograft models, these senescent cells were found to promote cancer fibrosis, enhance angiogenesis, and suppress cancer immune surveillance. Notably, the selective elimination of senescent cancer stromal cells improves the malignant TME, subsequently reducing tumor progression in PDAC. This highlights the anti-tumor efficacy of senolytic treatment alone and its synergistic effect when combined with conventional chemotherapy. Taken together, our findings suggest that the signaling crosstalk among senescent cancer stromal cells plays a key role in the progression of PDAC and may be a promising therapeutic target.

3. DPPA3 Disrupts UHRF1 Chromatin localization by Targeting the SRA Domain

Atsuya Nishiyama, Shota Tanimoto, Yoshie Chiba, Keita Sugimura, Ayana Ota, Kyohei Arita[1], Makoto Nakanishi

[1]Structural Biology laboratory, Yokohama City University

The E3 ubiquitin ligase UHRF1 binds specifically to hemimethylated DNA and is essential for recruiting DNA methyltransferase 1 (DNMT1) to DNA methylation sites. Recently, it was reported that DPPA3, a naturally disordered protein expressed in oocytes and early embryos, interacts with UHRF1, and suppresses its chromatin localization. We demonstrated that the addition of recombinant mouse DPPA3 (mDPPA3) to a cell-free system derived from *Xenopus* egg extracts strongly inhibited the chromatin binding of UHRF1. Furthermore, we reported that the interaction of mDPPA3 with the PHD domain of UHRF1 is critical for this inhibition. However, the precise mechanism by which mDPPA3 suppresses UHRF1 chromatin localization remained unclear.

We investigated the interaction between mDPPA3 and various deletion mutants of mouse UHRF1 (mUHRF1). Our results revealed that mDPPA3 interacts not only with the PHD domain but also with the SRA domain of mUHRF1. Moreover, amino acids 119–138 of mDPPA3 were identified as critical sequence for binding to the SRA domain of mUHRF1,

and mDPPA3 mutants lacking this sequence failed to inhibit UHRF1 chromatin binding. Next, we examined whether mDPPA3 inhibits the binding of UHRF1 to hemimethylated DNA. Incubation of hemimethylated DNA beads with egg extracts induced UHRF1 binding to the DNA, but the addition of recombinant DPPA3 inhibited this binding. mDPPA3 mutant, which lacked the SRA domain-binding sequence, did not suppress UHRF1 binding to hemimethylated DNA. Furthermore, we found that a highly conserved cysteine cluster in the C-terminal region of mDPPA3 coordinates a single Zn ion with H422 in the SRA domain of mUHRF1. This Zn coordination was shown to be essential for the formation of the mDPPA3-mUHRF1-SRA complex.

These findings strongly suggest that mDPPA3 suppresses UHRF1 chromatin binding by inhibiting its interaction with hemimethylated DNA through Zn-dependent binding to the SRA domain of UHRF1.

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Department of Cancer Biology

Division of Aging and Regeneration
老化再生生物学分野

Professor	Emi K. Nishimura, M.D., Ph.D.	教　授　博士(医学)	西　村　栄　美
Associate Professor	Takuma Shibata, Ph.D.	准教授　博士(医学)	柴　田　琢　磨
Assistant Professor	Yasuaki Mohri, Ph.D.	助　教　博士(農学)	毛　利　泰　彰
Assistant Professor	Kyosuke Asakawa, Ph.D.	助　教　博士(工学)	浅　川　杏　祐

Stem cell systems play fundamental roles in sustaining tissue turnover and homeostasis. Our goal is to understand the mechanisms of tissue homeostasis in mammals and to apply that knowledge to better understand the mechanisms underlying tissue/organ aging, cancer development and other relevant diseases associated with aging. We further aim to apply this knowledge to drug discovery, regenerative medicine and the prevention and treatment of age-associated diseases.

1. Stem cell fate governs hair graying and melanoma development

Yasuaki Mohri[1], Jialiang Nie[1], Hironobu Morinaga[2], Tomoki Kato[2], Takahiro Aoto[2], Takashi Yamanashi[3,4], Daisuke Nanba[1], Hiroyuki Matsumura[1], Sakura Okamoto[2], Kouji Kobiyama[5], Ken J Ishii[5], Masahiro Hayashi[6], Tamio Suzuki[6], Takeshi Namiki[7], Jun Seita[3,4], and Emi K Nishimura[1]

[1] Division of Aging and Regeneration, The Institute of Medical Science, The University ofTokyo, Japan
[2]Department of Stem Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.
[3]Advanced Data Science Project, RIKEN Information R&D and Strategy Headquarters, Tokyo, Japan.
[4] Center for Integrative Medical Sciences, RIKEN, Kanagawa, Japan.
[5]Division of Vaccine Science, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; International Vaccine Design Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
[6]Department of Dermatology, Faculty of Medicine, Yamagata University, Yamagata, Japan.
[7]Department of Dermatology, Tokyo Medical and Dental University Graduate School and Faculty of Medicine, Tokyo, Japan.

The accumulation of an individual's lifelong environmental exposure, known as the "exposome", has a significant impact on health. Somatic tissues undergo functional decline with age, exhibiting characteristic ageing phenotypes such as hair graying and cancer. However, specific genotoxins and signals driving each phenotype and their underlying cellular mechanisms remain largely unknown. Importantly, DNA damage foci are relatively frequently found in somatic stem cells in the skin during physiological aging. Using a DNA damage inducing model, we previously found that the induction of DNA double strand breaks (DSBs) advances the expression of aging phenotypes including hair graying. To study the fate and dynamics of DNA-damaged stem cells in tissues and the resultant impact in the expression of aging phenotypes, we first focused on the melanocyte lineage and traced the fate of melanocyte stem cells (McSCs) which acquired DNA DSBs and demonstrated that

those cells disappear from the niche, causing the loss of mature melanocytes for hair pigmentation.

We studied the impact of DSBs in McSCs and found that McSCs and their niche coordinately determine individual stem cell fate through antagonistic, stress-responsive pathways, depending on the type of genotoxic damage incurred. Chronological stem cell fate-tracking in mice revealed that McSCs undergo cellular senescence-associated differentiation (seno-differentiation) in response to DSBs and downstream signaling, resulting in their selective depletion and hair graying, effectively acting as a protective mechanism against melanoma development. Conversely, carcinogens can suppress McSC seno-differentiation, even in DSB-harboring cells, by activating KITL (KIT ligand), a master niche factor for McSC self-renewal. Collectively, our data demonstrate that the fate of individual stem cell clones - expansion versus exhaustion - cumulatively and antagonistically governs a degenerative ageing phenotype and/or cancer development through the stem cell niche, depending on the exposome. We are currently testing whether DNA DSBs in other stem cells similarly promotes degenerative tissue aging.

2. Elucidating the molecular mechanisms underlying Sialadenitis

Takuma Shibata[1], Kensuke Miyake[2], Nishimura EK[1].

[1] Division of Aging and Regeneration, The Institute of Medical Science, The University ofTokyo, Japan
[2] Division of Innatute Immunity, The Institute of Medical Science, The University ofTokyo, Japan

We humans are continuously exposed to diverse stressors such as radiation, ultraviolet light, and infections, and homeostasis is maintained by stress responses triggered by cells detecting these challenges. However, dysregulated or excessive stress responses can lead to various diseases and accelerate aging. Under stress conditions, characteristic molecular patterns that are normally absent in the body emerge. Pathogen-derived molecules are termed PAMPs (Pathogen-Associated Molecular Patterns), whereas self-derived molecules released during cell death are known as DAMPs (Damage-Associated Molecular Patterns). Nucleic acids and their metabolites also function as PAMPs or DAMPs, and their accumulation under such conditions gives rise to "nucleic acid stress." Our research has focused on nucleic acid sensors that detect this stress, and we have demonstrated that excessive activation of these sensors can lead to autoimmune diseases and histiocytosis. In contrast, the role of nucleic acid sensors in the aging process remains largely unknown. Recently, the lead author found that genetically engineered mice with hyperactivation of the single-stranded RNA sensor TLR7 readily develop sialadenitis. The reduction in saliva production directly leads to xerostomia (dry mouth), a hallmark of aging that contributes to dental caries, periodontal disease, and taste disorders, ultimately resulting in a significant long-term decline in quality of life (QOL). However, due to the unclear molecular mechanisms underlying xerostomia, current treatments for sialadenitis are limited to symptomatic management. Based on insights gained from these sialadenitis model mice, we aim to develop a comprehensive understanding of xerostomia and ultimately elucidate the molecular mechanisms underlying salivary gland aging.

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Sato R, Liu K, Shibata T, Hoshino K, Yamaguchi K, Miyazaki T, Hiranuma R, Fukui R, Motoi Y,Fukuda-Ohta Y, Zhang Y, Zhang Y, Reuter T, Ishida Y, Kondo T, Chiba T, Asahara H, Taoka M,Yamauchi Y, Isobe T, Kaisho T, Furukawa Y, Latz E, Nakatani K, Izumi Y, Nie Y, Taniguchi H,Miyake K."RNase T2 deficiency promotes TLR13-dependent replenishment of tissue-protective Kupffercells" Journal of Experimental Medicine. 2025 Mar; 222(3): e20230647. doi: 10.1084/jem.20230647.

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Department of Basic Medical Sciences

Division of Cell Signaling and Molecular Medicine

分子シグナル制御分野

Professor	Mutsuhiro Takekawa, M.D., Ph.D.	教　授	博士(医学)	武　川　睦　寛
Senior Assistant Professor	Yuji Kubota, Ph.D.	講　師	博士(理学)	久保田　裕　二
Assistant Professor	Hisashi Moriizumi, Ph.D.	助　教	博士(医科学)	森　泉　寿　士
Assistant Professor	Ryosuke Hiranuma, Ph.D.	助　教	博士(医科学)	平　沼　亮　祐

The aims of the ongoing research projects in our laboratory are to elucidate the regulatory mechanisms of intracellular signal transduction systems responsible for cell-fate decisions, such as MAP kinase cascades and Stress granules. Perturbation of these signaling systems is involved in a variety of life-threatening diseases, including cancer, autoimmune diseases, neurodegenerative disorders and type 2 diabetes. Our laboratory also aims to develop new diagnostic or therapeutic tools for currently intractable disorders in which these pathways are involved.

1. Regulation of the stress-responsive p38 and JNK MAPKs under stress conditions

Shiho Fujioka[1], Naoki Yasumoto[1], Shuri Komai[1], Yui Tanishiki[1], Saeko Kawataki[1], Noriko Nishizumi-Tokai[1], Ryosuke Hiranuma[1], Hisashi Moriizumi[1], Kotoe Katayama2, Yuji Kubota[1], Seiya Imoto3, and Mutsuhiro Takekawa[1,2]: [1]Division of Cell signaling and Molecular Medicine, IMUST, [2]Medical Proteomics Laboratory, IMSUT, [3]Laboratory of Sequence Analysis, Human Genome Center, IMSUT, [4]Division of Health Medical Intelligence, Human Genome Center, IMSUT

In mammalian cells, extracellular stimuli (e.g., growth factors and environmental stresses) activate specific intracellular signaling pathways that regulate diverse cellular processes, including cell proliferation, survival, and death. The MAPK pathways, which consist of three tiers of sequentially activating protein kinases (i.e., MAPKKK, MAPKK, and MAPK), are key signaling systems that govern cell fate decisions. In mammals, there are at least three distinct MAPK signaling pathways, namely p38, JNK, and ERK pathways. The p38 and JNK pathways preferentially respond to various stresses such as oxidative stress, heat shock, and high osmolality. Upon stress, one or more stress-responsive MAPKKKs are activated, which in turn phosphorylate and activate their cognate MAPKKs, leading to the activation of p38/JNK. Activated p38/JNK then phosphorylate various substrates, including transcription factors (e.g., Jun and ATF2), and modulate their transcriptional activity, thereby regulating gene expression and cellular stress responses (e.g., apoptosis and cytokine production). Notably, sustained activation of p38/JNK promotes apoptosis.

Previous studies have shown that more than a dozen stress-responsive MAPKKKs (e.g., MTK1, ASK1, TAK1, and ZAK) exist in mammalian cells. Although these MAPKKKs can be activated by distinct sets of stress stimuli, their precise roles remain ill-defined. We have previously identified the human stress-responsive MAPKKK, MTK1, and demonstrat-

ed that the GADD45 family proteins (GADD45α/β/γ) specifically activate MTK1. This year, by establishing various cell lines deficient in SAPK signaling molecules (e.g., GADD45, MTK1, SAPKKs, or others), we investigated their regulation and function, and uncovered their roles in carcinogenesis, DNA-damage response, inflammation, and cell growth control. In particular, we found that the GADD45β-MTK1 signaling axis mediates ERK-p38/JNK crosstalk under oncogenic stress conditions and plays a key role in tumor suppression. We demonstrated that, in normal cells, hyperactivation of ERK signaling by oncogenes induces GADD45β expression through the prolonged induction of the TF EGR1, and leads to MTK1-mediated, sustained p38/JNK activation. Transcriptome analyses revealed that this ERK-p38/JNK crosstalk upregulates a set of genes involved in apoptosis and immune response, thereby preventing carcinogenesis. Importantly, ERK-induced GADD45β expression and the resulting MTK1-p38/JNK activation are often abolished in cancer cells due to aberrant downregulation of EGR1, GADD45β, or/and MTK1. Dysregulation of GADD45β–MTK1 signaling impedes oncogenic stress-induced apoptosis in cancer cells, allowing tumor development and progression. Our findings delineate how cells sense and respond to oncogenic stress, and how this mechanism is disrupted in human cancer.

2. Identification of novel substrates of human mitogen-activated protein kinases and their roles in human cancer.

Yuto Ishii, Ryoko Ando, Yuji Kubota, and Mutsuhiro Takekawa

Mitogen-activated protein kinases (MAPKs) are key regulators of intracellular signaling pathways that orchestrate a wide range of cellular processes, including cell proliferation, differentiation, and survival. Among these, the ERK pathway transduces mitogenic signals and plays a pivotal role in a wide array of biological processes, including cell proliferation, differentiation, and carcinogenesis. Upon stimulation of cells with growth factors such as epidermal growth factor (EGF), their respective receptor tyrosine kinases (RTKs) activate Ras and recruit Raf family kinases to the plasma membrane, which promotes Raf activation. Activated Raf phosphorylates and activates MEK1/2, which in turn activate ERK1/2 by phosphorylation. A portion of the activated ERK then translocates to the nucleus where it phosphorylates and activates specific substrate proteins, including several TFs (e.g., ELK1 and Sp1), and promotes cell growth and tumorigenesis. Since ERK exerts is biological effects through the phosphorylation of its substrate proteins, the characterization of these substrates are crucial for understanding the regulatory mechanisms of critical biological phenomena and the etiology of human cancer. In addition to known ERK substrates (e.g., RSK, ELK1, and Sp1), recent evidence suggests that many unrecognized substrates remain to be discovered. Identifying these proteins can shed light on previously unexplored regulatory networks influencing cell cycle progression, RNA metabolism, and cell death pathways.

In our laboratory, we have employed multiple screening strategies to uncover novel ERK substrate proteins, including a yeast three-hybrid system and a Phos-tag SDS-PAGE analysis, Through these approaches, we have isolated previously uncharacterized substrates of ERK, sucha as MCRIP1, NELF-A, and others. These substrates proteins include signaling molecules involved in RNA metabolism, growth-promoting gene expression, and the regulation of cell fate decisions. We confirmed that each of these candidate substrates is directly phosphorylated by ERK both in vitro, using purified ERK and recombinant substrates, and in vivo, following mitogenic stimulation in cultured cells. Thus, these molecules are bona fide substrates of ERK. Ongoing research in our group focuses on defining the biological and pathological implications of these phosphorylation events. In particular, we are currently examining how altered phosphorylation states of these novel substrates in cancer influence tumor progression, metastatic behavior, and resistance to chemotherapeutic agents. Moreover, we are investigating whether modulating biological activity of these substrates could offer new therapeutic avenues in cancer or other human diseases.

3. Role of stress granule assembly in regulation of cellular stress ad response

Sayoko Akiike[1], Daisuke Yosioka[1], Takanori Nakamura[1], Hisashi Moriizumi, Noriko Nishizumi-Tokai[1], Yuji Kubota[1], and Mutsuhiro Takekawa[1,2]: [1]Division of Cell signaling and Molecular Medicine, IMUST, [2]Medical Proteomics Laboratory, IMSUT

In dealing with environmental stresses, human cells either activate defense mechanisms to survive or initiate cell death signaling, depending on the level and type of stress. One of the major cellular defense mechanisms is the assembly of stress granules (SGs). SGs are cytoplasmic ribonucleoprotein foci that appear when eukaryotic cells are exposed to specific types of stress such as ER stress, heat shock, hypoxia or viral infection. The core components of SGs are large aggregates of stalled translation pre-initiation complexes that contain mRNA, 40S ribosomal subunits, translation initiation factors and several RNA-binding proteins (RBPs). In general, the assembly of SGs is triggered by stress-induced phosphorylation of eIF2α, and requires self-oligomerization of certain RBPs such as G3BP. In cells under various stresses, eIF2α is phosphorylated by several different

stress-sensing kinases. Phosphorylation of eIF2α suppresses productive translation initiation by preventing formation of the eIF2-GTP-Met-tRNAi complex. Under the stress conditions, specific RBPs such as G3BP1/2, instead of the ternary complex, interact with an mRNA in the 43S complex, leading to the assembly of a translationally stalled 48S complex. Self-oligomerization of RBPs by liquid-liquid phase separation (LLPS) promotes the formation of discrete cytoplasmic foci termed SGs. Although SGs were initially considered to control RNA metabolism and translation reprograming under stress, their roles in these processes remain obscure. In contrast, increasing evidence shows that SGs function as signaling hubs by concentrating several signaling molecules into the granules, and promote adaptive stress responses such as the protection of cells from apoptosis and pyroptosis. However, the precise function of SGs in the regulation of cell-fate decisions under stress remains ill-defined.

Recent work in our laboratory has elucidated new connections between stress SGs, which promote cell survival during stress, and the NLRP3 inflammasome pathway, which orchestrates pyroptosis and inflammation in response to viral or other pathogenic signals. This year, we identified DHX33, a viral RNA sensor for the NLRP3 inflammasome, as a SG component, and the SG-nucleating protein G3BP as an NLRP3 inflammasome component. We also found that a decrease in intracellular potassium (K^+) concentration, a key common step in NLRP3 inflammasome activation, markedly inhibited SG assembly. This discovery suggests a mutually exclusive relationship between SGs and the NLRP3 inflammasome under certain stress conditions: when macrophages are exposed to stress stimuli with the potential to induce both SGs and the NLRP3 inflammasome, such as cytoplasmic poly(I:C) stimulation and viral infection, the cells preferentially form the NLRP3 inflammasome and avoid SG assembly by sequestering G3BP into the inflammasome and by inducing a reduction in intracellular K^+ levels. Thus, under such conditions, DHX33 is primarily utilized as a viral RNA sensor for the inflammasome. Our data illuminate a critical regulatory point in the interplay between cell survival mechanisms (SG formation) and inflammatory cell death (pyroptosis) during viral infection, and delineate a molecular mechanism that regulates cell-fate decisions and anti-viral innate immunity under stress.

4. Identification of genes whose expression is controlled by MAPK signaling pathways.

Shuri Komai, Junichiro Nashimoto, Ryosuke Hiranuma, Yuji Kubota, Noriko Nishizumi-Tokai, and Mutsuhiro Takekawa

Sequential activation of protein kinases within MAPK cascades is an evolutionary-conserved mechanism of intracellular signaling in the eukaryotic world. While the classical ERK MAPK is mainly activated by mitogenic stimuli, two relatively newly identified MAPKs, p38 and JNK, are preferentially activated by various environmental stresses. Therefore, p38 and JNK MAPKs are collectively called stress-activated protein kinases (SAPKs). Each of these MAPK cascades can regulate several different and sometimes overlapping biological functions. In general, the ERK pathway mediates growth-promoting and anti-apoptotic signaling, while the p38 and JNK pathways play pivotal roles in cellular stress responses such as growth arrest and apoptosis. In addition, the p38 and JNK pathways are involved in inflammatory responses. Perturbation of these crucial signal transduction pathways is involved in the pathophysiology of various life-threatening diseases, including cancer, autoimmune diseases, and neurodegenerative disorders.

The initial cellular response to various environmental cues, such as growth factors, environmental stresses, and cytokines, is the transcriptional regulation of a set of genes that control a wide variety of biological functions. MAPK signaling pathways are known to play crucial roles in this process. Previous studies have shown that MAPKs directly phosphorylate and activate a bunch of transcription factors and regulators. For instance, the transcription factor ELK-1, which is a member of the ternary complex factor (TCF) subfamily, is a substrate of ERK. TCFs interact with a second transcription factor, serum response factor (SRF), and these two transcription factors jointly bind and activate serum response elements (SREs) in the promoters of immediately early genes (IEGs). Moreover, upon stress stimulation, p38 and JNK MAPKs directly phosphorylate activating transcription factor 2 (ATF2). ATF2 binds either to CRE response elements as a homodimer, or to both AP-1 and CRE sequences as a heterodimer, in which ATF2 forms a complex with other members of the ATF family or with Jun/Fos family members, thereby inducing target gene expression.

We have comprehensively searched for human genes whose expression is transcriptionally regulated by the MAPK signaling pathways and have succeeded in identifying dozens of such genes. Interestingly, these transcripts include not only protein-coding mRNAs but also various non-coding, functional RNAs. We confirmed that some of these transcripts were indeed expressed preferentially in cancer cells with hyper-ERK activity or in cells treated with certain types of stresses. The roles of these MAPK-dependent transcripts in the regulation of cell fate decisions are currently under investigation in our laboratory.

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武川睦寛．非膜オルガネラによるストレス応答制御 実験医学「ストレス応答と相分離－環境感知・応答システムの新機構とその破綻による疾患－（武川睦寛編）」42巻13号 1992-1997（2024）

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Department of Basic Medical Sciences

Division of RNA and Gene Regulation
RNA 制御学分野

Professor	Toshifumi Inada, Ph.D.	教　授	博士(理学)	稲　田　利　文
Associate Professor	Yoshitaka Matsuo, Ph.D.	准教授	博士(理学)	松　尾　芳　隆
Assistant Professor	Toru Suzuki, Ph.D.	助　教	博士(理学)	鈴　木　　亨
Assistant Professor	Sihan Li, Ph.D.	助　教	博士(薬科学)	李　　思　涵

Quality control of translation eliminates aberrant proteins and maintains protein homeostasis and normal cell function. Improving the accuracy of translation and preventing the production of abnormal proteins is a practical approach for suppressing a series of neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases. We analyzed the molecular mechanism of quality control mechanisms that suppress abnormal proteins and clarified the molecular basis of drug discovery. We propose that the increase in translation accuracy and enhancement of translation quality control mechanisms are possible strategies to prevent abnormal protein production and prolong healthy life expectancy.

1. Quality control for translation abnormalities Molecular mechanism of RQC and NGD

Ribosome-associated Quality Control (RQC) is responsible for monitoring aberrant translation and for decomposing and removing abnormal proteins during their synthesis. RQC plays a crucial role in maintaining protein homeostasis at an early stage. We have previously reported a molecular mechanism that recognizes and dissociates stagnant ribosomes during translation elongation, which is an early stage of RQC. In recent years, we have identified the E3 ubiquitin ligase Hel2 and its mammalian homolog ZNF598 as essential for RQC. Additionally, we discovered a novel RQT complex involved in the dissociation of ubiquitinated ribosomes into subunits. Our work, along with that of the Hegde lab, has shown that the E3 ubiquitin ligase can recognize collided ribosomes (Disomes/Trisomes) and their specific structural features. We have previously reconstituted the ubiquitination of uS10 by Hel2 and identified an RQT complex that specifically dissociates ubiquitinated ribosomes into subunits, achieving in vitro reconstitution of this reaction.

In yeast, RQT complex components Cue3 and Rqt4 interact with K63-linked ubiquitin chains, facilitating the recruitment of the RQT complex to the ubiquitinated colliding ribosomes. The CUE domain of Cue3 and the N-terminal domain of Rqt4 bind independently to the K63-linked ubiquitin chain, and their deletion abolishes ribosomal dissociation mediated by the RQT complex. High-speed atomic force microscopy (HS-AFM) has revealed that the intrinsically disordered regions of Rqt4 allow for an expanded searchable area for interaction with the ubiquitin chain. These findings provide mechanistic insights into how the ubiquitin code is decoded for the clearance of colliding ribosomes by the RQT complex.

In mammals, uS10 is polyubiquitinated, while eS10 is preferentially mono-ubiquitinated by ZNF598. We characterized the ubiquitination activity of ZNF598 and its importance in human RQT-mediated subunit dissociation, using endogenous XBP1u and poly(A) translation stallers. Cryo-electron microscopy (Cryo-EM) analysis of human-collided disomes revealed a distinct composite interface with substantial differences from those of yeast collided disomes. Biochemical analysis showed that ZNF598 forms K63-linked polyubiquitin chains on uS10, which are crucial for initiating mammalian RQC. The human RQT

(hRQT) complex, consisting only of ASCC3, ASCC2, and TRIP4, dissociates collided ribosomes depending on the ATPase activity of ASCC3 and the ubiquitin-binding capacity of ASCC2. For the hRQT to mediate subunit dissociation, K63-linked polyubiquitination of uS10 is required; however, monoubiquitination of eS10 or uS10 alone is insufficient. Thus, we conclude that ZNF598 functionally marks collided mammalian ribosomes through K63-linked polyubiquitination of uS10, allowing for the trimeric hRQT complex to mediate subunit dissociation.

The collision of ribosomes also triggers No-Go Decay (NGD) quality controls in conjunction with RQC, which leads to endonucleolytic cleavage of mRNA in the collided ribosome. We reported two NGD pathways: one involving mRNA cleavage coupled to the dissociation of collided ribosomes in RQC (NGDRQC-) and another independent of RQC occurring near collided ribosomes (NGDRQC+). The ubiquitin-binding activity of Cue2 is necessary for NGDRQC-, but not for NGDRQC+. This activity involves the first two N-terminal Cue domains, while Trp122 of Cue2 is critical for NGDRQC+. Additionally, the colliding ribosome association factor Mbf1 and its interaction with uS3 are vital for NGDRQC+ via the SDD1-staller. We propose that for Cue2-dependent cleavage upstream of collided ribosomes (NGDRQC-), polyubiquitination of eS7a is recognized by the two N-terminal Cue domains of Cue2. Conversely, for cleavage within collided ribosomes (NGDRQC+), the UBA domain, Trp122, and the interaction between Mbf1 and uS3 are critical.

NEMF (the homolog of Rqc2 in yeast) interacts with 60S ribosome-nascent chain complexes (RNCs) and recruits Ltn1/Listerin, which ubiquitinates peptidyl-tRNA on dissociated 60S subunits. Within the 60S subunit, Rqc2 catalyzes the C-terminal extension of stalled tRNA-bound peptides with alanine and threonine residues (CAT tails) through a non-canonical, mRNA-independent elongation reaction. CAT tailing enables the degradation of substrates lacking an Ltn1p-accessible ubiquitination site by exposing a lysine residue that is typically sequestered in the ribosome exit tunnel. In the context of nascent chain degradation in budding yeast, CAT tailing acts as a fail-safe mechanism that broadens the range of substrates degradable by RQC. However, the physiological functions of CAT tailing remain elusive. We recently discovered that failure to degrade CAT-tailed proteins disrupts neuronal morphogenesis and cell survival. In mammals, NEMF modifies the translation products of nonstop mRNAs, which are a major type of erroneous mRNA, by adding a C-terminal tail composed mainly of alanine and several other amino acids.

1.1. Mechanisms of Translation-coupled Quality Control.

Toshifumi Inada[1], Roland Beckmann[2]
[1]Division of RNA and gene regulation, Institute of Medical Science, The University of Tokyo, Minato-Ku 108-8639, Japan.; [2]Department of Biochemistry, Gene Center, Feodor-Lynen-Str. 25, University of Munich, 81377 Munich, Germany.

The collision sensor Hel2 specifically recognizes colliding ribosomes and ubiquitinates the ribosomal protein uS10. This process leads to the noncanonical dissociation of ribosomal subunits through the ribosome-associated quality control trigger (RQT) complex. While the ubiquitination of uS10 is essential for rescuing stalled ribosomes, its exact function and recognition mechanisms are not fully understood. In this study, we demonstrated that the RQT complex components Cue3 and Rqt4 interact with the K63-linked ubiquitin chain, which accelerates the recruitment of the RQT complex to the ubiquitinated colliding ribosome. The CUE domain of Cue3 and the N-terminal domain of Rqt4 independently bind to the K63-linked ubiquitin chain. Deleting these domains abolished the ribosomal dissociation mediated by the RQT complex. High-speed atomic force microscopy (HS-AFM) revealed that the intrinsically disordered regions of Rqt4 enhance the interaction area with the ubiquitin chain. These findings offer mechanistic insight into how the ubiquitin code is decoded for the clearance of colliding ribosomes by the RQT complex.

Translation of aberrant messenger RNAs can cause ribosomal stalling, which results in ribosomal collisions. Collided ribosomes are specifically recognized to initiate stress responses and quality control pathways. Ribosome-associated quality control facilitates the degradation of incomplete translation products and requires the dissociation of stalled ribosomes. A central event in this process is the splitting of collided ribosomes by the ribosome quality control trigger complex (RQT), though the mechanism remains unclear. We show that RQT requires accessible mRNA and the presence of a neighboring ribosome. Cryogenic electron microscopy imaging of RQT-ribosome complexes reveals that RQT engages the 40S subunit of the leading ribosome and can switch between two conformations. We propose that the Ski2-like helicase 1 (Slh1) subunit of RQT applies a pulling force on the mRNA, leading to destabilizing conformational changes in the small ribosomal subunit, ultimately resulting in subunit dissociation. Our findings provide a conceptual framework for a helicase-driven mechanism of ribosomal splitting.

Ribosome-associated quality control (RQC) is a conserved process that degrades potentially toxic truncated nascent peptides. Malfunctions in this process contribute to neurodegeneration and proteostasis decline in aging. During RQC, the dissociation of stalled ribosomes is followed by the elongation of the nascent peptide with alanine and threonine residues, driven by Rqc2 independently of mRNA, the small

ribosomal subunit, and guanosine triphosphate (GTP)-hydrolyzing factors. The resulting carboxy-terminal (CAT) tails and subsequent ubiquitination by Ltn1 mark nascent peptides for proteasomal degradation. In this study, we present ten cryogenic electron microscopy (cryo-EM) structures that reveal the mechanistic basis for the individual steps of the CAT tailing cycle, including initiation, decoding, peptidyl transfer, and tRNA translocation. We identified eIF5A as a crucial eukaryotic RQC factor that facilitates peptidyl transfer. Additionally, we observed the dynamic behavior of RQC factors and tRNAs, which allows the CAT tailing cycle to occur without additional energy input. Together, these results elucidate key differences and common principles between CAT tailing and canonical translation.

1.2. Multiprotein bridging factor 1 is required for robust activation of the integrated stress response on collided ribosomes

Kyusik Q Kim[1], Jeffrey J Li[2,] Ankanahalli N Nanjaraj Urs1, Miguel E Pacheco[2], Victor Lasehinde[1], Timo Denk[3], Petr Tesina[3,] Shota Tomomatsu[4], Yoshitaka Matsuo[4], Elesa McDonald[1], Roland Beckmann[3], Toshifumi Inada[4], Rachel Green[5], Hani S Zaher[6]

[1]Department of Biology Washington University in St. Louis St. Louis MO 63130 USA.[2] Howard Hughes Medical Institute Department of Molecular Biology and Genetics Johns Hopkins University School of Medicine Baltimore MD 21205 USA. [3]Gene Center Department of Biochemistry Ludwig-Maximilians-Universität München München Germany. [4]Division of RNA and Gene Regulation Institute of Medical Science The University of Tokyo Minato-ku 108-8639 Japan. [5]Howard Hughes Medical Institute Department of Molecular Biology and Genetics Johns Hopkins University School of Medicine Baltimore MD 21205 USA. [6]Department of Biology, Washington University in St. Louis, St. Louis, MO 63130, USA.

In yeast multiprotein bridging factor 1 (Mbf1) has been proposed to function in the integrated stress response (ISR) as a transcriptional coactivator by mediating a direct interaction between general transcription machinery and the process's key effector Gcn4. However mounting evidence has demonstrated that Mbf1 (and its human homolog EDF1) is recruited to collided ribosomes a known activator of the ISR. In this study we connect these otherwise seemingly disparate functions of Mbf1. Our biochemical and structural analyses reveal that Mbf1 functions as a core ISR factor by interacting with collided ribosomes to mediate Gcn2 activation. We further show that Mbf1 serves no role as a transcriptional coactivator of Gcn4. Instead Mbf1 is required for optimal stress-induced eukaryotic initiation factor 2α (eIF2α) phosphorylation and downstream de-repression of GCN4 translation. Collectively our data establish that Mbf1 functions in ISR signaling by acting as a direct sensor of stress-induced ribosome collisions.

1.3. The UFM1 system: Working principles cellular functions and pathophysiology

Masaaki Komatsu[1] Toshifumi Inada[2] Nobuo N Noda[3]

[1]Department of Physiology, Juntendo University Graduate School of Medicine, Bunkyo-ku, Tokyo 113-8421, Japan; [2]Division of RNA and gene regulation, Institute of Medical Science, The University of Tokyo, Minato-Ku, 108-8639, Japan; [3]Institute for Genetic Medicine, Hokkaido University, Kita-Ku, Sapporo, 060-0815, Japan; [4]Institute of Microbial Chemistry (Bikaken), Shinagawa-ku, Tokyo, 141-0021, Japan.

Ubiquitin-fold modifier 1 (UFM1) is a ubiquitin-like protein covalently conjugated with intracellular proteins through UFMylation, a process similar to ubiquitylation. Growing lines of evidence regarding not only the structural basis of the components essential for UFMylation but also their biological properties shed light on crucial roles of the UFM1 system in the endoplasmic reticulum (ER), such as ER-phagy and ribosome-associated quality control at the ER, although there are some functions unrelated to the ER. Mouse genetics studies also revealed the indispensable roles of this system in hematopoiesis, liver development, neurogenesis, and chondrogenesis. Of critical importance, mutations of genes encoding core components of the UFM1 system in humans cause hereditary developmental epileptic encephalopathy and Schohat-type osteochondrodysplasia of the epiphysis. Here, we provide a multidisciplinary review of our current understanding of the mechanisms and cellular functions of the UFM1 system as well as its pathophysiological roles and discuss issues that require resolution.

2. Molecular mechanism of quality control NRD for deficient ribosomes.

The ribosome is the central machinery for protein synthesis and is responsible for accurate codon recognition and highly efficient peptide-bond formation. Ribosomes interact with various factors to perform essential functions in gene expression. Since abnormal ribosomes generated during the synthesis cause various expression abnormalities, cells have a quality control mechanism Nonfunctional Ribosomal RNA Decay (NRD) recognizes and eliminates functionally defective ribosomes. We recently analyzed the quality control of ribosomes deficient in function due to base substitution mutations conserved in all species, which are essential for accurate codon recognition in 18S

rRNA and ubiquitin at the K212 residue of ribosomal protein uS3 in yeast. We identified E3 ubiquitin ligases that are both essential and involved. We identified Fap1 as a stalling sensor that triggers 18S nonfunctional rRNA decay via polyubiquitination of uS3. Ribosome profiling revealed the enrichment of Fap1 at the translation initiation site and an association with elongating individual ribosomes. Cryo-EM structures of Fap1-bound ribosomes revealed that Fap1 probes the mRNA simultaneously at both the entry and exit channels, suggesting an mRNA stasis sensing activity and Fap1 sterically hinders the formation of canonical collided di-ribosomes. Our findings indicate that individual stalled ribosomes are the potential signal for ribosome dysfunction, leading to accelerated turnover of the ribosome itself. It was also revealed that the ubiquitinated stagnant 80S ribosome was dissociated into its subunits by Slh1, and then the abnormal 40S was degraded.

3. The Role of Ribosomal Dynamic Modification in Stress Response

The synthesis and modification of secretory proteins in the endoplasmic reticulum (ER) are essential functions for cells. When abnormal proteins accumulate in the ER, they can be harmful, prompting the cell to activate the unfolded protein response (UPR) pathway. In *Saccharomyces cerevisiae* (yeast), the membrane protein Ire1 is activated by ER stress, leading to the splicing of precursor mRNA for the transcription factor Hac1. The resultant Hac1 protein then induces the transcription of chaperones, which assist in protein folding. In higher eukaryotes, the protein PARK phosphorylates eIF2α, resulting in the suppression of global protein translation initiation. Our investigation into the physiological role of ribosomal ubiquitination revealed a novel translational regulator involved in the ER stress response. We identified a new mechanism of translational control during ER stress in *S. cerevisiae* and established that the ubiquitination of the ribosomal protein eS7, carried out by the E3 ubiquitin ligase Not4, is essential for this process.

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Department of Basic Medical Sciences

Division of Protein Metabolism
タンパク質代謝制御分野

Professor	Yasushi Saeki, Ph.D.	教　授	博士(薬学)	佐伯　泰
Associate Professor	Taeko Kobayashi, Ph.D.	准教授	博士(理学)	小林妙子
Assistant Professor	Takuya Tomita, Ph.D.	助　教	博士(薬科学)	冨田拓哉

The ubiquitin-proteasome system and lysosomes are the major proteolytic pathways and play a fundamental role in cellular protein homeostasis (proteostasis). Dysregulation of these pathways causes a plethora of diseases, including neurodegenerative disorders, but the detailed pathogenic mechanisms remain largely unknown. Our goal is to elucidate the basic molecular mechanisms governing proteostasis-related diseases by analyzing the regulation of protein metabolism, thereby providing the basis for therapeutic strategies.

1. Characterization of proteasomopathy model mice

Hikaru Tsuchiya[1], Takuya Tomita, Hiromichi Yonekawa[1], He Zhang, Taeko Kobayashi, Keiji Tanaka[1], Yasushi Saeki: [1]Laboratory of Protein Metabolism, Tokyo Metropolitan Institute of Medical Science

The proteasome plays a central role in many biological processes, including cell proliferation, transcriptional regulation, inflammation and proteostasis, by selectively degrading ubiquitinated proteins. Nevertheless, proteasome research at the whole-body level has lagged far behind because the proteasome is essential for the viability of all cells and knockout mice of the constitutive subunits are embryonic lethal. Recently, heterozygous mutations in *PSMD12*, a 19S subunit gene of the proteasome, have been identified in patients with developmental disorders and autism. Based on this finding, we have generated three lines of heterozygous mutant mice lacking the 3′ end of *Psmd12* at different lengths. The most deficient *Psmd12* mutant mice exhibited growth retardation, impaired liver function, and diaphragmatic hernia. Further behavioral analysis revealed delayed startle response and abnormal pain perception in the central nervous system. In addition, histological analysis revealed hepatocyte shedding and accumulation of ubiquitinated substrates in the cerebellum. The other two lines of mutant mice had mild or no phenotypes. Next, we performed transcriptome analysis and deep proteome analysis on the livers of mutant mice. RNA-seq data indicated increased expression of genes associated with oxidative stress, apoptosis, and molecular chaperones in these mutant mice. In contrast, the most pronounced proteome changes were observed in the mildest *Psmd12* mutant mice, with accumulation of proteins involved in mitochondrial dysfunction, DNA damage, and oxidative stress responses. These results demonstrate that systemic proteasome dysfunction exhibits diverse phenotypes, including impaired liver function, and highlight that even subtle decreases in proteasome activity can trigger significant proteostasis abnormalities.

2. ZFAND5/6 act as novel shuttling factors to suppress NF-κB signaling through the ubiquitin-proteasome pathway.

Liu Dongqi, Hikaru Tsuchiya[1], Takuya Tomita, Yasushi Saeki

The proteasome targets ubiquitinated substrates either directly or indirectly using extrinsic receptors

known as shuttling factors such as RAD23A/B, UBQLN1/2/4, and DDI2. Our laboratory has been investigating ZFAND (Zinc Finger AN1-Type containing) family proteins as potential members of a novel class of shuttling factors. Among the seven ZFAND family proteins, ZFAND5 and ZFAND6 share similar domain structures (an A20 domain for ubiquitin-binding and an AN1 domain for proteasome-binding). Through interactome analysis, we found that ZFAND5 and ZFAND6 interact with components of the NF-κB signaling pathway, including the E3 ligase BIRC2 (also known as cIAP1). Functional studies revealed that ZFAND5 and ZFAND6 negatively regulate NF-κB activation. Overexpression of ZFAND5/ZFAND6 inhibited IκB degradation, while ZFAND5 knockdown significantly upregulated NF-κB target genes. Furthermore, we observed that TNF-α stimulation enhances the degradation of ZFAND5 and ZFAND6. This suggests a potential self-regulatory mechanism where TNF-α disrupts the intramolecular interaction within ZFAND5/ZFAND6, leading to their degradation as well as the degradation of their target proteins within the NF-κB pathway. These findings highlight the crucial role of ZFAND5 and ZFAND6 in regulating NF-κB signaling and provide new insights into the mechanisms of protein degradation and cellular homeostasis.

3. A selective probe for evaluating hydrophobicity-exposed defective proteins in live cells

Yasuyuki Iwasa[2], Sohtaroh Miyata[2], Takuya Tomita, Yasushi Saeki, and Hiroyuki Kawahara[2]: [2]Laboratory of Cell Biology and Biochemistry, Tokyo Metropolitan University

The accumulation of defective polypeptides in cells is a major cause of various diseases. However, probing defective proteins is challenging because no currently available method can effectively retrieve unstable defective translational products in a soluble state. To overcome this issue, there is a need for a molecular device specifically designed to capture structurally defective polypeptides. In this study, we developed an artificial protein architecture comprising tandemly aligned BAG6 Domain I, a minimal substrate recognition platform responsible for protein quality control. This tandem-aligned entity demonstrated enhanced affinity not only for model defective polypeptides but also for endogenous polyubiquitinated proteins, which are sensitive to translational inhibition. Mass spectrometry analysis with this probe enabled the identification of endogenous defective proteins, including orphaned subunits derived from multi-protein complexes and misassembled transmembrane proteins. This probe is also useful for the real-time visualization of protein foci derived from defective polypeptides in stressed cells. Therefore, this "new molecular trap" is a versatile tool for evaluating currently "invisible" pools of defective polypeptides as tangible entities.

4. Delta/Notch-like EGF-related receptor (DNER) is involved in neural stem cell proliferation in the adult mouse brain.

Zhou Lu, Hiroshi Takeshima[3], Mineko Kengaku[4], Ryoichiro Kageyama[5], Koshi Imami[6], Yasushi Ishihama[3], Takuya Tomita, Yasushi Saeki, Taeko Kobayashi: [3] GSPS, Kyoto University, [4]iCeMS, Kyoto University, [5]RIKEN CBS, [6]RIKEN IMS

Adult neural stem cells (NSCs), located in the subventricular zone (SVZ) and dentate gyrus (DG), are mainly in a reversible state of quiescence. NSC activation from the quiescent state is required for their self-renew and differentiation toward neural and glial cells. The transition of NSCs between quiescent and activated states is regulated by local niche signals that promote quiescence (BMP, Delta/Notch) or cell proliferation (WNT, EGF, Notch). We demonstrated that Delta/Notch-like EGF-related receptor (DNER), identified as a lysosomal substrate and highly expressed in quiescent and reactivated NSCs, is a new regulator of NSC proliferation. DNER is a single-pass transmembrane protein previously reported to function as an inducer for functional differentiation of Bergmann glial cells via non-canonical Notch signaling. We found that DNER-KO reduced proliferating NSCs in the adult brain, effectively induced quiescence of NSCs *in vitro,* and increased Hes1 expression. DNER overexpression repressed Hes1 expression in a Notch-independent manner. These results suggest that DNER regulates the proliferative capacity of adult NSCs via Hes1 repression.

5. Multiple analyses to reveal regulation of quiescence maintenance in neural stem cells.

Yusuke Kihara, Rina Onishi, Takuya Nitta, He Zhang, Yuki Murai[7], Shenghuan Deng, Tianquan Cui, Risa Takamura[7], Marika Hirao[7], Takuya Tomita, Yasushi Saeki, Taeko Kobayashi: [7]GSB, Kyoto University,

Quiescence is a reversible arrest in the G0/G1 phase of the cell cycle and a strategy to maintain the quality of tissue stem cells for an extended period. Most adult neural stem cells in the brain keep the quiescent state and produce neurons and glial cells through differentiation after activating from the quiescent state to the proliferating state. In this process, proteostasis and organellostasis, including proteolysis and autophagy, associated with proteome remodeling are essential to transition between the quiescent and proliferating states. We have identified molecules involved in proteome remodeling between proliferating and quiescent states and analyzed them on multi-

ple aspects, including transcription, translation, receptor degradation, vesicular transport, and autophagy. We have revealed the numerous regulatory strategies crucial to maintaining quiescent neural stem cells.

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Human Genome Center

Laboratory of Molecular Medicine
ゲノム医科学分野

Professor	Tatsuhiro Shibata, M.D., Ph.D.	教　授	医学博士	柴　田　龍　弘
Senior Assistant Professor	Atsushi Niida, Ph.D.	講　師	博士(理学)	新井田　厚　司
Assistant Professor	Kazuki Takahashi, Ph.D.	助　教	博士(農学)	高　橋　数　冴

The Laboratory of Molecular Medicine focuses on comprehensive characterization of currently-untreatable diseases including cancer on the basis of molecular genomics and aims to make "breakthroughs for human health" by identifying novel disease-related genes/pathways, including potential therapeutic or preventive targets and biomarkers, and to understand human diseases as heterogeneous but intervention-able "biological systems". This group has also organized the facility for the analysis of next-generation high-performance sequencers.

1. Multiancestry comprehensive molecular analysis of gastric cancer

International differences in the incidence of many cancer types indicate the existence of carcinogen exposures that have not yet been identified by conventional epidemiology make a substantial contribution to cancer burden. In clear cell renal cell carcinoma, obesity, hypertension and tobacco smoking are risk factors, but they do not explain the geographical variation in its incidence. Underlying causes can be inferred by sequencing the genomes of cancers from populations with different incidence rates and detecting differences in patterns of somatic mutations. We sequenced 962 clear cell renal cell carcinomas from 11 countries with varying incidence. The somatic mutation profiles differed between countries. In Japan, a mutational signature of unknown cause was found in more than 70% of cases but in less than 2% elsewhere. A further mutational signature of unknown cause was ubiquitous but exhibited higher mutation loads in countries with higher incidence rates of kidney cancer. Known signatures of tobacco smoking correlated with tobacco consumption, but no signature was associated with obesity or hypertension, suggesting that non-mutagenic mechanisms of action underlie these risk factors. The results of this study indicate the existence of multiple, geographically variable, mutagenic exposures that potentially affect tens of millions of people and illustrate the opportunities for new insights into cancer causation through large-scale global cancer genomics.

2. Implementable assay for monitoring minimum residual disease after radical treatment for colorectal cancer.

Considering the cost and invasiveness of monitoring postoperative minimal residual disease (MRD) of colorectal cancer (CRC) after adjuvant chemoradiotherapy (ACT), we developed a favorable approach based on methylated circulating tumor DNA to detect MRD after radical resection. Analyzing the public database, we identified the methylated promoter regions of the genes FGD5, GPC6, and MSC. Using digital polymerase chain reaction (dPCR), we termed the "amplicon of methylated sites using a specific enzyme" assay as "AMUSE." We examined 180 and 114 pre- and postoperative serial plasma samples from 28 recurrent and 19 recurrence-free pathological stage III CRC patients, respectively. The results showed 22 AMUSE-positive of 28 recurrent patients (sensitivity, 78.6%) and 17 AMUSE-negative of 19 recurrence-free patients (specificity, 89.5%). AMUSE predicted recur-

rence 208 days before conventional diagnosis using radiological imaging. Regarding ACT evaluation by the reactive response, 19 AMUSE-positive patients during their second or third blood samples showed a significantly poorer prognosis than the other patients (p = 9E-04). The AMUSE assay stratified four groups by the altered patterns of tumor burden postoperatively. Interestingly, only 34.8% of cases tested AMUSE-negative during ACT treatment, indicating eligibility for ACT. The AMUSE assay addresses the clinical need for accurate MRD monitoring with universal applicability, minimal invasiveness, and cost-effectiveness, thereby enabling the timely detection of recurrences. This assay can effectively evaluate the efficacy of ACT in patients with stage III CRC following curative resection. Our study strongly recommends reevaluating the clinical application of ACT using the AMUSE assay.

3. Pancancer analysis of subclonal evolution

Cancer develops through the accumulation of cancer-related genomic mutations in normal cells. These mutations are acquired as the tumor grows, leading to the "evolution" of cancer towards increased malignancy and treatment resistance. This study aims to analyze evolutionary patterns across different cancer types and elucidate the differences and factors unique to each cancer type. It is known that a single tumor can contain genetically distinct cell populations (subclones). The presence of subclones derived from genomic mutations can be detected through genome sequencing data, and numerous studies have been conducted on this subject. However, many of these studies, including PCAWG study, have not estimated the selection pressure acting on the extracted subclones. In other words, it has not been determined whether the detected subclones emerged due to strong selection pressure or by chance through nearly neutral evolution. I am currently using whole-genome data from 10,000 cancer cases, sequenced at a high read depth of x120, to detect subclones and estimate their selection pressures. Through this data, I aim to elucidate the differences in selection pressures and evolutionary patterns of subclones among different cancer types. Furthermore, I seek to estimate the strength of driver genes by examining the differences in selection pressures exerted on subclones by driver mutations. This year, I conducted the pilot analysis for some cancer types as part of this cross-cancer evolutionary study.

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Human Genome Center

Laboratory of Genome Technology
シークエンス技術開発分野

Project Professor　Koichi Matsuda, M.D., Ph.D.　　特任教授　博士（医学）　松　田　浩　一

The major goal of our group is to identify genes of medical importance, and to develop new diagnostic and therapeutic tools. We have been attempting to isolate genes involving in carcinogenesis and also those causing or predisposing to various diseases as well as those related to drug efficacies and adverse reactions. By means of technologies developed through the genome project including a high-resolution SNP map, a large-scale DNA sequencing, and proteome/metabolome analysis, we have isolated a number of biologically and/or medically important genes, and are developing novel diagnostic and therapeutic tools.

1. Functional analysis of p53 signaling pathway Regulation of the innate immune response and gut microbiome by p53

p53 is a key tumor suppressor mutated in half of human cancers. In recent years, p53 was shown to regulate a wide variety of functions. From the transcriptome analysis of 24 tissues of irradiated mice, we identified 553 genes markedly induced by p53. Gene Ontology (GO) enrichment analysis found that the most associated biological process was innate immunity. 16S rRNA-seq analysis revealed that Akkermansia, which has anti-inflammatory properties and is involved in the regulation of intestinal barrier integrity, was decreased in p53-knockout ($p53^{-/-}$) mice after radiation. $p53^{-/-}$ mice were susceptible to radiation-induced GI toxicity and had a significantly shorter survival time than p53-wild-type ($p53^{+/+}$) mice following radiation. However, administration of antibiotics resulted in a significant improvement in survival and protection against GI toxicity. Mbl2 and Lcn2, which have antimicrobial activity, were identified to be directly transactivated by p53 and secreted by liver into the circulatory system. We also found the expression of MBL2 and LCN2 was decreased in liver cancer tissues with p53 mutations compared with those without p53 mutations. These results indicate that p53 is involved in shaping the gut microbiome through its downstream targets related to the innate immune system, thus protecting the intestinal barrier.

2. Analysis of host genetic factors of various diseases
Genotype imputation accuracy and the quality metrics of the minor ancestry in multi-ancestry reference panels

Large-scale imputation reference panels are currently available and have contributed to efficient genome-wide association studies through genotype imputation. However, whether large-size multi-ancestry or small-size population-specific reference panels are the optimal choices for under-represented populations continues to be debated. We imputed genotypes of East Asian (180k Japanese) subjects using the Trans-Omics for Precision Medicine reference panel and found that the standard imputation quality metric (Rsq) overestimated dosage r^2 (squared correlation between imputed dosage and true genotype) particularly in marginal-quality bins. Variance component analysis of Rsq revealed that the increased imputed-genotype certainty (dosages closer to 0, 1 or 2) caused upward bias, indicating some systemic bias in the imputation. Through systematic simulations using different template switching rates (θ value) in the hidden Markov model, we revealed that the lower θ

value increased the imputed-genotype certainty and Rsq; however, dosage r^2 was insensitive to the θ value, thereby causing a deviation. In simulated reference panels with different sizes and ancestral diversities, the θ value estimates from Minimac decreased with the size of a single ancestry and increased with the ancestral diversity. Thus, Rsq could be deviated from dosage r^2 for a subpopulation in the multi-ancestry panel, and the deviation represents different imputed-dosage distributions. Finally, despite the impact of the θ value, distant ancestries in the reference panel contributed only a few additional variants passing a predefined Rsq threshold. We conclude that the θ value substantially impacts the imputed dosage and the imputation quality metric value.

Identification of an novel genetic variant associated with osteoporosis: insights from the Taiwan Biobank Study

Purpose

The purpose of this study was to identify new independent significant SNPs associated with osteoporosis using data from the Taiwan Biobank (TWBB).

Material and Methods

The dataset was divided into discovery (60%) and replication (40%) subsets. Following data quality control, genome-wide association study (GWAS) analysis was performed, adjusting for sex, age, and the top 5 principal components, employing the Scalable and Accurate Implementation of the Generalized mixed model approach. This was followed by a meta-analysis of TWBB1 and TWBB2. The Functional Mapping and Annotation (FUMA) platform was used to identify osteoporosis-associated loci. Manhattan and quantile–quantile plots were generated using the FUMA platform to visualize the results. Independent significant SNPs were selected based on genome-wide significance ($P < 5 \times 10^{-8}$) and independence from each other ($r^2 < 0.6$) within a 1 Mb window. Positional, eQTL(expression quantitative trait locus), and Chromatin interaction mapping were used to map SNPs to genes.

Results

A total of 29 084 individuals (3154 osteoporosis cases and 25 930 controls) were used for GWAS analysis (TWBB1 data), and 18 918 individuals (1917 cases and 17 001 controls) were utilized for replication studies (TWBB2 data). We identified a new independent significant SNP for osteoporosis in TWBB1, with the lead SNP rs76140829 (minor allele frequency = 0.055, *P*-value = 1.15×10^{-08}). Replication of the association was performed in TWBB2, yielding a *P*-value of 6.56×10^{-3}. The meta-analysis of TWBB1 and TWBB2 data demonstrated a highly significant association for SNP rs76140829 (*P*-value = 7.52×10^{-10}). In the positional mapping of rs76140829, 6 genes (*HABP2*, *RP11-481H12.1*, *RNU7-165P*, *RP11-139 K1.2*, *RP11-57H14.3*, *and RP11-214 N15.5*) were identified through chromatin interaction mapping in mesenchymal stem cells.

Conclusions

Our GWAS analysis using the Taiwan Biobank dataset unveils rs76140829 in the *VTI1A* gene as a key risk variant associated with osteoporosis. This finding expands our understanding of the genetic basis of osteoporosis and highlights the potential regulatory role of this SNP in mesenchymal stem cells.

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Human Genome Center

Laboratory of Functional Analysis *In Silico*
機能解析イン・シリコ分野

Professor	Kenta Nakai, Ph.D.		教　授	博士(理学)　中　井　謙　太
Associate Professor	Sung-Joon Park, Ph.D.		准教授	博士(工学)　朴　　聖　俊
Assistant Professor	Martin Loza, Ph.D.		助　教	博士(生命機能学)　ロサ　マルティン

Laboratory of Genome Database
ゲノムデータベース分野

Professor	Kenta Nakai, Ph.D.	教　授	博士(理学)　中　井　謙　太

Our laboratory's mission is to conduct computational ("in silico") studies on the functional aspects of genome information. At present, we mainly focus on analyzing regulatory information about gene expression in the non-coding region using various next-generation sequencing (NGS) data. We also actively collaborate with researchers from various fields.

1. A graph-embedding approach to dissecting proximal and distal gene regulators

Sung-Joon Park and Kenta Nakai

The spatial organization of the genome plays a critical role in mediating the functional effects of distal chromosomal interactions. In particular, enhancer-promoter interactions have been intensively studied using advanced computational algorithms. However, our understanding of how enhancer signals are transmitted to their target promoters through complex regulatory networks remains limited. In this study, we developed a novel computational framework that combines a regression model, which predicts gene expression by identifying key promoter-distal and -proximal regulators, with a graph-embedding algorithm designed to detect cell-type-specific and conserved regulatory interactions within complex gene regulatory networks. We applied this method to human naïve and germinal center B cells and, as a result, identified sets of promoter-distal transcription factors and architectural cofactor proteins, which are co-regulated to maintain cellular stability and prevent malignancy. These findings emphasize the importance of understanding both cis- and trans-regulatory interactions in the transcriptional machinery. Our approach provides a valuable alternative for studying enhancer biology and its mediation through protein-protein interactions within the context of 3D genome organization.

2. Epigenetic profiling of housekeeping core promoters in the human genome

Martin Loza, Alexis Vandenbon[1] and Kenta Nakai

[1]Institute for Life and Medical Sciences, Kyoto University, Japan

This research investigates the role of HK-CREs in gene regulation and cancer development. We identify approximately 11,000 HK-CREs across the human genome, demonstrating their widespread influence beyond housekeeping gene expression. These elements are enriched in unmethylated CpG sites and exhibit extensive interactions with other genes. Our analysis reveals that aberrant methylation of HK-CREs, particularly those associated with zinc finger genes (ZNFGs), is prevalent in various cancer types. We observe a strong correlation between the expression of genes linked to these HK-CREs and patient survival, suggesting their critical role as tumor suppressors. Then, we use HiC data to explore the relationship between HK-CREs and zinc finger genes (ZNFGs). Despite transcriptional differences likely due to heterochromatin marks, HK-CREs and ZNFGs exhibit remarkable similarities in their epigenetic profiles. Both gene sets display extensive promoter-promoter interactions, suggesting a previously unrecognized relationship between them.

3. TF-EPI: an interpretable enhancer-promoter interaction detection method based on Transformer

Bowen Liu, Weihang Zhang, Xin Zeng, Martin Loza, Sung-Joon Park and Kenta Nakai

In this study, we developed TF-EPI, a deep-learning model based on a Transformer architecture to detect enhancer-promoter interactions solely from DNA sequences. The performance of TF-EPI surpassed that of other state-of-the-art methods on multiple benchmark datasets. Importantly, by utilizing the attention mechanism of the Transformer, we identified distinct cell type-specific motifs and sequences in enhancers and promoters, which were validated against databases such as JASPAR and UniBind, highlighting the potential of our method in discovering new biological insights. Moreover, our analysis of the transcription factors (TFs) corresponding to these motifs and short sequence pairs revealed the heterogeneity and commonality of gene regulatory mechanisms and demonstrated the ability to identify TFs relevant to the source information of the cell line. Overall, our work unveils important sequence information for the investigation of enhancer-promoter pairs based on the attention mechanism of the Transformer, providing an important milestone in the investigation of cis-regulatory grammar.

4. HyGAnno: Hybrid graph neural network-based cell type annotation for single-cell ATAC sequencing data

Weihang Zhang, Yang Cui, Bowen Liu, Martin Loza, Sung-Joon Park and Kenta Nakai

Reliable cell type annotations are crucial for investigating cellular heterogeneity in single-cell omics data. Although various computational approaches have been proposed for single-cell RNA sequencing (scRNA-seq) annotation, high-quality cell labels are still lacking in single-cell ATAC sequencing (scATAC-seq) data because of extreme sparsity and inconsistent chromatin accessibility between datasets. Here, we present a novel automated cell annotation method called HyGAnno that transfers cell type information from a well-labeled scRNA-seq reference to an unlabeled scATAC-seq target via a parallel graph neural network in a semi-supervised manner. Unlike existing methods that utilize only gene expression or gene activity features, HyGAnno integrates genome-wide accessibility peak features to facilitate the training process. In addition, HyGAnno reconstructs a reference-target cell graph that can be used to detect cells with low prediction reliability according to their specific graph connectivity patterns. HyGAnno was tested using large datasets and demonstrated the advantages of accurate cell annotation, interpretable cell embedding, robustness to noisy reference data, and adaptability to tumor tissues.

5. Spatial transcriptomics analysis via image-aided graph contrastive learning for domain exploration and alignment-free integration

Yitao Yang, Yang Cui, Xin Zeng, Yubo Zhang, Martin Loza, Sung-Joon Park and Kenta Nakai

Spatial transcriptomics is an essential application for investigating cellular structures and interactions and requires multimodal information to study spatial domains precisely. Here, we propose STAIG, a novel deep-learning model that integrates gene expression, spatial coordinates, and histological images using graph-contrastive learning coupled with high-performance feature extraction. STAIG can integrate tissue slices without pre-alignment and remove batch effects. Moreover, it was designed to accept data acquired from various platforms, with or without histological images. By performing extensive benchmarks, we demonstrated the capability of STAIG to recognize spatial regions with high precision and uncover new insights into tumor microenvironments, highlighting its promising potential in deciphering spatial biological intricates.

6. SCOIGET: a tool for predicting spatial tumor evolution patterns by inferring spatial copy number variation distributions

Yujia Zhang[2], Yitao Yang, Kenta Nakai and Hui Lu[2,3]

[2]School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, PR China
[3]AI Institute Shanghai Jiao Tong University, PR China

A comprehensive spatiotemporal map of tumor heterogeneity is essential for understanding tumor evolution, with copy number variation (CNV) as a significant feature. Existing studies often rely on tools originally developed for single-cell data, which fail to utilize spatial information. Here, we introduce SCOIGET (Spatial COpy number Inference by Graph on Evolution of Tumor), a novel framework using graph neural networks with graph attention layers to learn spatial neighborhood gene expression features and infer copy number variations. Our model significantly improves the efficiency and accuracy of depicting tumor evolution, capturing detailed spatial and temporal changes within the tumor microenvironment. It is highly versatile, showing strong generalizability across various spatial omics technologies and cancer types, making it applicable to diverse downstream tasks. This performance enhances research efficiency and offers valuable insights into tumor progression. In conclusion, SCOIGET integrates multiple features with advanced algorithms to provide a detailed and accurate representation of tumor heterogeneity and evolution, supporting the development of personalized cancer treatment strategies.

7. Comparative single-cell transcriptomic analysis reveals putative differentiation drivers and potential origin of vertebrate retina

Xin Zeng, Fuki Gyoja[4], Yang Cui, Martin Loza, Takehiro Kusakabe[4] and Kenta Nakai
[4]Faculty of Science and Engineering, Konan University

Despite known single-cell expression profiles in vertebrate retinas, understanding their developmental and evolutionary expression patterns among homologous cell types remains limited. We examined and compared approximately 240,000 retinal cells from four species and found significant similarities among homologous cell types, indicating inherent regulatory patterns. To understand these shared patterns, we constructed gene regulatory networks for each developmental stage for three of these species. We identified 690 regulons governed by 530 regulators across three species, 10 common cell class-specific regulators, and 16 highly preserved regulons. RNA velocity analysis pinpointed conserved putative driver genes and regulators to retinal cell differentiation in both mouse and zebrafish. Investigation of the origins of retinal cells by examining conserved expression patterns between vertebrate retinal cells and invertebrate *Ciona intestinalis* photoreceptor-related cells implied functional similarities in light transduction mechanisms. Our findings offer insights into the evolutionarily conserved regulatory frameworks and differentiation drivers of vertebrate retinal cells.

8. Computational analysis reveals MHC-II expressing tumor cells influence immune surveillance and prognostic outcomes in triple-negative breast

Yang Cui, Weihang Zhang, Xin Zeng, Yitao Yang, Sung-Joon Park and Kenta Nakai

Triple-negative breast cancer (TNBC) is an aggressive subtype with poor prognosis and therapy resistance, driven partly by the tumor microenvironment (TME). In this study, we combined scRNA-seq and bulk RNA-seq data from TNBC patients and identified two TME-based subtypes: tumor-dense (TD) and non-tumor-dense (nonTD). TD is associated with a more malignant phenotype, reduced immune cell infiltration, and poor prognosis, while nonTD shows an active immune response and better outcomes. We identified a tumor cell subgroup, C3, with high MHC-II pathway activity, which interacts with CD4+ T cells. C3 cells were almost absent in TD but prevalent in nonTD, suggesting their role in immune responses. Spatial transcriptomics revealed that C3 cells co-localize with immune-infiltrated regions, indicating their role in recruiting immune cells to tumors. Finally, we developed survival and immune infiltration prediction models based on C3 gene signatures, achieving high accuracy. This work enhances understanding of TNBC biology and offers potential for improving clinical outcomes.

9. Integrative analysis of cancer multimodality data identifying COPS5 as a novel biomarker of diffuse large B-cell lymphoma

Yutong Dai, Jingmei Li[5], Keita Yamamoto[5], Martin Loza, Sung-Joon Park, Susumu Goyama[5] and Kenta Nakai
[5]Department of Computational Biology and Medical Science, The University of Tokyo, Kashiwa, Japan

Accurate clinical biomarkers are essential for preventing, diagnosing, and treating diseases; however, identifying them remains challenging. Advanced computational methods have accelerated biomarker discovery from complex multimodal data, but managing sparse data with missing information still limits performance and interpretability. To address this, we developed a pipeline that combines joint non-negative matrix factorization (JNMF) to identify key features in sparse, high-dimensional data with biological pathway analysis to interpret these features by detecting activated pathways. Applying this pipeline to large-scale cancer datasets, we identified genomic features relevant to specific cancers as common pat-

tern modules (CPMs) of JNMF. We found COPS5 to be a potential upstream regulator of pathways linked to diffuse large B-cell lymphoma (DLBCL). COPS5 demonstrated co-overexpression with DLBCL markers MYC, TP53, and BCL2, and its high expression correlated with lower survival probabilities in patients. Using CRISPR-Cas9, we confirmed COPS5's role in promoting tumor growth, suggesting it as a novel prognostic biomarker for DLBCL. This work demonstrates that integrating and simplifying complex data can uncover hidden biological insights, advancing the discovery of clinical biomarkers.

10. In-silico analysis revealed Marco (SR-A6) and Abca1/2 as potential regulators of lipid metabolism in M1 macrophage hysteresis

Yubo Zhang, Wenbo Yang, Yutaro Kumagai[6], Martin Loza, Yitao Yang, Sung-Joon Park and Kenta Nakai
[6]National Institute of Advanced Industrial Science and Technology (AIST)

Macrophages undergo polarization, resulting in distinct phenotypes. These transitions, including de-/repolarization, lead to hysteresis, where cells retain genetic and epigenetic signatures of previous states, influencing macrophage function. We previously identified a set of interferon-stimulated genes (ISGs) associated with high lipid levels in macrophages that exhibited hysteresis following M1 polarization, suggesting potential alterations in lipid metabolism. In this study, we applied weighted gene co-expression network analysis (WGCNA) and conducted comparative analyses on 162 RNA-seq samples from de-/repolarized and lipid-loaded macrophages, followed by functional exploration. Our results demonstrate that during M1 hysteresis, the sustained high expression of Marco (SR-A6) enhances lipid uptake, while the suppression of Abca1/2 reduces lipid efflux, collectively leading to elevated intracellular lipid levels. This accumulation may compensate for reduced cholesterol biosynthesis and provide energy for sustained inflammatory responses and interferon signaling. Our findings elucidate the relationship between M1 hysteresis and lipid metabolism, contributing to understanding the underlying mechanisms of macrophage hysteresis.

11. RNA secondary structure prediction by conducting multi-class classifications

Jiyuan Yang, Kengo Sato[7], Martin Loza, Sung-Joon Park and Kenta Nakai
[7]School of Life Science and Technology, Tokyo Institute of Technology

Predicting the RNA secondary structure based on the RNA sequence is challenging, as valid predictions should follow various constraints. While several deep learning methods have been developed for predicting RNA secondary structures, they commonly adopt post-processing steps to adjust the model output to produce valid predictions, which are complicated and could limit performance. In this research, we propose a simple method by considering RNA secondary structure prediction as multiple multi-class classifications, eliminating the need for those complicated post-processing steps. We use this method to train and evaluate our model based on the attention mechanism and the convolutional neural network. Besides, we introduce two additional methods, including data augmentation to improve further the within-RNA-family performance and a method to alleviate the performance drop in the cross-RNA-family evaluation. We could produce valid predictions and perform better without complex post-processing steps. We show that our additional methods benefit the performance of within-RNA-family and cross-RNA-family evaluations.

12. Characterization of trans-spliced chimeric RNAs: insights into the mechanism of trans-splicing

Rui Yokomori, Takehiro G. Kusakabe[4] and Kenta Nakai

Trans-splicing is a post-transcriptional processing event that joins exons from separate RNAs to produce a chimeric RNA. However, the detailed mechanism of *trans*-splicing remains poorly understood. Here, we characterize *trans*-spliced genes and provide insights into the mechanism of *trans*-splicing in the tunicate *Ciona*. Tunicates are the closest invertebrates to humans, and their genes frequently undergo trans-splicing. Our analysis revealed that, in genes that give rise to both trans-spliced and non-trans-spliced messenger RNAs, trans-splice acceptor sites were preferentially located at the first functional acceptor site, and their paired donor sites were weak in both *Ciona* and humans. Additionally, we found that *Ciona trans*-spliced genes had GU- and AU-rich 5′ transcribed regions. Our data and findings are not only useful for the *Ciona* research community but may also aid in a better understanding of the *trans*-splicing mechanism, potentially advancing the development of gene therapy based on *trans*-splicing.

13. Developing an open-access repository for the multi-dimensional genome structure data

Sung-Joon Park, Katsuhiko Shirahige[8], Shoji Takada[9] and Tomoko Nishiyama[10]
[8]Institute for Quantitative Biosciences, The University of Tokyo
[9]Department of Biophysics, Graduate School of Science, Kyoto University

[10]Division of Biological Sciences, Graduate School of Science, Kyoto University

The community-wide effort to characterize 3D genome organization has underscored the significance of functional connections between genetic and epigenetic processes and the physical properties of DNA, such as stiffness, torsion, and supercoiling. However, the mechanisms that establish functional genome structures remain poorly understood, highlighting the need for comprehensive and integrative approaches. In this context, we are developing a data repository system, the Genome Modality Suite, as part of the research project Genome Modality. The system is designed to handle heterogeneous and multi-dimensional data, including RNA-seq and ChIP-seq signal tracks (1D data), Hi-C contact matrices (2D data), and XYZ-coordinate genome structures (3D data). Additionally, we have successfully launched the system for public use, leveraging PHP, MySQL, and JavaScript libraries, and we are continuously refining its features. Our system aims to accelerate progress in understanding the multi-dimensional properties of the genome.

14. Single-cell transcriptome analysis of ocular-like cell lineages derived from human pluripotent stem cells

Sung-Joon Park, Laura Howard[11], Andrew J Quantock[11] and Ryuhei Hayashi[12]
[11]School of Optometry and Vision Sciences, Cardiff University
[12]Department of Stem Cells and Applied Medicine, Osaka University Graduate School of Medicine

The generation of self-formed, ectodermal, autonomous multi-zone structures, known as SEAM, from human induced pluripotent stem cells (hiPSCs) provides a unique opportunity to explore the dynamics of ocular cell differentiation. In this study, we used single-cell transcriptomics to study ectodermally-derived ocular cell populations that emerge during SEAM formation. Our analysis reveals the interdependence between early eye tissues and outlines the sequential formation of specific cell types over a 12-week period. We demonstrate a progression from pluripotency to ocular tissue specification and differentiation, including cornea, conjunctiva, lens, and retina. These findings not only enhance our understanding of ocular development in a human stem cell-based model but also establish a robust methodology for investigating cellular and molecular dynamics during SEAM formation at single-cell resolution. Furthermore, they underscore the potential of hiPSC-derived systems as powerful platforms for modeling human eye development and disease.

15. Advances in immunology: insights into B cell dynamics, antibody discovery, and TCR repertoires in autoimmunity

Melissa Garcia-Vega[13], Llamas-Covarrubias Mara A[14], Martin Loza, Monica Resendiz-Sandoval[13], Diana Hinojosa-Trujillo[13], Edgar Melgoza-Gonzalez[13], Olivia Valenzuela[13], Bowen Liu, Wanzhe Zang, Atsushi Tanaka[15], Diego Diez[16], Zichang Xu, Ee Lyn Lim[17], Shunsuke Teraguchi[18], Daron Standley[14], Shimon Sakaguchi[15], Jesus Hernandez[13] and Kenta Nakai
[13]Laboratorio de Inmunologia, Centro de Investigacion en Alimentacion y Desarrollo
[14]Laboratory of Systems Immunology, WPI Immunology Frontier Research Center, Osaka University
[15]Department of Experimental Pathology, Institute for Frontier Medical Sciences, Kyoto University
[16]Quantitative Immunology Research Unit, Immunology Frontier Research Center, Osaka University
[17]Laboratory of Experimental Immunology, WPI Immunology Frontier Research Center, Osaka University
[18]Department of Genome Informatics, Research Institute for Microbial Diseases (RIMD), Osaka University

This research explores immune cell behavior and innovative approaches to address infectious and autoimmune diseases. In COVID-19 patients, single-cell transcriptomics revealed proinflammatory signatures in B cells correlated with disease severity and uncovered a developmental pathway linking atypical memory B cells to conventional memory B cells. Key genes, such as ZFP36 and DUSP1, were identified as drivers of differentiation and activation, shedding light on their roles in immune recovery and response to SARS-CoV-2. A bioinformatics pipeline was developed to accelerate antibody discovery using machine learning and AI, integrating sequencing data to predict neutralizing antibodies. This scalable approach reduces the resources needed for therapeutic development and offers potential applications for other diseases. In parallel, analysis of T cell receptor (TCR) repertoires in ZAP-70 mutant mice revealed how attenuated TCR signaling promotes self-reactive Th17 cells in inflamed joints. This disruption shifts regulatory T cells (Tregs) toward a conventional T cell-like repertoire, increasing autoimmune susceptibility. These findings provide critical insights into the mechanisms of self-tolerance breakdown, with implications for rheumatoid arthritis and related autoimmune diseases. Together, these studies enhance our understanding of immune dynamics and demonstrate how advanced technologies can drive new therapeutic interventions in immunology.

16. Exploration for the critical quality attributes (CQA) of chondrocytes derived from polydactyly patients with guaranteed efficacy

Yasuhisa Ishikawa, Eriko Toyoda[19], Sung-Joon Park, Kenta Nakai and Masato Sato[19,20]
[19]Department of Orthopaedic Surgery, Surgical Science, Tokai University School of Medicine
[20]Center for Musculoskeletal Innovative Research and Advancement (C-MiRA), Tokai University

Osteoarthritis of the knee (OAK) is a degenerative disease commonly affecting middle-aged and elderly women over 40. It progresses gradually due to factors such as lower limb deformation (e.g., bow legs), genetic predisposition, aging, obesity, muscle weakening, joint inflammation, or trauma from heavy work. OAK significantly reduces activities of daily living and quality of life, presenting a major challenge in aging societies by shortening healthy life expectancy. Despite its high prevalence, no curative treatment exists, and advanced cases often require artificial joint replacement. To address this, Tokai University has pioneered articular cartilage regeneration by transplanting chondrocyte sheets in OAK patients. Clinical studies have shown that both autologous and allogeneic chondrocyte sheet transplants can repair and regenerate OAK cartilage defects with hyaline cartilage, restoring original joint function. As part of this initiative, our research focuses on developing Critical Quality Attributes (CQAs) for allogeneic raw cells using bioinformatics tools, leveraging data provided by Tokai University. These CQAs are vital for ensuring the consistency, comparability, and scalability of allogeneic cell sheets for mass production. This approach aims to provide a curative treatment for OAK, enabling patients to preserve their natural joints throughout life without artificial replacements.

17. Resting heart rate and risk of dementia: a mendelian randomization study in the international genomics of Alzheimer's project and UK biobank

Xingxing Chen[21], Yi Zheng, Jun Wang[22], Blake Yue[23], Xian Zhang[24], Kenta Nakai and Lijing L. Yan[21]
[21]School of Public Health, Wuhan University
[22]Huazhong University of Science and Technology
[23]School of Business and Law, Edith Cowan University
[24]Duke Kunshan University, Global Health Research Center

Observational studies suggest a higher resting heart rate (RHR) is linked to an increased risk of dementia, but the causal relationship remains unclear. This study used two-sample Mendelian randomization to assess whether genetically predicted higher RHR influences Alzheimer's disease (AD) risk. Summary statistics from genome-wide association studies (GWAS) were analyzed using the generalized summary Mendelian randomization (GSMR) approach. Outcomes included AD diagnosis, maternal and paternal family history of AD (from UK Biobank), and a combined meta-analysis of these GWAS results. Further adjustments were made to account for RHR-modifying medications. The GSMR analysis found no significant causal relationship between genetically predicted higher RHR and AD risk (β_{GSMR} = 0.12, P = 0.30), maternal family history (β_{GSMR} = −0.18, P = 0.13), or paternal family history (β_{GSMR} = −0.14, P = 0.39). These findings remained consistent after adjusting for medication effects (β_{GSMR} = −0.03, P = 0.72). This study concludes that RHR does not causally influence dementia risk, suggesting that previous observational associations likely result from shared correlations between RHR and cardiovascular conditions.

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Human Genome Center

Department of Public Policy
公共政策研究分野

Professor	Kaori Muto, Ph.D.	教　授　博士（保健学）	武　藤　香　織
Associate Professor	Izen Ri, Ph.D.	准教授　博士（学際情報学）	李　　怡　然

The Department of Public Policy contributes to accomplishment of the following major missions: research ethics consultation to help scientists to comply with ethical guidelines and build public trust; public policy science studies of translational research and its societal impact; and promotion of patient and public involvement/ engagement in research and health care. Through qualitative and quantitative social science studies and policy analysis, we facilitate discussion of the challenges posed by the advances in medical science.

1. (Not So) Lost in Translation: Considering the GA4GH Diversity in Datasets Policy in the Japanese Context

The genomics community has long acknowledged the lack of diversity in datasets used for research, prompting various stakeholders to confront this issue. In response, the Global Alliance for Genomics and Health (GA4GH) formulated a policy framework that recognizes the multiplicity of perspectives on diversity and proposed a systemic approach for more optimal data diversity. Given the importance of the research context, assessing this policy's applicability within countries where diversity is less discussed is important. This study investigated the feasibility of implementing the GA4GH policy in Japan, a nation with a smaller genetic diversity than many Western countries. As the proportion of East Asian genomic research is limited internationally, focusing on the Japanese genome contributes to enhancing diversity. Meanwhile, labelling findings as "Japanese" can inadvertently reinforce perceptions of homogeneity and overlook ethnic minorities. Regions and socioeconomic status are also recognized as substantial factors of diversity within academia, yet concerns persist among the public regarding the heritability of stigmatized conditions. Social inclusion of sexual minorities has begun in Japan, but research surveys generally still use binary sex and gender categories, which underscores the need for additional variables. This study found that both academia and the public need to confront the overemphasis on homogeneity within Japanese society and hesitancy in addressing genetic factors. By doing so, more inclusive and diverse datasets can advance the field both ethically and scientifically. Perhaps the most important impact of the GA4GH policy will be to draw greater attention to the complex diversity challenges ahead in Japan.

2. Attitudes of patients with IVF/ICSI toward human embryo in vitro culture beyond 14 days

When the International Society for Stem Cell Research revised its 2021 guidelines, it reversed its ban on the in vitro culture of human embryos beyond 14 days. However, despite widespread recognition of the importance of public debate on embryo research, it remains unclear how patients who have undergone in vitro fertilization (IVF) and/or intracytoplasmic sperm injection (ICSI) perceive this change in the guidelines. Three focus group interviews were conducted with IVF/ICSI patients to understand their opinions on extending the in vitro culture of human embryos beyond 14 days. Thematic analysis revealed a primarily favorable attitude toward the extension of in vitro embryo culture, identifying six reasons for

this positive perspective. However, two reasons for negative attitudes were identified, along with some concerns that need to be addressed. To facilitate an open discussion, the following suggestions were made to the government and scientific community. The government and scientific community should provide sufficient knowledge to IVF/ICSI patients about research before discussions. It's important to consider diverse views on embryo models, including distrust and resistance. Ensuring IVF/ICSI patients' psychological safety is essential. "Public conversations" with citizens, including IVF/ICSI patients, should be promoted, and their opinions should be considered as part of a broader public spectrum.

3. Opinions on research involving human embryo models by researchers and the general public

Rules and ethical considerations regarding research on embryo models have been debated across numerous countries. In this paper, we provide insights from our attitude survey conducted among Japanese researchers, including members of the Japanese Society for Regenerative Medicine, and among the general public residing in Japan, the US, the UK, Canada, and Australia. Our survey revealed that many researchers expressed the need for clear guidelines for embryo model research. Furthermore, a minority but significant portion of the general public in each country expressed opposition to research on embryo models but did not oppose research involving real embryos.

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Human Genome Center

Division of Medical Data Informatics
医療データ情報学分野

Professor	Tetsuo Shibuya, Ph.D.	教　授　博士(理学)	渋　谷　哲　朗
Assistant Professor	Robert Daniel Barish, Ph.D.	助　教　博士(学術)	ロバート　ダニエル　バリッシュ

The objective of Division of Medical Data Informatics is to develop fundamental data informatics technologies for medical data, including algorithm theory, big data technologies, artificial intelligence, data mining, and privacy preserving technologies. Medical data, especially genome data are increasing exponentially from basics to clinical research in medical science. Our aim is to innovate medical science with novel data informatics solutions.

1. Development of Privacy Preserving Technologies for Medical Data

a. Privacy-Optimized Randomized Response for Sharing Multi-Attribute Data

Akihito Yamamoto[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

With the increasing amount of data in society, privacy concerns in data sharing have become widely recognized. Particularly, protecting personal attribute information is essential for a wide range of aims from crowdsourcing to realizing personalized medicine. Although various differentially private methods based on randomized response have been proposed for single attribute information or specific analysis purposes such as frequency estimation, there is a lack of studies on the mechanism for sharing individuals' multiple categorical information itself. The existing randomized response for sharing multi-attribute data uses the Kronecker product to perturb each attribute information in turn according to the respective privacy level but achieves only a weak privacy level for the entire dataset. Therefore, in this study, we propose a privacy-optimized randomized response that guarantees the strongest privacy in sharing multi-attribute data. Furthermore, we present an efficient heuristic algorithm for constructing a near-optimal mechanism [1]. The time complexity of our algorithm is $O(k^2)$, where k is the number of attributes, and it can be performed in about 1 second even for large datasets with k = 1,000. The experimental results demonstrate that both of our methods provide significantly stronger privacy guarantees for the entire dataset than the existing method. Overall, this study is an important step toward trustworthy sharing and analysis of multi-attribute data. In addition, we show an analysis example using genome statistics to confirm the high utility of our method, along with supplemental materials.

b. Generalization and Enhancement of Piecewise Mechanism for Collecting Multidimensional Data

Akihito Yamamoto[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

As the amount of data in society increases, the importance of collecting and storing data while protecting privacy also increases. In particular, protecting personal numeric data is essential for crowdsourcing and big data analytics. Although various methods have been proposed for specific analysis purposes

such as mean estimation, methods for storing numeric values themselves are still lacking. Furthermore, no method that can flexibly collect all data information with multiple attributes exists. Therefore, this study first generalizes the piecewise mechanism (PM), the state-of-the-art method in collecting a single numeric value, and proposes a new mechanism that achieves a truly smaller variance of the collected private values than the original one. Subsequently, we enhance our generalized PM for collecting multidimensional numeric data while considering a situation in which each attribute information has its own privacy level [2]. The proposed mechanism is optimal in terms of privacy guarantees for the entire dataset, and is highly advisable for collecting all information with high privacy assurance. We further evaluate our mechanism both theoretically and experimentally and show that it outperforms existing methods. We measure the accuracy of the collected private values using real census data as well, demonstrating the utility of our mechanism.

c. Differentially Private Selection using Smooth Sensitivity

Akihito Yamamoto[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

With the growing volume of data in society, the need for privacy protection in data analysis also rises. In particular, private selection tasks, wherein the most important information is retrieved under differential privacy are emphasized in a wide range of contexts, including machine learning and medical statistical analysis. However, existing mechanisms use global sensitivity, which may add larger amount of perturbation than is necessary. Therefore, this study proposes a novel mechanism for differentially private selection using the concept of smooth sensitivity and presents theoretical proofs of strict privacy guarantees. Simultaneously, given that the current state-of-the-art algorithm using smooth sensitivity is still of limited use, and that the theoretical analysis of the basic properties of the noise distributions are not yet rigorous, we present fundamental theorems to improve upon them. Furthermore, new theorems are proposed for efficient noise generation [3]. Experiments demonstrate that the proposed mechanism can provide higher accuracy than the existing global sensitivity-based methods. Finally, we show key directions for further theoretical development.

d. Cycle Counting under Local Differential Privacy for Degeneracy-bounded Graphs

Quentin Hillebrand[1], Vorapong Suppakitpaisarn[2], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo, [2]Graduate School of Information Science and Technology, The University of Tokyo

We propose an algorithm for counting the number of cycles under local differential privacy for degeneracy-bounded input graphs. Numerous studies have focused on counting the number of triangles under the privacy notion, demonstrating that the expected ℓ_2-error of these algorithms is $\Omega(n^{1.5})$, where n is the number of nodes in the graph. When parameterized by the number of cycles of length four (C_4), the best existing triangle counting algorithm has an error of $O(n^{1.5}+C_4^{0.5}) = O(n^2)$. In this study, we introduce an algorithm with an expected ℓ_2-error of $O(\delta^{1.5}n^{0.5} + \delta^{0.5}d_{\max}{}^{0.5}n^{0.5})$, where δ is the degeneracy and $d_{\max}$ is the maximum degree of the graph. For degeneracy-bounded graphs ($\delta \in \Theta(1)$) commonly found in practical social networks, our algorithm achieves an expected ℓ_2-error of $O(d_{\max}{}^{0.5}n^{0.5}) = O(n)$. Our algorithm's core idea is a precise count of triangles following a preprocessing step that approximately sorts the degree of all nodes. This approach can be extended to approximate the number of cycles of length k, maintaining a similar ℓ_2-error, namely $O(\delta^{(k-2)/2} d_{\max}{}^{0.5}n^{(k-2)/2} + \delta^{k/2}n^{(k-2)/2})$ or $O(d_{\max}{}^{0.5}n^{(k-2)/2}) = O(n^{(k-1)/2})$ for degeneracy-bounded graphs.

2. Development of Biomedical Database Technologies

a. KEGG: Biological Systems Database as a Model of the Real World

Mari Ishiguro-Watanabe[1], Minoru Kanehisa[2]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo, [2]Institute for Chemical Research, Kyoto University.

KEGG is a database resource for representation and analysis of biological systems [5]. Pathway maps are the primary dataset in KEGG representing systemic functions of the cell and the organism in terms of molecular interaction and reaction networks. The KEGG Orthology (KO) system is a mechanism for linking genes and proteins to pathway maps and other molecular networks. Each KO is a generic gene identifier and each pathway map is created as a network of KO nodes. This architecture enables KEGG pathway mapping to uncover systemic features from KO assigned genomes and metagenomes. Additional roles of KOs include characterization of conserved genes and conserved units of genes in organism groups, which can be done by taxonomy mapping. A new tool has been developed for identifying conserved gene orders in chromosomes, in which gene orders are treated as sequences of KOs. Furthermore, a new dataset called VOG (virus ortholog group) is computationally generated from virus proteins and expanded to proteins of cellular organisms, allowing gene orders to be compared as VOG sequences as

well. Together with these datasets and analysis tools, new types of pathway maps are being developed to present a global view of biological processes involving multiple organism groups.

b. Fair Selection of Clearing Schemes for Kidney Exchange Markets

Robert Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

For the Kidney Exchange Problem (KEP), one has a barter exchange market represented by a digraph with vertices corresponding to either immunologically incompatible donor-acceptor pairs, non-directed donors, cadavers, or unpaired recipients, and directed edges corresponding to possible kidney exchanges. The objective is then to solve the associated clearing problem of finding an above threshold weight partition of the network into vertex-disjoint transplant cycles and/or paths. In this study, with a primary motivation being the broad applicability of the KEP model to barter exchange markets of indivisible goods, we conduct a theoretical investigation of the problem of uniformly, and in this sense "fairly", sampling witnesses for a formalization of the KEP we denote KEP-(Lc, Lp, Υ), where we have cycle and path vertex-wise length constraints Lc and Lp, respectively, and where we require that the sum of all edge weights in a partition is at least $\Upsilon \in \mathrm{N}_0$ [6]. Here, for KEP- (∞,∞, 0), we provide an $O(4^g \cdot n^4 \cdot m)$ time uniform sampling scheme (assuming access to an idealized coin flipping oracle) for networks on n vertices and m edges admitting bimodal embeddings (i.e., embeddings where each set of edges oriented away from a given vertex occur contiguously in a rotational ordering of edges incident to the vertex) on genus $\leq g$ surfaces, as well as a Fully Polynomial-time Almost Uniform Sampling (FPAUS) scheme for arbitrary genus digraphs. Subsequently, taking inspiration from recent rapid experimental advances in using boson sampling (respectively, Guassian boson sampling) to approximate the permanents (respectively, hafnians) of complex matrices, we reduce the uniform sampling problem for KEP-(Lc, Lp, Υ) on networks with n vertices and m edges to calculating at most $O(n \cdot m)$ permanents of hollow Hermitian {−1, 0, 1} matrices. However, we moderate this latter finding by ruling out (unless NP = RP) a Fully Polynomial-time Randomized Approximation Scheme (FPRAS) for the permanent of such matrices.

c. String Editing under Pattern Constraints

Robert Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

We introduce the novel Nearest Pattern Constrained String (NPCS) problem of finding a minimum set Q of character mutation, insertion, and deletion edit operations sufficient to modify a string χ to contain all contiguous substrings in a pattern set P and no contiguous substrings in a forbidden pattern set F. Letting Σ be the alphabet of allowed characters, and letting η and Υ be the longest string length and sum of all string lengths in $P \cup F$, respectively, we show that NPCS is fixedparameter tractable in $|P|$ with time complexity $O(2^{|P|} \cdot \Upsilon \cdot |\Sigma| \cdot (|P| + \eta)(|\chi|+1))$. Additionally, we consider a generalization of the NPCS problem in which we allow for constraints based on the membership of substrings in regular languages. In particular, we introduce a problem we denote String Editing under Substring in Language Constraints (StrEdit-SILC), where provided a wildcardfree string $\chi \in \Sigma^*$, a finite set of regular languages $R = \{L1, L2, \ldots\}$, and a regular language L_F, the objective is to find a minimum cost set of mutation, insertion, and deletion edit operations Q that suffice to convert the input string χ into a string $\chi' \in \Sigma^*$, where no substring has membership in L_F, and $\forall Li \in R$, there exists a substring in Li. Here, letting Ψ and ϖ be the sum of all regular expression lengths and longest regular expression length for languages in $R \cup \{L_F\}$, respectively, and letting $C_{mid} \in \mathbb{N}$ be the maximum cost of an edit operation, we show that StrEdit-SILC is fixed-parameter tractable with respect to Ψ, having time complexity $O(2^{\Psi} \cdot |\chi| \cdot (\varpi \cdot |\Sigma| + C_{mid}))$. However, we also show that StrEdit-SILC is MAX-SNP-hard and otherwise difficult to approximate under stringent constraints.

d. Affine Optimal *k*-proper Connected Edge Colorings

Robert Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

We introduce *affine optimal k-proper connected edge colorings* as a variation on Fujita's notion of *optimal k-proper connected colorings* with applications to the frequency assignment problem [8]. Here, for a simple undirected graph G with edge set E_G, such a coloring corresponds to a decomposition of E_G into color classes $C_1, C_2, \ldots, C_n$, with associated weights $w_1, w_2, \ldots, w_n$, minimizing a specified affine function $A := \Sigma w_i \cdot |C_i|$, while also ensuring the existence of k vertex disjoint *proper paths* (i.e., simple paths with no two adjacent edges in the same color class) between all pairs of vertices. In this context, we define $\zeta^k(A, G)$ as the minimum possible value of A under a k-proper connectivity requirement. For any fixed number of color classes, we show that computing $\zeta^k(A', G)$ is treewidth fixed parameter tractable. However, we also show that determining $\zeta^k(A', G)$ with the affine function $A' := |C_2|$ is *NP*-hard for 2-connected planar graphs in the case

where $k = 1$, cubic 3-connected planar graphs for $k = 2$, and k-connected graphs $\forall k \geq 3$. We also show that no fully polynomial-time randomized approximation scheme can exist for approximating $\zeta^k(A', G)$ under any of the aforementioned constraints unless $NP = RP$.

e. Proper Colorability of Segment Intersection Graphs

Robert Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

We consider the vertex proper coloring problem for highly restricted instances of geometric intersection graphs of line segments embedded in the plane [9]. Provided a graph in the class UNIT-PURE-k-DIR, corresponding to intersection graphs of unit length segments lying in at most k directions with all parallel segments disjoint, and provided explicit coordinates for segments whose intersections induce the graph, we show for $k = 4$ that it is NP-complete to decide if a proper 3-coloring exists, and moreover, #P-complete under many-one counting reductions to determine the number of such colorings. In addition, under the more relaxed constraint that segments have at most two distinct lengths, we show these same hardness results hold for finding and counting proper $(k-1)$-colorings for every $k \geq 5$. More generally, we establish that the problem of proper 3-coloring an arbitrary graph with m edges can be reduced in $O(m^2)$ time to the problem of proper 3-coloring a UNIT-PURE-4-DIR graph. This can then be shown to imply that no $2^{o(\sqrt{n})}$ time algorithm can exist for proper 3-coloring PURE-4-DIR graphs under the Exponential Time Hypothesis (ETH), and by a slightly more elaborate construction, that no $2^{o(\sqrt{n})}$ time algorithm can exist for counting the such colorings under the Counting Exponential Time Hypothesis (#ETH). Finally, we prove an NP-hardness result for the optimization problem of finding a maximum order proper 3-colorable induced subgraph of a UNIT-PURE-4-DIR graph.

f. Counting on Rainbow k-Connections

Robert Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

For an undirected graph imbued with an edge coloring, a rainbow path (resp. proper path) between a pair of vertices corresponds to a simple path in which no two edges (resp. no two adjacent edges) are of the same color. In this context, we refer to such an edge coloring as a rainbow k-connected w-coloring (resp. k-proper connected w-coloring) if at most w colors are used to ensure the existence of at least k internally vertex disjoint rainbow paths (resp. k internally vertex disjoint proper paths) between all pairs of vertices. At present, while there have been extensive efforts to characterize the complexity of finding rainbow 1-connected colorings, we remark that very little appears to known for cases where k >1. In this work, we first show that the problems of counting rainbow k-connected w-colorings and counting k-proper connected w-colorings are both linear time treewidth Fixed Parameter Tractable (FPT) for every k>0 and w>0. Subsequently, and in the other direction, we extend prior NP-completeness results for deciding the existence of a rainbow 1-connected w-coloring for every w>1, in particular, showing that the problem remains NP-complete for every k>0 and w>1. This yields as a corollary that no Fully Polynomial-time Randomized Approximation Scheme (FPRAS) can exist for approximately counting such colorings in any of these cases (unless NP = RP). Next, concerning counting hardness, we give the first #P-completeness result we are aware of for rainbow connected colorings, proving that counting rainbow k-connected 2-colorings is #P-complete for every k>0 [10].

g. Counting 2-Factors of 4-Regular Bipartite Graphs

Robert Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

We prove that counting 2-factors (i.e., spanning 2-regular subgraphs or vertex disjoint cycle covers) of 4-regular bipartite graphs is #P-complete under many-one counting (i.e., weakly parsimonious) reductions. This resolves a missing case in a proof by Felsner et al. that counting 2-factors of k-regular bipartite graphs is #P-complete for cases k >5 and k=3. Due to a bijective correspondence, it establishes the same hardness result for counting the Eulerian orientations of 4-regular bipartite graphs [11].

h. Recognition and Proper Coloring of Unit Segment Intersection Graphs

Robert Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

In this work, we concern ourselves with the fine-grained complexity of recognition and proper coloring problems on highly restricted classes of geometric intersection graphs of "thin" objects (i.e., objects with unbounded aspect ratios) [12]. As a point of motivation, we remark that there has been significant interest in finding algorithmic lower bounds for classic decision and optimization problems on these types of graphs, as they appear to escape the net of known planar or geometric separator theorems for "fat" objects (i.e., objects with bounded aspect ratios). In particu-

lar, letting n be the order of a geometric intersection graph, and assuming a geometric ply bound, per what is known as the "square root phenomenon", these separator theorems often imply the existence of $2^{o(\sqrt{n})}$ algorithms for problems ranging from finding proper colorings to finding Hamiltonian cycles. However, in contrast, it is known for instance that no $2^{o(\sqrt{n})}$ time algorithm can exist under the Exponential Time Hypothesis (ETH) for proper 6-coloring intersection graphs of line segments embedded in the plane. We begin by establishing algorithmic lower bounds for proper k-coloring and recognition problems of intersection graphs of line segments embedded in the plane under the most stringent constraints possible that allow either problem to be non-trivial. In particular, we consider the class UNIT PURE-k-DIR of unit segment geometric intersection graphs, in which segments are constrained to lie in at most k directions in the plane, and no two parallel segments are permitted to intersect.

Here, under the ETH, we show for every $k \geq 3$ that no $2^{o(\sqrt{n/k})}$ time algorithm can exist for either recognizing or proper k-coloring UNIT-PURE-k-DIR graphs of order n. In addition, for every $k \geq 4$, we establish the same algorithmic lower bound under the ETH for the problem of proper $(k-1)$-coloring UNIT-PURE-k-DIR graphs when provided a list of segment coordinates specified using $O(n \cdot k)$ bits witnessing graph class membership. As a consequence of our approach, we are also able to show that the problem of properly 3-coloring an arbitrary graph on m edges can be reduced in $O(m^2)$ time to the problem of properly $(k-1)$-coloring a UNIT-PURE-k-DIR graph. Finally, we consider a slightly less constrained class of geometric intersection graphs of lines (of unbounded length) in which line-line intersections must occur on any one of $(r = 3)$ parallel planes in R3. In this context, for every $k \geq 3$, we show that no $2^{o(n/k)}$ time algorithm can exist for proper k-coloring these gr*aphs unless the ETH is false.*

i. Polyhedral roll-connected colorings of partial tiling

Robert Barish[1], Tetsuo Shibuya[1]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo

We consider the problem of coloring the faces of an edge-to-edge partial tiling T such that a specified face-colored polyhedron P "rolling" over this tiling – where each time a face of the polyhedron is congruent with a polygonal tile in T, both the face and the tile must have the same coloration – is able to reach any tile from any other tile [13]. Here, for P corresponding to any Platonic solid, we show that the existence of such a coloring, using at most $w \geq 1$ distinct colors, can be decided in $O(T)$ time. On the other hand, when we require at least two internally disjoint manners of rolling from any starting location to any ending location, we show for P corresponding to the cube and for $w = 3$ that deciding the existence of and counting such colorings becomes NP-hard and #P-hard, respectively.

3. Survey on Cutting-Edge Quantum Machine Learning Technologies

Yaswita Gujju[1], Atsushi Matsuo[2], Rudy Raymond[3,4,5]: [1]Division of Medical Data Informatics, Institute of Medical Science, The University of Tokyo, [2]IBM Quantum, IBM Research, [3]Department of Computer Science, The University of Tokyo, [4]Global Technology and Applied Research, J. P. Morgan Chase & Co., [5]Quantum Computing Center, Keio University

The past decade has witnessed significant advancements in quantum hardware, encompassing improvements in speed, qubit quantity, and quantum volume—a metric defining the maximum size of a quantum circuit effectively implementable on near-term quantum devices. This progress has led to a surge in quantum machine learning (QML) applications on real hardware, aiming to achieve quantum advantage over classical approaches. Our survey [14] focuses on selected supervised and unsupervised learning applications executed on quantum hardware, specifically tailored for real-world scenarios. The exploration includes a thorough analysis of current QML implementation limitations on quantum hardware, covering techniques like encoding, ansatz structure, error mitigation, and gradient methods to address these challenges. Furthermore, the survey evaluates the performance of QML implementations in comparison to classical counterparts. In conclusion, we discuss existing bottlenecks related to applying QML on real quantum devices and propose potential solutions to overcome these challenges in the future.

Publications

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2. Akito Yamamoto and Tetsuo Shibuya, "Generalization and Enhancement of Piecewise Mechanism for Collecting Multidimensional Data", *Proc. 17th IEEE International Conference on Security, Privacy and Anonymity in Computation, Communication and Storage*, in press.

3. Akito Yamamoto and Tetsuo Shibuya, "Differentially Private Selection using Smooth Sensitivity", *Proc. 43rd IEEE International Performance, Computing and Communications Conference*, in press.
4. Quentin Hillebrand, Vorapong Suppakitpaisarn, and Tetsuo Shibuya, "Cycle Counting under Local Differential Privacy for Degeneracy-bounded Graphs", *Proc. the 42nd International Symposium on Theoretical Aspects of Computer Science*, in press.
5. Minoru Kanehisa, Miho Furumichi, Yoko Sato, Yuriko Matsuua, Mari Ishiguro-Watanabe, "KEGG: biological systems database as a model of the real world", *Nucleic Acids Research*, 2024, gkae909.
6. Robert Barish, Tetsuo Shibuya, "Fair Selection of Clearing Schemes for Kidney Exchange Markets", *Proc. 17th International Conference on Combinatorial Optimization and Applications*, in press.
7. Robert Barish, Tetsuo Shibuya, "String editing under pattern constraints", Theoretical Computer Science, *Theoretical Computer Science*, 1022, 2024, 114889, ISSN 0304-3975.
8. Robert Barish, Tetsuo Shibuya, "Affine optimal k-proper connected edge colorings", *Optimization Letters*, S11590-024-021, Springer, 2024.
9. Robert Barish, Tetsuo Shibuya, "Proper colorability of segment intersection graphs", *Journal of Combinatorial Optimization*, 47(4), 2024.
10. Robert Barish, Tetsuo Shibuya, "Counting on rainbow *k*-connections", In: Chen, X., Li, B. (eds) Theory and Applications of Models of Computation (TAMC 2024), *Lecture Notes in Computer Science*, vol. 14637, Springer, 2024, pp 272–283.
11. Robert Barish, Tetsuo Shibuya, "Counting 2-factors of 4-regular bipartite graphs is #P-complete", *Lecture Notes in Computer Science*, Springer, in press.
12. Robert D. Barish and Tetsuo Shibuya. "Recognition and Proper Coloring of Unit Segment Intersection Graphs". *Proc. 19th Scandinavian Symposium and Workshops on Algorithm Theory*. Leibniz International Proceedings in Informatics (LIPIcs), 294, , 2024, pp. 5:1-5:19.
13. Robert Barish, Tetsuo Shibuya, "Polyhedral roll-connected colorings of partial tilings", *Proc. Canadian Conference on Computational Geometry*, July 17-19, 2024, pp. 317-324.
14. Yaswitha Gujju, Atsushi Matsuo, Rudy Raymond, "Quantum Machine Learning on Near-Term Quantum Devices: Current State of Supervised and Unsupervised Techniques for Real-World Applications", *Phys. Rev. Applied,* 21, 067001.

Human Genome Center

Division of Health Medical Intelligence
健康医療インテリジェンス分野

Professor	Seiya Imoto, Ph.D.	教　授	博士(数理学)	井　元　清　哉
Project Associate Professor	Yao-zhong Zhang, Ph.D.	准教授	博士(情報理工学)	張　　耀　中
Assistant Professor	Noriaki Sato, Ph.D.	助　教	博士(医学)	佐　藤　憲　明
Project Assistant Professor	Satoshi Ito	特任助教		伊　東　　聰
Project Assistant Professor	Yusri Dwi Heryanto, Ph.D.	特任助教	博士(医学)	ユスリ ドウィ ヘリヤント

Laboratory of Sequence Analysis
シークエンスデータ情報処理分野

Professor	Seiya Imoto, Ph.D.	教　授	博士(数理学)	井　元　清　哉
Associate Professor	Kotoe Katayama, Ph.D.	准教授	博士(情報学)	片　山　琴　絵

Our mission is to realize genomic medicine based on the integrated data analysis of whole genomes of human and commensal microbiota by supercomputing. Development of computational data analysis methods including artificial intelligence for genomic, health, and medical big data is one of our main focuses. We promote integrative analysis of human whole genome, RNA and other omics data, commensal microbiota including bacteriome and virome, and health and medical-related big data. Furthermore, health medical intelligence aims at using the analysis results of such big data to create personalized health-medical action plan of individuals.

1. Whole Genome Sequencing and Genomic Medicine

a. Creating New Genomic Medicine by Integrating Human Whole Genome and Commensal Microbiota

Katayama K, Sato N, Shimizu E, Kasajima R, Yamaguchi K, Yokoyama K, Yadome M, Hyugaji T, Komura M, Yamamoto M, Saito A, Zhang Y-Z, Fujimoto K, Kobayashi M, Ogawa M, Takei T, Yasui H, Yuji K, Takane K, Ikenoue T, Robert B, Shibuya T, Hiroshima Y, Hasegawa T, Miyagi Y, Muto K, Goyama S, Shida D, Boku N, Kawabata K, Miyano S, Yamaguchi R, Uematsu S, Kumasaka N, Takahashi S, Nanya Y, Furukawa Y, Imoto S

Using state-of-the-art genome analysis and artificial intelligence, our mission is to implement "new genomic medicine" by integrating human genome information and human symbiotic microbial metagenome information.

In Japan, gene panel testing was covered by national health insurance from Jun 2019, however, it

analyzed several hundreds of genes, which were known cancer-related genes. Since the gene panel has trivial limitation due to its focused genes, Japanese government considered to extend the gene panel to whole genome. However, it remains a question that whether the whole genome sequence information is enough to realize precision medicine.

Although human genome has 20 thousand genes, intestinal microbiota has 20 million genes, and they work together with human genes for keeping homeostasis of our lives. In recent years, with the advancement of sequencing technology, we could have a whole figure of intestinal microbiota and found its dysbiosis leads to various diseases. We are proceeding a research for utilizing the information of intestinal microbiota (meta-genome) and human genome to create new genomic medicine in Society5.0. For this purpose, we need to establish an artificial intelligence to translate the information of human genome and meta-genome to clinical actions of physicians.

b. Establishment of Data Analysis Center in Action Plan for Whole Genome Analysis of Ministry of Health, Labour and Welfare

Katayama K, Shibuya T, Yamaguchi R, Kumasaka N, Matsuda K, Miyo K[1], Okamura H[2], Ota K[2], Shintani A[2], Shiraishi Y[3], Kohno T[3], Kato M[3], Okada Y[4], Fujimoto A[4], Kasai S[5], Imoto S: [1]National Center for Global Health and Medicine, [2]Osaka Metropolitan University, [3]National Cancer Center, Japan, [4]University of Tokyo School of Medicine, Japan, [5]Information-Technology Promotion Agency, Japan

Based on the Whole Genome Analysis Action Plan (Version 1) formulated on December 20, 2019 by the Ministry of Health, Labour and Welfare, the AMED project was launched in 2021 aiming at returning the result of WGS analysis to the patients as medical actions. This national project covers a wide range of intractable cancers, including gastrointestinal, hematological, pediatric, rare, gynecological, and respiratory cancers. A total of 9,900 patients will be subjected to whole genome sequencing analysis with depth of 30x for normal and 120x for tumor samples, and RNA sequencing will also be conducted.

Our team (PI: Prof. Seiya Imoto of IMSUT) is building the Analysis Data Center to collect and compile a database of genomic data and clinical information of these cancer patients. The mission of the Analysis Data Center is to construct a unified analysis pipeline for primary analysis of genomic data, to collect clinical information, to build a reporting system that can be used in expert panels, to build a secure data sharing system, and to build an analysis environment that can perform advanced secondary analysis in a hybrid computational environment of on-premises and cloud.

2. Metagenome Analysis of Intestinal Microbiota

a. Unveiling viral dark matter by whole metagenome analysis of bacteriome and virome

Fujimoto K, Kimura Y, Shimohigoshi M, Sato N, Zhang Y-Z, Katayama K, Satoh M, Sato S, Tremmel G, Uematsu M, Kawaguchi Y, Usui Y, Nakano Y, Hayashi T, Kashima K, Yuki Y, Yamaguchi K, Furukawa Y, Kakuta M, Akiyama Y[4], Yamaguchi R, Crowe SE[5], Ernst PB[6], Miyano S, Kiyono H, Imoto S, Uematsu S: [4]Department of Computer Science, Tokyo Institute of Technology, Japan, [5]Department of Medicine, University of California, San Diego, USA, [6]CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines, University of California San Diego, USA.

The application of bacteriophages (phages) is proposed as a highly specific therapy for intestinal pathobiont elimination. However, the infectious associations between phages and bacteria in the human intestine, which is essential information for the development of phage therapies, have yet to be fully elucidated. Here, we report the intestinal viral microbiomes (viromes), together with bacterial microbiomes (bacteriomes), in 101 healthy Japanese individuals. Based on the genomic sequences of bacteriomes and viromes from the same fecal samples, the host bacteria-phage associations are illustrated for both temperate and virulent phages. To verify the usefulness of the comprehensive host bacteria-phage information, we screened Clostridioides difficile-specific phages and identified antibacterial enzymes whose activity is confirmed both in vitro and in vivo. These comprehensive metagenome analyses reveal not only host bacteria-phage associations in the human intestine but also provide vital information for the development of phage therapies against intestinal pathobionts.

b. An enterococcal phage-derived enzyme suppresses graft-versus-host disease

Fujimoto K, Hayashi T[7], Yamamoto M, Sato N, Shimohigoshi M[7], Miyaoka D[7], Yokota C[7], Watanabe M[7], Hisaki Y[7], Kamei Y[7], Yokoyama Y[7], Yabuno T[7], Hirose A[7], Nakamae M[7], Nakamae H[7], Uematsu M, Sato S[7], Yamaguchi K, Furukawa Y, Akeda Y[8], Hino M[7], Imoto S, Uematsu S: [7]Osaka Metropolitan University, [8]National Institute of Infectious Diseases.

Changes in the gut microbiome have pivotal roles in the pathogenesis of acute graft-versus-host disease (aGVHD) after allogenic haematopoietic cell transplantation (allo-HCT). However, effective methods for safely resolving gut dysbiosis have not yet been established. An expansion of the pathogen Enterococcus faecalis in the intestine, associated with dysbiosis,

has been shown to be a risk factor for aGVHD. Here we analyse the intestinal microbiome of patients with allo-HCT, and find that E. faecalis escapes elimination and proliferates in the intestine by forming biofilms, rather than by acquiring drug-resistance genes. We isolated cytolysin-positive highly pathogenic E. faecalis from faecal samples and identified an anti-*E. faecalis* enzyme derived from *E. faecalis*-specific bacteriophages by analysing bacterial whole-genome sequencing data. The antibacterial enzyme had lytic activity against the biofilm of *E. faecalis* in vitro and in vivo. Furthermore, in aGVHD-induced gnotobiotic mice that were colonized with *E. faecalis* or with patient faecal samples characterized by the domination of *Enterococcus*, levels of intestinal cytolysin-positive *E. faecalis* were decreased and survival was signiffcantly increased in the group that was treated with the *E. faecalis*-specific enzyme, compared with controls. Thus, administration of a phage-derived antibacterial enzyme that is specific to biofilm-forming pathogenic *E. faecalis*—which is difficult to eliminate with existing antibiotics—might provide an approach to protect against aGVHD.

3. Health Medical Data Science

a. stana: an R package for metagenotyping analysis and interactive application based on clinical data

Sato N, Katayama K, Miyaoka D, Uematsu M, Saito A, Fujimoto K, Uematsu S, Imoto S

Metagenotyping of metagenomic data has recently attracted increasing attention as it resolves intraspecies diversity by identifying single nucleotide variants. Furthermore, gene copy number analysis within species provides a deeper understanding of metabolic functions in microbial communities. However, a platform for examining metagenotyping results based on relevant grouping data is lacking. Here, we have developed the R package, stana, for the processing and analysis of metagenotyping results. The package consists of modules for preprocessing, statistical analysis, functional analysis and visualization. An interactive analysis environment for exploring the metagenotyping results was also developed and publicly released with over 1000 publicly available metagenome samples related to human diseases. Three examples exploring the relationship between the metagenotypes of the gut microbiome and human diseases are presented-end-stage renal disease, Crohn's disease and Parkinson's disease. The results suggest that stana facilitated the confirmation of the original study's findings and the generation of a new hypothesis.

b. Predicting cell types with supervised contrastive learning on cells and their types

Heryanto YD, Zhang YZ, Imoto S.

Single-cell RNA-sequencing (scRNA-seq) is a powerful technique that provides high-resolution expression profiling of individual cells. It significantly advances our understanding of cellular diversity and function. Despite its potential, the analysis of scRNA-seq data poses considerable challenges related to multicollinearity, data imbalance, and batch effect. One of the pivotal tasks in single-cell data analysis is cell type annotation, which classifies cells into discrete types based on their gene expression profiles. In this work, we propose a novel modeling formalism for cell type annotation with a supervised contrastive learning method, named SCLSC (Supervised Contrastive Learning for Single Cell). Different from the previous usage of contrastive learning in single cell data analysis, we employed the contrastive learning for instance-type pairs instead of instance-instance pairs. More specifically, in the cell type annotation task, the contrastive learning is applied to learn cell and cell type representation that render cells of the same type to be clustered in the new embedding space. Through this approach, the knowledge derived from annotated cells is transferred to the feature representation for scRNA-seq data. The whole training process becomes more efficient when conducting contrastive learning for cell and their types. Our experiment results demonstrate that the proposed SCLSC method consistently achieves superior accuracy in predicting cell types compared to five state-of-the-art methods. SCLSC also performs well in identifying cell types in different batch groups. The simplicity of our method allows for scalability, making it suitable for analyzing datasets with a large number of cells. In a real-world application of SCLSC to monitor the dynamics of immune cell subpopulations over time, SCLSC demonstrates a capability to discriminate cell subtypes of CD19+ B cells that were not present in the training dataset.

c. Biotextgraph: graphical summarization of functional similarities from textual information

Sato N, Zhang YZ, Gu Z[9], Imoto S: [9]National Center for Tumor Diseases, Heidelberg, Germany

Functional interpretation of biological entities such as differentially expressed genes is one of the fundamental analyses in bioinformatics. The task can be addressed by using biological pathway databases with enrichment analysis. However, textual description of biological entities in public databases is less explored and integrated in existing tools and it has a potential to reveal new mechanisms. Here, we present a new R package biotextgraph for graphical sum-

marization of omics' textual description data which enables assessment of functional similarities of the lists of biological entities. We illustrate application examples of annotating gene identifiers in addition to enrichment analysis. The results suggest that the visualization based on words and inspection of biological entities with text can reveal a set of biologically meaningful terms that could not be obtained by using biological pathway databases alone. The results suggest the usefulness of the package in the routine analysis of omics-related data. The package also offers a web-based application for convenient querying.

4. COVID-19

a. Statistically and functionally fine-mapped blood eQTLs and pQTLs from 1,405 humans reveal distinct regulation patterns and disease relevance

Wang QS, Hasegawa T, Namkoong H, Saiki R, Edahiro R, Sonehara K, Tanaka H, Azekawa S, Chubachi S, Takahashi Y, Sakaue S, Namba S, Yamamoto K, Shiraishi Y, Chiba K, Tanaka H, Makishima H, Nannya Y, Zhang Z, Tsujikawa R, Koike R, Takano T, Ishii M, Kimura A, Inoue F, Kanai T, Fukunaga K, Ogawa S, Imoto S, Miyano S, Okada Y, Japan COVID-19 Task Force

Studying the genetic regulation of protein expression (through protein quantitative trait loci (pQTLs)) offers a deeper understanding of regulatory variants uncharacterized by mRNA expression regulation (expression QTLs (eQTLs)) studies. Here we report *cis*-eQTL and *cis*-pQTL statistical fine-mapping from 1,405 genotyped samples with blood mRNA and 2,932 plasma samples of protein expression, as part of the Japan COVID-19 Task Force (JCTF). Fine-mapped eQTLs ($n = 3,464$) were enriched for 932 variants validated with a massively parallel reporter assay. Fine-mapped pQTLs ($n = 582$) were enriched for missense variations on structured and extracellular domains, although the possibility of epitope-binding artifacts remains. *Trans*-eQTL and trans-pQTL analysis highlighted associations of class I HLA allele variation with KIR genes. We contrast the multi-tissue origin of plasma protein with blood mRNA, contributing to the limited colocalization level, distinct regulatory mechanisms and trait relevance of eQTLs and pQTLs. We report a negative correlation between *ABO* mRNA and protein expression because of linkage disequilibrium between distinct nearby eQTLs and pQTLs.

b. Quantitative association of SARS-CoV-2 in wastewater and clinically confirmed cases in different areas of the Tokyo 2020 Olympic and Paralympic Village

Kitajima M[9], Murakami M[10], Ando H[11], Kadoya SS[9], Iwamoto R[12], Kuroita T[12], Yamaguchi K, Kobayashi H[12], Okabe S[11], Katayama H[9], Imoto S: [9]Graduate School of Engineering, The University of Tokyo, [10]Osaka University, [11]Hokkaido University, [12]Shionogi & Co. Ltd.

International mass gathering events, such as the Olympic and Paralympic Games, face the risk of cross-border transmission of infectious diseases. We previously reported that wastewater-based epidemiology (WBE), which has attracted attention as a COVID-19 surveillance tool, was implemented in the Tokyo 2020 Olympic and Paralympic Village to gain a comprehensive understanding of COVID-19 incidence in the village. In the present study, we explored the quantitative association of wastewater viral load and clinically confirmed cases in various areas of the village. From July 14 through September 8, 2021, 360 passive samples and 329 grab samples were collected from seven distinct areas within the village through manholes and examined for SARS-CoV-2 RNA by the Efficient and Practical virus Identification System with Enhanced Sensitivity (EPISENS) methods. The detection rates of SARS-CoV-2 RNA in passive and grab samples showed a significant association ($P < 0.001$, $\varphi = 0.32$, chi-square test), with passive sampling showing higher positive rate. Based on the Receiver Operating Characteristic (ROC) curve analysis on the wastewater viral load and clinically confirmed cases, the most sensitive cutoff point was judged to be the limit of quantification (LOQ) for the passive three-day samples. Under this optimal condition, the sensitivity and specificity were 0.78 and 0.40, respectively. The present study demonstrated the effectiveness of passive sampling for building-level wastewater surveillance based on the quantitative analysis of wastewater viral load and reported cases. Wastewater surveillance can be a powerful tool to monitor the incidence of infectious diseases among temporary residents, such as tourists and participants in international mass gathering events, provided that proper analytical methods and quantitative cutoff point are employed.

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Division of Metagenome Medicine
メタゲノム医学分野

Project Professor	Satoshi Uematsu, M.D., Ph.D.		特任教授	博士（医学）	植　松　　智
Project Associate Professor	Kosuke Fujimoto, M.D., Ph.D.		特任准教授	博士（医学）	藤　本　康　介

Abnormal compositions of intestinal microbiota have been reported to be associated with various diseases. We analyze intestinal bacteriome and virome in various diseases and search for "pathobiont" that causes the diseases. By making use of bioinformatics, we are constructing an analysis pipeline for intestinal microbiome, conducting comprehensive metagenomic analysis, and developing phage therapy for the specific control of pathobionts.

1. Analysis of skin microbiota in axillary osmidrosis.

Kosuke Fujimoto[1], Seiya Imoto[2] and Satoshi Uematsu[1]

[1]Division of Metagenome Medicine, Human Genome Center, The Institute of Medical Science, The University of Tokyo. [2]Division of Health Medical Inteligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo. [3]Division of Metagenome Medicine, Human Genome Center, The Institute of Medical Science, The University of Tokyo.

About 10% of the Japanese population is said to have axillary odor. The odor is caused by apocrine gland secretions contained in axillary sweat. They are odorless immediately after secretion, but are transformed into malodorous metabolites when metabolized by indigenous bacteria in the skin. Each axillary odor has its own characteristics, and about 90% of people can be divided into, in order of prevalence, milk-like odor (Type M), acid-like odor (Type A), and curry-spice-like odor (Type C). In a joint study with Mandom Corporation, we collected body fluid samples extracted from the axillae of 20 healthy adult males and classified them into 11 C-type and 9 M-type individuals based on the judgment of odor judges. Analysis of metabolites in the samples showed an increase in precursors of odor-causing metabolites in the C group. Next, shotgun metagenomic analysis of axillary skin flora showed that *Streptococcus hominis*, which is involved in the production of odorant precursors in type C, was significantly increased, indicating that it plays an important role in the production of odorant substances. Furthermore, we searched for a specific bacteriolysis enzyme for *S. hominis* using metagenomic data and succeeded in obtaining a new bacteriolysis enzyme sequence that could be purified. We also confirmed that this bacteriolysis enzyme has no bacteriolytic effect on typical skin-dwelling bacteria other than the targeted *S. hominis*. The results of this study may be a useful tool for specifically lysing *S. hominis*, which is involved in the production of odor substance. In the future, we plan to develop phage deodorants for axillary odors.

2. The development of a new therapeutic agent for GVHD

Kosuke Fujimoto[1], Seiya Imoto[2] and Satoshi Uematsu[1]

[1]Division of Metagenome Medicine, Human Genome Center, The Institute of Medical Science, The University of Tokyo. [2]Division of Health Medical Inteligence, Human Genome Center, The Institute of

Medical Science, The University of Tokyo. [3]Division of Metagenome Medicine, Human Genome Center, The Institute of Medical Science, The University of Tokyo.

In recent years, it has become clear that "dysbiosis" is found in a variety of diseases, due to improved genome analysis technology. In organ transplantation, immune cells attack the transplanted organ as a foreign body, resulting in rejection. In hematopoietic stem cell transplantation for the treatment of leukemia and other diseases, immune cells derived from the transplanted hematopoietic stem cells may develop graft-versus-host disease (GVHD), in which immune cells attack the transplant patient's organ as if it were a foreign body. Previous studies have reported that GVHD is exacerbated when the balance of the intestinal microflora is disturbed during the treatment process of hematopoietic stem cell transplantation and *Enterococcus spp*. increase. We performed a metagenomic analysis of fecal samples from 46 hematopoietic stem cell transplant (allogeneic transplant) patients at Osaka Metropolitan University Hospital and found not only an increase in *Enterococcus spp.* in 30 of the 46 patients, but also the presence of highly toxic *Enterococcus faecalis*, involved in the development of GVHD. During the treatment of hematopoietic stem cell transplantation, antimicrobial agents are used to protect against infection, and it was thought that this highly toxic *E. faecalis* escaped from the antimicrobial agents by forming biofilms in the intestinal tract, thereby proliferating. In addition, it was found that GVHD worsened in the gnotobiotic mice in which highly toxic *E. faecalis* was established. Therefore, we tried to couduct a metagenomic analysis of *E. faecalis* to search for a bacteriolysis enzyme that specifically acts on *E. faecalis*. As a result, we successfully identified the sequence of a novel bacteriolysis enzyme, endolysin and synthesized the enzyme according to the sequence. This enzyme nicely lysed *E. faecalis*. Furthermore, the endolysin destroyed biofilme of *E. faecalis*, *in vitro* and *in vivo*. When this bacteriolysis enzyme was administered to GVHD model mice in which highly toxic *E. faecalis* was established, we confirmed that it inhibited the worsening of GVHD and significantly improved the mortality rate. The phage-derived bacteriolysis enzyme obtained in this study is expected to lead to the development of new therapeutic agents for GVHD in the future.

3. Development of a microbiome digital twin to predict disease states based on metagenome analysis of intestinal microflora

Kosuke Fujimoto[1], Seiya Imoto[2] and Satoshi Uematsu[1]
[1]Division of Metagenome Medicine, Human Genome Center, The Institute of Medical Science, The University of Tokyo. [2]Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo. [3]Division of Metagenome Medicine, Human Genome Center, The Institute of Medical Science, The University of Tokyo.

We are developing a digital twin that predicts disease states using metagenomic data of the intestinal microbiota and gene pathway analysis data as teaching data in collaboration with Fujitsu Limited. For this purpose, we collected fecal samples from 10 Crohn's disease patients and 18 Parkinson's disease patients and performed metagenomic analysis. We compared these data with metagenomic data of 100 healthy subjects and have performed machine learning and deep learning. We are tuning to further develop it into an XAI.

4. Development of next-generation mucosal vaccine against infectious diseases

Kosuke Fujimoto[1], Satoshi Uematsu[1]
[1]Division of Metagenome Medicine, Human Genome Center, The Institute of Medical Science, The University of Tokyo.

A next-generation vaccine strategy capable of inducing both systemic and mucosal immunity is awaited. We showed that intramuscular vaccination with a combination of CpG oligodeoxynucleotides and curdlan as adjuvants systemically induced antigen-specific IgA and IgG production in mice. After priming, markedly high titers and long-lasting antigen-specific IgA and helper T-cell responses including Th1 and Th17 responses in the mucosa were acquired by antigen boosting of the target organs. This immunization effectively regulated *Streptococcus pneumoniae* infection in mice. The patent of this new vaccine strategy was granted in 2019 in Japan, in 2020 in US and in 2021 in Europe. We have conducted monkey experiments for formulation in human by using PspA, a universal Ag of *S. pneumoniae*. Although vaccination is recommended for protection against invasive pneumococcal disease, the frequency of pneumococcal pneumonia is still high worldwide. In fact, no vaccines are effective for all pneumococcal serotypes. Fusion pneumococcal surface protein A (PspA) has been shown to induce a broad range of cross-reactivity with clinical isolates and afford cross-protection against pneumococcal challenge in mice. Furthermore, we developed prime-boost-type mucosal vaccines that induce both antigen-specific IgG in serum and antigen-specific IgA in targeted mucosal organs in previous studies. We investigated whether our prime-boost-type immunization with a fusion PspA was effective against pneumococcal infection in mice and cynomolgus macaques. C57BL/6 mice were intramuscularly injected with fusion PspA combined with CpG oligodeoxynucleotides and/or curdlan. Six

weeks later, PspA was administered intranasally. Blood and bronchoalveolar lavage fluid were collected and antigen-specific IgG and IgA titers were measured. Some mice were given intranasal *Streptococcus pneumoniae* and the severity of infection was analyzed. Macaques were intramuscularly injected with fusion PspA combined with CpG oligodeoxynucleotides and/or curdlan at week 0 and week 4. Then, 13 or 41 weeks later, PspA was administered intratracheally. Blood and bronchoalveolar lavage fluid were collected and antigen-specific IgG and IgA titers were measured. Some macaques were intranasally administered *S. pneumoniae* and analyzed for the severity of pneumonia. Serum samples from mice and macaques injected with antigens in combination with CpG oligodeoxynucleotides and/or curdlan contained antigen-specific IgG. Bronchial samples contained antigen-specific IgA after the fusion PspA boosting. This immunization regimen effectively prevented *S. pneumoniae* infection. Prime-boost-type immunization with a fusion PspA prevented *S. pneumoniae* infection in mice and macaques. Unlike mice, primates were able to induce sIgA sufficiently with emulsion and curdlan, and we would like to create a new emulsion that compensates for the action of CpG DNA and consider using it as a new vaccine adjuvant.

Publications

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Human Genome Center

Division of Digital Genomics
デジタル・ゲノミクス分野

Professor Natsuhiko Kumasaka, Ph.D.
教　授　博士（理学）　熊　坂　夏　彦

A genome-wide association study (GWAS) is a powerful approach for identifying genetic variants and related genes involved in the molecular mechanisms of common complex traits, such as diabetes and human height. As of April 2024, the GWAS Catalogue reports 691,532 genetic associations discovered for 36,643 common complex traits. The Division of Digital Genomics aims to identify these genetic associations of common complex traits and uncover their molecular mechanisms using cutting-edge molecular biology assays and integrated mathematical and statistical approaches.

1. Discovery of genetic determinants for child health and development

Natsuhiko Kumasaka

Understanding the influence of both genetics and environment on human health, especially early in life, is essential for shaping long-term health. Here, I utilize a population-based prospective birth cohort, the Japan Environment and Children's Study (JECS), to conduct a large-scale genetic study using questionnaire surveys and biological and physical measurements collected from both parents and their children since the participant mothers were pregnant.

JECS is a large-scale birth cohort study established by the Ministry of the Environment, Government of Japan, to evaluate the effects of environmental chemicals on children's health and development. Over 100,000 pregnant women were enrolled at 15 geographically different regional centers across Japan, representing the comprehensive genetic diversity of the Japanese population. Detailed data from questionnaires, biological and physical measurements have been collected with additional surveys every six months still being conducted on average 70% of the child participants until the age of 4.

My role is to conduct genome-wide association studies using the various child health and developmental outcomes and to make the results available to the public. As of December 2024, genome-wide genotyping analyses have been performed on 80,638 child participants with parental consent and sufficient DNA from cord blood samples. Systematic genome-wide association studies of 1,163 child health and developmental traits (including, for example, food allergy or ASQ-3 developmental screening) and parental environmental exposure traits identified 4,985 common genomic loci, of which a part of loci represented novel associations not previously reported. The results have been tailored as the flagship publication, entitled 'Genome-wide association study on longitudinal and cross-sectional traits of child health and development in a Japanese population', which is currently in the process of paper submission and will appear on bioRxiv in early 2025 prior to full peer review.

2. Development of a novel in-vitro approach to validate and elucidate underlying molecular mechanisms of genetic associations discovered through GWAS

Yuji Miyatake, Kotoe Katayama[1], Seiya Imoto[1], Ka-

zuaki Yokoyama[2], Yasuhito Nannya[2], Atsushi Fukuda[3], Natsuhiko Kumasaka
[1]Division of Health Medical Intelligence, Human Genome Center, IMSUT, [2]Department of Hematology/Oncology, IMSUT, [3]School of Medicine, Tokai University

Although Genome-wide association studies (GWAS) have identified hundreds of thousands of genetic associations in common complex traits, more than 90% of genetic variants discovered by GWAS (referred to as GWAS variants) are located in non-coding regions. This poses a significant challenge in our efforts to identify putative causal variants and functional genes involved in a regulatory cascade of disease onset and progression. In addition, the target cell type(s) and cellular states in which these GWAS variants affect gene expression often remain unknown, limiting our ability to identify molecular mechanisms of GWAS susceptibility loci in follow-up studies.

Expression quantitative trait locus (eQTL) mapping is a powerful approach to gain insight into the role of non-coding variants in gene regulation. It allows us to discover genes that are regulated by these GWAS variants and helps elucidate downstream consequences. In addition, the recent advances in single-cell genomics allow us to identify target cell types and cellular states in which GWAS variants modulate gene expression through eQTLs. However, the study of eQTLs can often prove to be cost-ineffective and labor-intensive, especially when sample collection is challenging, such as in the case of in vivo brain samples collected from hundreds of patients undergoing neurosurgery, or in the case of differentiated neurons derived from hundreds of human pluripotent stem cell (hPSC) lines.

Recently, an alternative approach combining single-cell RNA-seq (scRNA-seq) with massively parallel CRISPR interference (CRISPRi) screening has been proposed to map eQTLs. This approach relies on CRISPR-mediated perturbations instead of natural genetic variation and is theoretically feasible from a single donor's sample. However, even though this approach significantly reduces the cell culture and experimental burden, it has not yet been applied to any brain cell type implicated in neuropsychiatric and neurodegenerative diseases.

Indeed, a combination of a flexible in vitro system and a robust in silico approach is lacking. Although the use of hPSC is a valuable tool to generate different types of mature cells, it is not trivial to maintain a sufficient gRNA repertoire through cell expansion and differentiation processes due to the selection pressure of specific CRISPR-mediated perturbations (*i.e.*, only cells with specific gRNA expand faster and take over other cells). From a data analysis perspective, the identification of dynamic genetic effects that manifest only during specific phases of cellular transition has historically been challenging, due to the absence of robust machine learning approaches until very recently.

We combine the unique expertise of machine learning/bioinformatics and stem cell biology/gene editing to develop a novel CRISPR perturbation system based on midbrain organoids established from hPSC lines, coupled with a state-of-the-art machine learning technique using Gaussian processes. We have already established a comprehensive computational approach, GASPACHO (GAuSsian Processes for Association mapping leveraging Cell HeterOgeneity), for mapping eQTLs along dynamic cellular states, which can be readily applicable to CRISPR-mediated eQTL mapping as if presence/absence of a gRNA in a cell were different genotypes at a natural genetic variant. We also have established several hPSC lines for CRISPR screening with inducible expression of dCas9-KRAB or dCas9-P300 by doxycycline (Dox), which ensures a more stable cell culture and allows to introduce perturbations at any desired time point of cell differentiation. In addition, we have extensive experience in two-dimensional neuronal differentiation and development of brain organoids.

As of December 2024, we have established both the Dox-inducible CRISPRi and CRISPRa iPS cell lines. Using the CRISPRi line, we developed a midbrain organoid suitable for studying Parkinson's disease (PD) GWAS loci. We have sequenced approximately 160K cells at the first two different time points of organoid development. This year we plan to sequence a further 160K cells at the two later time points as well as 4 different time points using the CRISPRa line to fully investigate the genetic role of PD-associated variants in the non-coding gene regulatory mechanism.

3. Development of novel statistical approaches to map genetic associations using Gaussian Processes

Yuji Miyatake and Natsuhiko Kumasaka

The Gaussian Process (GP) is a powerful approach for modelling non-linear phenomena in scientific fields such as genomics and genetics. This project focuses on the use of GPs for genetic association mapping. The aim is to identify genetic variants that affect gene regulation across continuous cellular states at the molecular level, and disease susceptibility over time and space at the population level. We are currently developing a robust and sensitive method for mapping dynamic genetic effects based on a quasi-Poisson generalized linear mixed model. This method can be applied to different outcomes, includ-

ing non-Gaussian outcomes, to estimate latent factors embedded in a data matrix and map genetic associations with appropriate statistical calibration.

Publications

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Center for Experimental Medicine and Systems Biology

Laboratory of Innate Immunity
自然免疫研究分野

Professor Kensuke Miyake, M.D., Ph.D.

教 授 医学博士 三 宅 健 介

Pathogen sensors, such as Toll-like receptor (TLR), play sentinel roles in detecting pathogenic ligands during infection and induce both innate and acquired immune responses. Meanwhile, excessive TLR responses are strongly associated with fatal diseases such as septic shock and autoimmune diseases. For this reason, immune system must strictly control TLR responses to avoid disruption of homeostasis. However, molecular mechanisms involved in TLR regulation are not fully elucidated. We have previously shown that TLRs are regulated by various TLR associating molecules including MD-2, PRAT4A and Unc93B1. Our goal is to uncover molecular mechanism that is indispensable for appropriate TLR responses using genetically engineered mice.

1. Targeting the nucleic acids-sensing TLRs for therapeutic intervention in autoimmune diseases

Yuji Motoi[1], Ryutaro Fukui[1], Takuma Shibata[1], Kensuke Miyake[1,2]
[1]Division of Innate Immunity, Department of Microbiology and Immunology, [2]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, TOKYO1208-8639, Japan.

TLR7 senses microbial-derived RNA in endolysosome, but can also erroneously respond to self-derived RNA. In fact, it has been reported that TLR7-dependent signaling promote autoimmune diseases. Thus, TLR7 can be therapeutic target. Although antibodies (Abs) are powerful tools for therapeutic intervention, TLR7 has been excluded from targets for Ab-mediated intervention because of its lack of cell surface expression. Despite this expectation, we found an anti-TLR7 Ab dose-dependently inhibits TLR7 responses in dendritic cells, macrophages and B cells. For this reason, we evaluated the therapeutic effect of anti-TLR7 Ab in *Unc93b1*$^{D34A/D34A}$ mice that cause thrombocytopenia, splenomegaly and chronic active hepatitis due to TLR7 hyper-responsiveness, and found that thrombocytopenia in *Unc93b1*$^{D34A/D34A}$ mice was significantly improved by the treatment with anti-TLR7 mAb. Furthermore, splenomegaly and hepatitis in mice treated with the anti-TLR7 mAb were also significantly remedy compared with control antibody.

On basis of these results, we established anti-human TLR7 Ab for blocking human TLR7 responses in vitro. Moreover, we generated human TLR7 transgenic (huTLR7 Tg) mice. We plan to use HuTLR7 Tg mice to evaluate the effects of anti-human TLR7 Ab *in vivo*.

In addition, TLR8 also recognize mouse TLR7 ligands in human and is involved in exacerbation of Rheumatoid Arthritis. Thus, in case of human disease, the anti-human TLR8 Ab that inhibits human TLR8 responses might work in clinical application. For this reason, we also constructed both anti-human TLR8 Abs and human TLR8 transgenic mice to verify our hypothesis.

2. RNaseT2-deficiency promotes TLR13-dependent replenishment of tissue-protective Kupffer cells

Ryota Sato[1], Kaiwen Liu[1], Takuma Shibata[1], Katsuaki Hoshino[2, 3], Kiyoshi Yamaguchi[4], Toru Miyazaki[5], Ryosuke Hiranuma[1], Ryutaro Fukui[1], Yuji Motoi[1], Yuri Fukuda-Ohta[6], Yun Zhang[1], Tatjana Reuter[7], Yuko Ishida[8], Toshikazu Kondo[8], Tomoki Chiba[9], Hiroshi Asahara[9, 10], Masato Taoka[11], Yoshio Yamauchi[11], Toshiaki Isobe[11], Tsuneyasu Kaisho[3, 6], Yoichi Furukawa[4], Eicke Latz[7, 12], Kohta Nakatani[13], Yoshihiro Izumi[13], Yunzhong Nie[14], Hideki Taniguchi[14], Kensuke Miyake[1]

[1] Division of Innate Immunity, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo; Minato-ku, Tokyo 108-8639, Japan. [2] Department of Immunology, Faculty of Medicine, Kagawa University, Miki, Kagawa 761-0793, Japan. [3] Laboratory for Inflammatory Regulation, RIKEN Center for Integrative Medical Science (IMS-RCAI), Yokohama, Kanagawa 230-0045, Japan. [4] Division of Clinical Genome Research, The Institute of Medical Science, The University of Tokyo; Minato-ku, Tokyo 108-8639, Japan. [5] The Institute for AIM Medicine, Tokyo 162-8666, Japan. [6] Department of Immunology, Institute of Advanced Medicine, Wakayama Medical University, Kimiidera, Wakayama 641-8509, Japan. [7] Institute of Innate Immunity, University Hospital Bonn, University of Bonn, 53127 Bonn, Germany. [8] Department of Forensic Medicine, Wakayama Medical University, Kimiidera, Wakayama 641-8509, Japan. [9] Department of Systems Biomedicine, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo 113-8501, Japan. [10] Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA. [11] Department of Chemistry, Graduate School of Science, Tokyo Metropolitan University; Tokyo 192-0397, Japan. [12] Deutsches Rheuma Forschungszentrum Berlin (DRFZ), 10117 Berlin, Germany. [13]Division of Metabolomics, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. [14] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo; Minato-ku, Tokyo 108-8639, Japan.

Lysosomal stress due to accumulation of nucleic acids (NAs) activates endosomal TLRs in macrophages. Here, we show that lysosomal RNA stress, caused by the lack of RNaseT2, induces macrophage accumulation in multiple organs such as the spleen and liver through TLR13 activation by microbiota-derived ribosomal RNAs. TLR13 triggered emergency myelopoiesis, increasing the numbers of myeloid progenitors in the bone marrow and spleen. Splenic macrophages continued to proliferate and mature into macrophages expressing the anti-inflammatory cytokine IL-10. In the liver, TLR13 activated monocytes/macrophages to proliferate and mature into monocyte-derived KCs (moKCs), in which, liver X receptor (LXR) was activated. In accumulated moKCs, tissue clearance genes such as MerTK, AXL, and apoptosis inhibitor of macrophage (AIM) were highly expressed, while TLR-dependent production of pro-inflammatory cytokines was impaired. Consequently, *Rnaset2*$^{-/-}$ mice were resistant against acute liver injuries elicited by acetaminophen (APAP) and LPS with D-Galactosamine. These findings suggest that TLR13 activated by lysosomal RNA stress promotes replenishment tissue-protective Kupffer cells.

3. CD20 and CD19 promote proliferation driven by the IgM-TLR9-L265P Myd88 complex

Yohei Kobayashi [1], Ryota Sato [1], Yuri Shimizu [1], Ryutaro Fukui [1], Takuma Shibata[1], Hiroki Tsukamoto [2], Takeshi Shibata [3], Kensuke Miyake [1]

[1]Division of Innate Immunity, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Japan. [2]Department of Pharmaceutical Sciences, School of Pharmacy at Fukuoka, International University of Health and Welfare, Okawa, Japan. [3]Department of Pathology, Nihon University School of Dentistry, Tokyo, Japan.

The cancer driver mutation L265P MyD88 is found in approximately 30 % of cases in the activated B cell-like subgroup of diffuse large B cell-like lymphoma (ABC DLBCL). L265P MyD88 forms a complex with TLR9 and IgM, referred to as the My-T-BCR complex, to drive proliferation. We here show that the B cell surface molecules CD19 and CD20 enhance proliferation mediated by the My-T-BCR complex. Using the IL-3-dependent Ba/F3 line transduced to express the IgM complex (IgM, CD79a, and CD79b) and TLR9, we observed proliferation in the presence of anti-IgM antibody and the TLR9 ligand CpG-B. TLR9 was constitutively associated with IgM and L252P MyD88. CD19 promoted proliferation with anti-IgM and CpG-B specifically in L252P MyD88-expressing Ba/F3 cells, while CD20 enhanced the proliferation in both wild-type- and L252P MyD88-expressing Ba/F3 cells. Additionally, CD20 uniquely enabled IgM-mediated proliferation in L252P MyD88-expressing Ba/F3 cells. Although CpG-B was not required for this proliferation, TLR9 expression remained indispensable. In the ABC DLBCL line TMD8, anti-IgM Ab mediated growth was impaired by the lack of CD20 and CD19 or of TLR9. Mechanistically, CD19 promoted IgM-dependent AKT phosphorylation, whereas CD20 increased expression of cell surface IgM, thereby enhancing the formation of the IgM-TLR9 complex. These findings suggest that CD19 and CD20 differentially contribute to the proliferation driven by the My-T-BCR complex.

Publications

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Center for Experimental Medicine and Systems Biology

Laboratory of Reproductive Systems Biology
生殖システム研究分野

Project Professor	Masahito Ikawa, Ph.D.	特任教授 博士(薬学)	伊 川 正 人
Associate Professor	Manabu Ozawa, Ph.D.	准教授 博士(農学)	小 沢 学

In the "post-genome project era," genetically modified animals play a key role in basic molecular biological investigations and act as models of human disease. Our laboratory studies the mechanisms underlying the mammalian reproductive system in gene-manipulated mice. We are the first group in the world to generate transgenic mice expressing GFP throughout the body (Green mice). We also established the ES cells that give green fluorescent spermatozoa to trace their movement and acrosome reaction during fertilization. Another tool invented in our laboratory is the placenta-specific gene manipulation system using lentiviral (LV) vectors. Using these techniques, we are trying to elucidate the mechanism underlying gametogenesis, fertilization, implantation, and placentation. Our recent interest is using the CRISPR/Cas9 system as a genome-editing tool. The combination of GWAS studies with genome editing will pave the way to understand and control human fertility problems.

1. FBXO24 deletion causes abnormal accumulation of membraneless electron dense granules in sperm flagella and male infertility

Yuki Kaneda[1,2], Haruhiko Miyaza[1], Zoulan Xu[1,2], Keisuke Shimada[1], Maki Kamoshita[1], Tatsuya Nakagawa[1,2], Chihiro Emori[1], Masahito Ikawa[1,2,3,4]: [1]Research Institute for Microbial Diseases, Oaka University. [2]Graduate School of Pharmaceutical Sciences,Osaka University. [3]Center for Infectious Disease Education and Research(CiDER), Osaka Univercity. [4]Center for Advanced Modalities and DDS(CAMaD), Osaka University.

Ribonucleoprotein (RNP) granules are membraneless electron-dense structures rich in RNAs and proteins, and involved in various cellular processes. Two RNP granules in male germ cells, intermitochondrial cement and the chromatoid body (CB), are associated with PIWI-interacting RNAs (piRNAs) and are required for transposon silencing and spermatogenesis. Other RNP granules in male germ cells, the reticulated body and CB remnants, are also essential for spermiogenesis. In this study, we disrupted FBXO24, a testis-enriched F-box protein, in mice and found numerous membraneless electron-dense granules accumulated in sperm flagella. *Fbxo24* knockout (KO) mice exhibited malformed flagellar structures, impaired sperm motility, and male infertility, likely due to the accumulation of abnormal granules. The amount and localization of known RNP granule-related proteins were not disrupted in *Fbxo24* KO mice, suggesting that the accumulated granules were distinct from known RNP granules. Further studies revealed that RNAs and two importins, IPO5 and KPNB1, abnormally accumulated in *Fbxo24* KO spermatozoa and that FBXO24 could ubiquitinate IPO5. In addition, IPO5 and KPNB1 were recruited to stress granules, RNP complexes, when cells were treated with oxidative stress or a proteasome inhibitor. These results suggest that FBXO24 is involved in the degradation of IPO5, disruption of which may lead to the accumulation of abnormal RNP granules in sperm flagella.

2. MYCBPAP is a central apparatus protein required for centrosome-nuclear envelope docking and sperm tail biogenesis in mice

Haoting Wang[1.2], Hiroko Kobayashi [1.2], Keisuke Shimada[1], Seiya Oura[1.2], Yuki Oyama[1.2], Hiroaki Kitakaze[1.5], Taichi Noda[1.6.7], Norikazu Yabuta[1], Haruhiko Miyata[1], Masahito Ikawa[1.2.3.5.]:[5]Graduate School of Medicine, Osaka University, [6]Institute of Resource Development and Analysis, Kumamoto University, [7]Priority Organization for Innovation and Excellence, Kumamoto University, Kumamoto0

The structure of the sperm flagellar axoneme is highly conserved across species and serves the essential function of generating motility to facilitate the meeting of spermatozoa with the egg. During spermiogenesis, the axoneme elongates from the centrosome, and subsequently the centrosome docks onto the nuclear envelope to continue tail biogenesis. Mycbpap is expressed predominantly in mouse and human testes and conserved in Chlamydomonas as FAP147. A previous cryo-electron microscopy analysis has revealed the localization of FAP147 to the central apparatus of the axoneme. Here, we generated Mycbpap-knockout mice and demonstrated the essential role of Mycbpap in male fertility. Deletion of Mycbpap led to disrupted centrosome-nuclear envelope docking and abnormal flagellar biogenesis. Furthermore, we generated transgenic mice with tagged MYCBPAP, which restored the fertility of Mycbpap-knockout males. Interactome analyses of MYCBPAP using Mycbpap transgenic mice unveiled binding partners of MYCBPAP including central apparatus proteins, such as CFAP65 and CFAP70, which constitute the C2a projection, and centrosome-associated proteins, such as CCP110. These findings provide insights into a MYCBPAP-dependent regulation of the centrosome-nuclear envelope docking and sperm tail biogenesis.

3. Golgi associated RAB2 interactor protein family contributes to murine male fertility to various extents by assuring correct morphogenesis of sperm heads

Haoting Wang[1.2], Rie Iida-Norita[1] , Daisuke Mashiko[1], Anh Hoang Pham[1.2] , Haruhiko Miyata[1], Masahito Ikawa[1.2.3.5]

Sperm heads contain not only the nucleus but also the acrosome which is a distinctive cap-like structure located anterior to the nucleus and is derived from the Golgi apparatus. The Golgi Associated RAB2 Interactors (GARINs; also known as FAM71) protein family shows predominant expression in the testis and all possess a RAB2-binding domain which confers binding affinity to RAB2, a small GTPase that is responsible for membrane transport and vesicle trafficking. Our previous study showed that GARIN1A and GARIN1B are important for acrosome biogenesis and that GARIN1B is indispensable for male fertility in mice. Here, we generated KO mice of other Garins, namely Garin2, Garin3, Garin4, Garin5a, and Garin5b (Garin2-5b). Using computer-assisted morphological analysis, we found that the loss of each Garin2-5b resulted in aberrant sperm head morphogenesis. While the fertilities of Garin2-/- and Garin4-/- males are normal, Garin5a-/- and Garin5b-/- males are subfertile, and Garin3-/- males are infertile. Further analysis revealed that Garin3-/- males exhibited abnormal acrosomal morphology, but not as severely as Garin1b-/- males; instead, the amounts of membrane proteins, particularly ADAM family proteins, decreased in Garin3 KO spermatozoa. Moreover, only Garin4 KO mice exhibit vacuoles in the sperm head. These results indicate that GARINs assure correct head morphogenesis and some members of the GARIN family function distinctively in male fertility.

4. Multiple ageing effects on testicular/epididymal germ cells lead to decreased male fertility in mice

Tsutomu Endo[1.8.11.12], Kiyonori Kobayashi[1.9], Takafumi Matsumura[1.2], Chihiro Emori[1], Manabu Ozawa, Shimpei Kawamoto[1], Daisuke Okuzaki[1], Keisuke Shimada[1], Haruhiko Miyata[1], Kentaro Shimada[1.2], Mayo Kodani[1.2], Yu Ishikawa-Yamauchi[10], Daisuke Motooka[1], Eiji Hara[1.5.9.11], Masahito Ikawa[1.2.5.11] :[8]Graduate School of Agricultural and Life Sciences, The University of Tokyo, [9]Graduate School of Frontier Biosciences, Osaka University, [10]Department of Regenerative Medicine, Yokohama City University Graduate School of Medicine, [11]Immunology Frontier Research Center, Osaka University, [12]Department of Experimental Animal Model for Human Disease, Center for Experimental Animals, Tokyo Medical and Dental University, Tokyo,

In mammals, females undergo reproductive cessation with age, whereas male fertility gradually declines but persists almost throughout life. However, the detailed effects of ageing on germ cells during and after spermatogenesis, in the testis and epididymis, respectively, remain unclear. Here we comprehensively examined the in vivo male fertility and the overall organization of the testis and epididymis with age, focusing on spermatogenesis, and sperm function and fertility, in mice. We first found that in vivo male fertility decreased with age, which is independent of mating behaviors and testosterone levels. Second, overall sperm production in aged testes was decreased; about 20% of seminiferous tubules showed abnormalities such as germ cell depletion, sperm release failure, and perturbed germ cell associations, and the remaining 80% of tubules contained lower number of germ cells because of decreased prolifera-

tion of spermatogonia. Further, the spermatozoa in aged epididymides exhibited decreased total cell numbers, abnormal morphology/structure, decreased motility, and DNA damage, resulting in low fertilizing and developmental rates. We conclude that these multiple ageing effects on germ cells lead to decreased in vivo male fertility. Our present findings are useful to better understand the basic mechanism behind the ageing effect on male fertility in mammals including humans.

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Center for Experimental Medicine and Systems Biology

Division of Genome Engineering
ゲノム編集研究分野

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　士
Associate Professor	Kazuto Yoshimi, Ph.D.	准教授	博士(医科学)	吉　見　一　人

Genome engineering technologies, such as clustered regularly interspaced short palindromic repeats (CRISPR)-associated (Cas) nucleases (CRISPR-Cas), have been widely used in life sciences and medicine. We have developed a novel genome editing tool, CRISPR-Cas3, to overcome the technical and patent limitations of the CRISPR-Cas9 system. We are investigating the molecular mechanisms underlying Cas3-mediated genome editing in human cells and optimizing this tool for translational research applications, including gene therapy and viral diagnostics. Additionally, we are developing efficient genome editing strategies using these tools in rodent models. These technologies enable straightforward and versatile gene editing in living organisms.

Genome editing using type I-E CRISPR-Cas3 in mice and rat zygotes

Kazuto Yoshimi [1], Akihiro Kuno [2], Yuko Yamauchi [3], Kosuke Hattori [3], Hiromi Taniguchi [3], Kouya Mikamo [3], Ryuya Iida [3], Saeko Ishida [3], Motohito Goto [4], Kohei Takeshita [5], Ryoji Ito [4], Riichi Takahashi [4], Satoru Takahashi [2], Tomoji Mashimo [1]

1, Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo; Division of Genome Engineering, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo

2, Department of Anatomy and Embryology, Faculty of Medicine, University of Tsukuba

3, Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo

4, Central Institute for Experimental Medicine and Life Science

5, Life Science Research Infrastructure Group, Advanced Photon Technology Division, RIKEN SPring-8 Center

The type I CRISPR system has recently emerged as a promising tool, especially for large-scale genomic modification, but its application to generate model animals by editing zygotes had not been established. In this study, we demonstrate genome editing in zygotes using the type I-E CRISPR-Cas3 system, which efficiently generates deletions of several thousand base pairs at targeted loci in mice with 40%-70% editing efficiency without off-target mutations. To overcome the difficulties associated with detecting the variable deletions, we used a newly long-read sequencing-based multiplex genotyping approach. Demonstrating remarkable versatility, our Cas3-based technique was successfully extended to rats as well as mice, even by zygote electroporation methods. Knockin for SNP exchange and genomic replacement with a donor plasmid were also achieved in mice. This pioneering work with the type I CRISPR zygote editing system offers increased flexibility and broader applications in genetic engineering across different species.

CRISPR Diagnostics for Quantification and Rapid Diagnosis of Myotonic Dystrophy Type 1 Repeat Expansion Disorders.

Koji Asano [1], Kazuto Yoshimi [1 2], Kohei Takeshita [3], Satomi Mitsuhashi [4], Yuta Kochi [5], Rika Hirano [1], Zong Tingyu [1], Saeko Ishida [1], Tomoji Mashimo [1 2]

1, Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo

2, Division of Genome Engineering, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo

3, Life Science Research Infrastructure Group, Advanced Photon Technology Division, RIKEN Spring-8 Center

4, Department of Neurology, St. Marianna University School of Medicine

5, Department of Genomic Function and Diversity, Medical Research Laboratory, Institute of Integrated Research, Institute of Science Tokyo

Repeat expansion disorders, exemplified by myotonic dystrophy type 1 (DM1), present challenges in diagnostic quantification because of the variability and complexity of repeat lengths. Traditional diagnostic methods, including PCR and Southern blotting, exhibit limitations in sensitivity and specificity, necessitating the development of innovative approaches for precise and rapid diagnosis. Here, we introduce a CRISPR-based diagnostic method, REPLICA (repeat-primed locating of inherited disease by Cas3), for the quantification and rapid diagnosis of DM1. This method, using in vitro-assembled CRISPR-Cas3, demonstrates superior sensitivity and specificity in quantifying CTG repeat expansion lengths, correlated with disease severity. We also validate the robustness and accuracy of CRISPR diagnostics in quantitatively diagnosing DM1 using patient genomes. Furthermore, we optimize a REPLICA-based assay for point-of-care-testing using lateral flow test strips, facilitating rapid screening and detection. In summary, REPLICA-based CRISPR diagnostics offer precise and rapid detection of repeat expansion disorders, promising personalized treatment strategies.

Sustainable and portable CRISPR-based diagnostics for high-sensitivity Mpox detection

Rika Hirano1, Kazuto Yoshimi1,2, Koji Asano1, Kohei Takeshita3, Ken J. Ishii4, Kei Sato5, Tomoji Mashimo1,2

1 Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo

2 Division of Genome Engineering, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo

3 Life Science Research Infrastructure Group, Advanced Photon Technology Division, RIKEN Spring-8 Center

4 Division of Vaccine Science, Department of Microbiology and Immunology, Institute of Medical Science, The University of Tokyo

5 Division of Systems Virology, Department of Microbiology and Immunology, Institute of Medical Science, The University of Tokyo

Mpox has emerged as a critical public health challenge, creating an urgent need for rapid, reliable, and field-deployable diagnostic tools for outbreak settings. Here, we present Kairo-CONAN, a novel CRISPR-Cas3-based point-of-care (POC) diagnostic platform for Mpox, engineered for sustainability and portability. This system leverages a disposable hand warmer (Kairo) as a stable heat source and incorporates freeze-dried reagents for ambient temperature stability, enabling device-free, sensitive detection through lateral flow assay strips. Utilizing CRISPR-Cas3's unique DNA-targeting and cleavage properties, we optimized probe DNA configurations for high specificity and designed clade-specific target crRNAs. Kairo-CONAN demonstrated rapid, high-sensitivity, and specific detection of Mpox virus (MPXV) DNA across multiple clades, including Clade Ia (Congo), Clade Ib (synthetic DNA), and Clade IIb (Tokyo). By addressing logistical and environmental challenges, Kairo-CONAN offers a sustainable, cost-effective, and field-adapted solution for infectious disease diagnostics, aligning with the 100-day mission framework to enhance global outbreak response efforts.

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Center for Experimental Medicine and Systems Biology

Division of Cell Regulation
細胞制御研究分野

Professor	Satoshi Yamazaki, Ph.D.		教　授	博士(生命科学)	山　﨑　　聡
Associate Professor	Yosuke Tanaka, Ph.D.		准教授	博士(医学)	田　中　洋　介
Assistant Professor	Hans Jiro Becker, M.D., Ph.D.		助　教	博士(医学)	ベッカーハンス次郎
Project Assistant Professor	Hyojung Jeon, Ph.D.		特任助教	博士(医学)	全　　孝　静

Our studies focus mainly on investigation of stem cell biology using the hematopoietic stem cell (HSC) as a research model. Recent identification of a variety of stem cell sources including embryonic and somatic (tissue-specific) stem cells has brought about substantial progress in the field of stem cell research.

1. Purging myeloma cell contaminants and simultaneous expansion of peripheral blood-mobilized stem cells

Kantaro Ishitsuka, Hidekazu Nishikii, Takaharu Kimura, Ayano Sugiyama-Finnis, Satoshi Yamazaki

Human hematopoietic stem cells (HSCs) are widely used as a cellular source for hematopoietic stem cell transplantation (HSCT) in the clinical treatment of hematological malignancies. After transplantation therapy, delays in hematopoietic recovery due to insufficient donor-derived HSCs can lead to increased risks of life-threatening infections and bleeding. Our previous studies developed an efficient ex vivo expansion culture medium (3a medium) for umbilical cord blood-derived HSCs (CBSCs), offering a potential solution to this problem. Nevertheless, the broader applicability of our culture method to alternative cell sources and, of greater significance, its efficacy in eliminating potentially disease-associated contaminated tumor cells, especially in autologous transplantation, raise critical clinical questions. In this study, we modified the 3a medium by incorporating UM729 to replace UM171, adding FMS-like tyrosine kinase 3 (Flt3) ligand, and adjusting the concentrations of butyzamide, 740Y-P, polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (PCL-PVAc-PEG, Soluplus) to create the modified-3a medium. This sophistication allowed the efficient expansion of not only CBSCs but also peripheral blood-mobilized HSCs (PBSCs). Additionally, we successfully removed contaminated myeloma cells by adding bortezomib and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) at appropriate concentrations, although we maintained HSCs through the addition of lenalidomide. Our research findings present the potential for widespread clinical application of the modified-3a medium and suggest a safe ex vivo culture technique for expanding human HSCs within peripheral blood-derived donor grafts used for autologous HSCT.

2. Activated mesenchymal stem/stromal cells promote myeloid cell differentiation via CCL2/CCR2 signaling

Satoshi Yamazaki, Yo Mabuchi, Takaharu Kimura, Eriko Grace Suto, Daisuke Hisamatsu, Yuna Naraoka, Ayako Kondo, Yuzuki Azuma, Riko Kikuchi, Hidekazu Nishikii, Soji Morishita, Marito Araki, Norio Komatsu, Chihiro Akazawa

Myeloid cells, which originate from hematopoietic

stem/progenitor cells (HSPCs), play a crucial role in mitigating infections. This study aimed to explore the impact of mesenchymal stem/stromal cells (MSCs) on the differentiation of HSPCs and progenitors through the C-C motif chemokine CCL2/CCR2 signaling pathway. Murine MSCs, identified as PDGFRα^+Sca-1$^+$ cells (PαS cells), were found to secrete CCL2, particularly in response to lipopolysaccharide stimulation. MSC-secreted CCL2 promoted the differentiation of granulocyte/macrophage progenitors into the myeloid lineage. MSC-derived CCL2 plays an important role in the early phase of myeloid cell differentiation in vivo. Single-cell RNA sequencing analysis confirmed that CCL2-mediated cell fate determination was also observed in human bone marrow cells. These findings provide valuable insights for investigating the in vivo effects of MSC transplantation.

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Center for Experimental Medicine and Systems Biology

Core Laboratory for Developing Advanced Animal Models
先進モデル動物作製コア

Professor	Satoshi Yamazaki, Ph.D.	教　授	博士(生命科学)	山　﨑	聡
Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下	知　士
Visiting Professor	Kimi Araki, Ph.D.	客員教授	博士(理学)	荒　木	喜　美
Associate Professor	Manabu Ozawa, Ph.D.	准教授	博士(農学)	小　沢	学
Project Assistant Professor	Jumpei Taguchi, Ph.D.	特任助教	博士(医科学)	田　口	純　平

The Core Laboratory for Developing Advanced Animal Models supports basic sciences in the life science field by producing and providing gene-manipulated mice or rats such as human disease models or gene KO/KI models. Using cutting-edge genome editing techniques, we make various types of gene-manipulated animals, including indel mutation, large fragment deletion, SNPs, conditional Cre/loxP, drug inducible gene expression/silencing, reporter gene KI, or gene conversion for making humanized mice or rat models either by direct gene editing in zygote or highly efficient ES cell-mediated gene targeting followed by chimera animal productions.
https://www.ims.u-tokyo.ac.jp/cemsb/public_html/index.html

Laboratories that consist of the Core

'Core Laboratory for Developing Advanced Animal Models' was launched in 2020 to provide gene-manipulated mouse or rat models to domestic or international academic institutions. One division, the Division of Genome Engineering, and two laboratories, the Laboratory of Reproductive Systems Biology and the Laboratory of Genetically Engineered Mouse Research, all of which belong to the Center for Experimental Medicine and Systems Biology, comprise the Core.

Cutting-edge genome editing techniques

For making indel mutants, large deletion, or short DNA fragment KI such as SNPs or peptide tags, we offer direct genome editing using mouse or rat zygotes through NEPA electroporation systems (NEPA Gene). In mice, embryos from C57BL/6J strain are routinely served for genome editing, but other strains, such as C57BL/6N or BDF1, are also applicable if necessary. In the rat, F344/Jcl strain is served for zygote genome editing. For large-size gene manipulations in mice, such as Cre/loxP conditional allele, fluorescein reporters KI, gene conversion from mice to human, or drug-inducible Tet-on/off system, we offer CRISPR/Cas9-assisted plasmid KI using ES cells through Neon Electroporation system (ThermoFisher) followed by blastocyst injection for developing chimeric mice. ES cells from C57BL/6J, C57BL/6N, 129, B6129F1, or BALB/c strains are available for chimera productions. The direct zygote genome editing technique, termed Combi-CRISPR, is applicable for producing large-size gene-manipulated rats, e.g., reporter KI or humanized rat models.

Supporting gene-manipulated mouse or rat model production through the core lab and AdAMS platform

We provide cutting-edge animal production techniques through our core lab and Advanced Animal Model Support, AdAMS. Our Core is a member of AdAMS, which belongs to the Committee on Promoting Collaboration in Life Science, MEXT, and is an ac-

ademic platform for producing gene-manipulated animals. Therefore, researchers earning KAKENHI, Grant-in-Aid for Scientific Research, can apply to this platform.

Number of mice or rat strains we developed in 2022

In 2024, our Core provided 2 or 11 strains of gene-manipulated mice through the core lab or Ad-AMS, respectively. In the rat case, 7 strains of gene-manipulated rats have also been provided through AdAMS.

Advanced Clinical Research Center

Division of Infectious Diseases
感染症分野

Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳　　宏
Senior Assistant Professor	Michiko Koga, M.D., D.M.Sc.	講　師	博士(医学)	古　賀　道　子
Assistant Professor	Makoto Saito, M.D., D.Phil.	助　教	博士(医学)	齋　藤　　真
Assistant Professor	Aya Ishizaka, Ph.D.	助　教	博士(理学)	石　坂　　彩
Assistant Professor	Yoshiaki Kanno, M.D., D.M.Sc.	助　教	博士(医学)	菅　野　芳　明

Our overall goal is medical sciences on infectious diseases in two directions, from clinic to bench and from bench to clinic. Our main subjects have been immunopathogenesis of HIV-1 infection in addition to other viruses, especially hepatitis viruses. Since the emergence of SARS-CoV-2, we have proceeded the basic and clinical research using clinical samples obtained from SARS-CoV-2-infected patients and vaccinated individuals.

1. Clinic and basic research for the control of COVID-19 including vaccination.

Michiko Koga, Makoto Saito, Aya Ishizaka, Yoshiaki Kanno, Eisuke Adachi[1], Taketoshi Mizutani[2,3], Ai Tachikawa-Kawana[4], Ken Ishii[5], Yoshihiro Kawaoka[6], Fumitaka Nagamura[7], Hiroshi Yotsuyanagi
[1] Department of Infectious Diseases and Applied Immunology, IMSUT hospital, IMSUT
[2] Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo
[3] Center for Emergency Preparedness and Response, National Institute of Infectious Diseases
[4] AIDS Research Center, National Institute of Infectious Diseases
[5] Division of Vaccine Science, IMSUT
[6] Division of Virology, IMSUT
[7] Center for Translational Research, IMSUT Hospital, IMSUT

Several laboratories at IMSUT and external institutes have continued COVID-19-related research during 2022, and we have been working in collaboration with some of these laboratories. Our main mission is to obtain and share clinical samples from COVID-19 patients and vaccinated indivisuals, but we are also conducting basic research by ourselves such as microbiomes and intestinal DNA phage in those patients. Additionally, we are attempting to perform high-resolution transcriptomic analysis of blood immune cells from disease progression to recovery in COVID-19 in order to enhance a better understanding of the protective and pathogenic immune responses of the disease. Specifically, we are performing gut microbiome and DNA phage analysis as well as single-cell RNA sequencing (scRNA-seq) to obtain a bias-free and comprehensive imaging of immune responses in peripheral blood mononuclear cells (PBMCs) from patients with COVID-19.

We have also conducted the investigator-initiated clinical trial and other clinical trial on the COVID-19 vaccine and analyzed changes in the nasal microbiome after vaccination (jRCT2031210503, jRCT10312 40457).

2. Immune escape mutation in hepatitis B virus (HBV) isolated from HBsAg+ donated blood.

Ayako Sedohara, Kazuaki Takahashi, Kazuhiko

Ikeuchi[1], Yoshiaki Kanno Eisuke Adachi[1], Michiko Koga, Takeya Tsutsumi[1], Hiroshi Yotsuyanagi

HB vaccines target small protein, and it is known that if mutations occur in the HBV DNA that encodes small protein, the HBV can escape from the immune system (vaccine escape mutation).

Universal vaccination with HB vaccines has been introduced in many countries around the world, but the relationship between the appearance of vaccine escape mutations and HB vaccines is unknown. In Japan, universal vaccination with the HB vaccine began in 2016, but most of the people aged 16 years or older who can donate blood do not have antibodies against HBV. To investigate the relationship between vaccine escape mutations and the HB vaccine, it is important to monitor whether the incidence of vaccine escape mutations will change in the future. The purpose of this study was to investigate the incidence of vaccine escape mutations in HBs antigen-positive (HBsAg+) blood donation samples and to provide a baseline for future research.

HBV DNA was isolated from 58 of 169 HBsAg+ blood samples donated to the Japanese Red Cross Society between 2021 and 2022. PCR was performed to amplify small protein coding regions, and the resulting purified PCR products were sequenced and analyzed for amino acid sequence. According to the mutation analysis, vaccine escape mutations were detected in 6 of the 58 samples (10.3%). We plan to continue our research in the future.

3. Analysis of the HIV-associated gut microbiome

Aya Ishizaka, Michiko Koga, Taketoshi Mizutani[3,4], Eisuke Adachi[1], Tetsuro Matano[8,9], Hiroshi Yotsuyanagi
[8] Department of AIDS Vaccine Development, IMSUT Hospital, IMSUT
[9] National Institute of Infectious Diseases

Loss of gut mucosal barrier function persists during HIV infection and allows translocation of gut-derived bacteria as well as microbial products into circulation. We reported the relevance between gut dysbiosis and chronic inflammation in people living with HIV infection (PLWH). We are currently analyzing gut microbiota alterations longitudinally to elucidate the causal relationship between HIV-specific gut microbiota dysbiosis and the incidence of age-related diseases in PLWH.

4. Evaluation of the durability of HA vaccine for HIV-MSM and analysis on NAFLD/MASLD in HIV infected patients

Michiko Koga, Takeya Tsutsumi, Aya Ishizaka, Taketoshi Mizutani[2], Megumi Kubota, Tomoe Senkoji, Kazuaki Takahashi, Amato Otani[1], Kazuhiko Ikeuchi[1], Tadashi Kikuchi[1], Eisuke Adachi[1], Hiroshi Yotsuyanagi

Hepatitis A (HA) is vaccine-preventable, with men who have sex with men (MSM) as key affected populations in regions with good sanitation. During the 2018-2019 HA outbreak in Japan, MSM living with HIV (MSM-LWHIV) were vaccinated with Aimmugen. While their antibody seroconversion rates were lower than healthy individuals, the durability of Aimmugen in MSM-LWHIV remains unclear. We evaluated antibody attenuation after a one-series vaccination and related factors. Anti-HA immunoglobulin G (IgG) titers and clinical data of MSM-LWHIV who seroconverted after Aimmugen vaccination and were tested ≥2 years post-vaccination were retrospectively analyzed. Among 51 MSM-LWHIV, seropositivity after three doses was 100% (median titer: 10.1 s/co). After 45 months, seropositivity declined to 90% (median titer: 4.4 s/co). Lower baseline B cell counts ($p = 0.049$) and lower post-vaccination IgG levels ($p = 0.002$, $p = 0.003$) were linked to seronegativity. Anti-HA-IgG titers in vaccinated MSM-LWHIV attenuate over time, suggesting the need for booster doses.

We have started to evaluate NAFLD of HIV infected patients. Of the 102 HIV-infected patients, the prevalence of NAFLD was estimated to be 53.9% from the elastography CAP value, and 7.8% were suspected of progressing fibrosis and immediate improvement in metabolic risk factors as desired. We are also conducting research from the perspective of the microbiome.

5. Analysis of the genetic sequence of hepatitis viruses.

Ayako Sedohara, Kazuaki Takahashi, Kazuhiko Ikeuchi[1], Yoshiaki Kanno Eisuke Adachi[1], Michiko Koga, Takeya Tsutsumi[1], Hiroshi Yotsuyanagi

We sometimes see patients with acute hepatitis at IMSUT Hospital. Most of the causes are viral hepatitis induced by hepatitis A, B, C, or E virus. In fact, in 2018, there was an outbreak of hepatitis A in HIV-infected patients, and until 2021, we sometimes observed some patients with hepatitis A. Concerning hepatitis B and C, owing to the similar route of infection, every year, we observed some patients who were also infected with HIV. Using sera and/or stools obtained from these patients, we cloned a total or part of the viral genome and determined the genetic sequence of the viruses to identify the transmission route of the viruses and drug-resistant mutations or vaccine escape mutations. Concerning hepatitis B, we have also been examining HBV-positive samples derived from blood donors who were accidentally found to be HBsAg positive. For hepatitis E, we are collaborating with outside researchers in Tokyo and Hokkaido, where hepatitis E virus infection is sometimes ob-

served. By cloning the virus from samples derived from not only patients but also suspectable foods or wild animals, we investigated the transmission routes of the virus.

6. Exploratory research of the malignancy with HIV infected hemophilia patients

Michiko Koga, Akari Fukuda, Takahiro Tanaka, Aya Ishizaka, Takashi Hosaka, Hiroshi Yotsuyanagi,

It is speculated that hemophiliacs infected with HIV due to chemical damage are more likely to get malignancy due to aging and immune dysfunction. Since April 2021, we have started this research with the following four objectives. 1. Construction and operation of a system design for a health examination. 2. Medical support at the time of diagnosis of malignancy and after diagnosis. 3. Mental care associated with malignancy. 4. Public relations regarding support and diseases.

Non-acquired immunodeficiency syndrome-defining malignancies (NADMs) are the crucial cause of mortality in people living with haemophilia and human immunodeficiency virus (PLWHH). We aimed to analyse the types and characters of NADMs in PLWHH after approval of direct-acting antivirals (DAA), considering that most PLWHH are infected with hepatitis C virus (HCV). We conducted a nationwide questionnaire mail survey across 395 HIV core facilities in Japan between May 2022 and February 2023. Eight-year data from 64 respondent hospitals (n = 328 PLWHH; 2015-2022) were collected; 35 NADM cases were identified and analysed. Standardised cancer incidence ratios (SCIRs) were calculated. The median age of PLWHH with NADMs was 51 years (interquartile range: 47-62 years); the SCIR was 2.08 (95% confidence interval [CI]: 1.48-2.90) for all malignancies (including carcinoma in situ). Liver cancer accounted for most NADMs (43% [15/35]). The SCIRs of liver cancer (23.09 [95% CI: 13.92- 38.30]) and papillary thyroid cancer (9.38 [2.35-37.50]) significantly increased after adjusting for general Japanese male sex and age. Among PLWHH with liver cancers, 73% (11/15) achieved HCV-sustained virological response. Notably, for patients aged ≤ 50 years, 47% (7/15) were affected by liver cancers, and 27% (4/15) succumbed to NADMs. This study presents the largest survey of NADMs in PLWHH after DAA approval. Our findings emphasised the elevated risk of malignancies in PLWHH, underscoring the need for early cancer screening and preventive measures, particularly against liver cancers, even in younger PLWHH.

7. Analysis of antibody acquisition by hepatitis B vaccine in HIV-infected patients

Yoshiaki Kanno, Ayako Sedohara, Michiko Koga, Eisuke Adachi, Hiroshi Yotsuyanagi

Although hepatitis B (HB) vaccination is recommended for HBV-uninfected people with HIV (PWH), HB surface antibody (HBsAb) acquisition rate by HB vaccination has been reported to be lower in PWH than in people without HIV. However, the acquisition rate in PWH who suppressed HIV under current antiretroviral therapy (ART) has not well been evaluated. We investigated the acquisition and maintenance of HBsAb by HB vaccine (Heptavax -II) among PWH attending IMSUT hospital. Among 22 patients who had HBsAb assay after the third dose, 21 were male and the median age was 41. All were on ART, had HIV-1 viral load of 20 copies/mL or less, and had median CD4+ T cell count of 599/μL. HBsAb acquisition rate among PWH in the present study was 81.8%, which was not significantly different from that in the population without HIV. And the titers dropped to about one-tenth in the first year. HIV infection alone may not have a significant effect on antibody production in patients suppressing HIV with unreduced number of CD4+ T cells. With increasing number of ART regimens without anti-HBV activity, this result may provide further rationale for recommending HB vaccination of HBV-uninfected PWH.

8. Identification of HAND biomarkers via neuroexosomes isolated from people living with HIV-1.

Kotaro Arizono, Ayako Sedohara, Takahiro Tanaka, Michiko Koga, Yoshiaki Kanno, Eisuke Adachi[1], Hiroshi Yotsuyanagi

ART suppresses HIV-1 proliferation and improves the prognosis of people living with HIV. However, chronic inflammation caused by persistent HIV-1 infection can lead to various complications. HIV-associated neurocognitive disorder (HAND) is one such complication, and it can significantly impair the quality of life of people living with HIV. Intervention is necessary before patients find it difficult to lead a social life, but HAND is different from general dementia, so diagnosis is not easy. The only way to diagnose HAND is to carry out a battery tests by a clinical psychologist, but this is not practical for all patients because the tests are time-consuming and place a heavy burden on the people living with HIV. If HAND can be diagnosed by blood tests, intervention before serious neurological symptoms appear is possible. Therefore, this study aimed to identify biomarkers for HAND.

Exosomes contain miRNAs, which are organ specific. In neuroexosomes secreted from the central nervous system of Alzheimer's disease patients, several miRNAs related to dementia have been identified, including a decrease in hsa-miR-132 and hsa-miR-212. In this study, neuroexosomes were isolated from the plasma of people living with HAND, and the miRNA transcriptome was analized. As a result,

we identified several miRNAs whose expression is increased in HAND. The target genes of these miRNAs were found to be related to vesicle transport. If vesicle transport is impaired in nerve cells, neurotransmission may be impaired. In addition, since these miRNAs do not match those identified in dementia, HAND is predicted to develop through a different mechanism than dementia.

9. HIV-1 infection affects follicular helper T (Tfh) cell function.

Ayako Sedohara, Yoshiaki Kanno, Michiko Koga, Eisuke Adachi[1], Hiroshi Yotsuyanagi

The seroconversion rate after HBV vaccination in people living with HIV-1 is reportedly lower than that in non-HIV-1 controls. HIV-1 infects follicular helper T (Tfh) cells, which are essential for humoral immunity. However, how this affects Tfh cell function was unknown. In this study, the number and function of circulating Tfh (cTfh) cells were examined in people living with HIV-1 who became S antibody positive after receiving three doses of HBV vaccine (HBsAb+ HIV group) and in people living with HIV who became S antibody negative (HBsAb- HIV group).

The results showed that the frequency of cTfh cells increased after immune stimulation in the non-HIV control group and the HBsAb+ HIV group, but not in the HBsAb- HIV group. Furthermore, the production of IL-21, which is essential for plasma cell differentiation, was lower in the HBsAb- HIV group than in the non-HIV control or the HBsAb+ HIV group. These results suggest that the poor response to HBV vaccination in people living with HIV-1 could be due to the reduction in Tfh cell function caused by HIV-1 infection.

10. Characteristics of Transmitted Drug-Resistant HIV-1 in Recently Infected Treatment-Naive Patients in Japan.

Michiko Koga, Megumi Kubota, Tomoe Senkoji, Yoshiaki Kanno, Eisuke Adachi[1], Tadashi Kikuchi[1], Hiroshi Yotsuyanagi

Progress in antiretroviral treatment has led to fewer virological failure cases, but about 10% of treatment-naive HIV/AIDS cases are reported to harbor drug-resistant strains (RS), suggesting transmission of drug-resistant HIV. We have determined the trend in prevalence of transmitted drug-resistant (TDR) HIV in Japan from 2003.

Drug-resistance test had been performed on national-wide HIV-1-infected cases newly diagnosed. The overall prevalence of TDR was about 9.4% in 2023.

11. Identification of drugs which reactivate latent HIV-1 reservoir

Ayako Sedohara, Michiko Koga, Makoto Saito, Kazuhiko Ikeuchi, Eisuke Adachi, Tomohiko Koibuchi, Hiroshi Yotsuyanagi

Antiviral therapy (ART) suppresses HIV-1 replication and restores the immune system that has been impaired by HIV-1 infection. However, HIV-1 integrates into the genome of host CD4 T cells and becomes a provirus. Even if ART suppresses HIV-1 production from the provirus, latent infected cells that harbor the provirus continue to persist. Therefore, the eradication of HIV-1 infected cells from people living with HIV is extremely important for an HIV-1 cure. One strategy for eradicating latently HIV-1-infected cells is shock and kill. This approach aims to eliminate the cells through host immunity by reactivating the latent HIV-1-infected cells via latency-reversing factors (LRAs) and expressing HIV-1 antigens derived from proviruses on the surface of CD4 T cells. Enhancer of zest homolog 2 (EZH2) is a component of polycomb protein complex 2 (PRC2) and methylates lysine 27 of the histone H3 protein (H3K27). Trimethylation of H3K27 (H3K27me3) is a marker of gene silencing regions and is mainly observed around the 5'LTR, which is a transcriptional regulatory region of the provirus. EZH2 is involved in the maintenance of latent HIV-1 infection, and selective inhibitors of EZH2, GSK127 and E7438, are known as LRAs. EHZ1 is known to work in concert with EZH2, and even if EZH2 is suppressed, its function is compensated for by EZH1. Therefore, it is necessary to simultaneously inhibit EZH1 and EHZ2. In this study, we examined the effects of the novel EZH1/2 dual inhibitor valemetostat/DS-3201/(R)-OR-S2 on latent HIV-1-infected cells. Compared with the selective EZH2 inhibitors GSK126 and E7438, valemetostat efficiently reactivated HIV-1-infected cells. To clarify the mechanism by which valemetostat affects on HIV-1-infected cells, we analyzed the transcriptomes of CD4 T cells isolated from people living with HIV and treated with valemetostat. The results revealed that the gene expression patterns of valemetostat was very similar to that of E7438, but different from that of GSK126.

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Advanced Clinical Research Center

Division of Clinical Genome Research
臨床ゲノム腫瘍学分野

Professor	Yoichi Furukawa, M.D., Ph.D.	教　授	博士(医学)　古　川　洋　一
Associate Professor	Kiyoshi Yamaguchi, Ph.D.	准教授	博士(薬学)　山　口　貴世志
Assistant Professor	Kiyoko Takane, M.D., Ph.D.	助　教	博士(医学)　高　根　希世子
Assistant Professor	Saya Nakagawa, Ph.D.	助　教	博士(医科学)　中　川　沙　弥

Research Projects
The aim of our research is the application of findings in basic cancer research to clinics. Currently, we are working on the following six projects: 1) identification of novel molecular targets for the treatment of colorectal cancer, 2) understanding the role of Wnt/β-catenin signaling pathway in human carcinogenesis, 3) discovery of Wnt inhibitors through a screening of large-scale chemical libraries, 4) establishment of intrahepatic cholangiocarcinoma mouse model by orthotopic transplantation of syngeneic tumor cells, 5) elucidation of the genetic features of rare cancers and the mechanisms of their development, and 6) clinical sequencing for the implementation of genomic medicine.

1. Identification of novel molecular targets for the treatment of colorectal cancer

Kiyoshi Yamaguchi, Saya Nakagawa, Manabu Ozawa[1], Yoichi Furukawa: [1]Laboratory of Reproductive Systems Biology, Center for Experimental Medicine and Systems Biology, IMSUT

Epigenetic modifications such as DNA methylation and histone modification change in gene expression with global dynamics of chromatin structure. Accumulated evidence has demonstrated that aberrant epigenetic modifications are involved in carcinogenesis. Bromodomains have been known as a protein-interaction module that recognizes acetylated lysine residues. Through this interaction, protein containing a bromodomain(s) directs the assembly of nuclear factor complexes to their target sites on chromatin, resulting in the transcriptional activation. Recently, we found that bromodomain containing 8 (BRD8) was frequently accumulated in colorectal cancer. Transcriptome analysis coupled with genome-wide mapping of BRD8-binding sites disclosed that BRD8 regulates the expression of multiple subunits of the pre-replicative complex in concert with the activator protein-1. Depletion of BRD8 induced cell-cycle arrest at the G1 phase and suppressed cell proliferation. We also showed that the bromodomain of BRD8 is indispensable for not only the interaction with histone H4 or transcriptional regulation but also its own protein stability. These findings highlight the importance of bromodomain as a therapeutic target.

Since BRD8 is abundantly expressed in the testis, we additionally generated testis-specific Brd8-knockout mice to investigate its roles in male fertility. These knockout mice exhibited reduced testis size and abnormal testis morphology. Intensive analyses of these mice should provide novel insights into the biological functions of BRD8.

2. Understanding the role of Wnt/β-catenin signaling pathway in human carcinogenesis

Kiyoshi Yamaguchi, Saya Nakagawa, Yoichi Furukawa

Aberrant Wnt/β-catenin signaling has been found in the various types of cancer, including colon and liver cancer. This activation leads to the accumulation of β-catenin in the nucleus, where it functions as a transcriptional co-activator of the TCF/LEF family. Therefore, comprehensive understanding of genes regulated by the heterodimeric β-catenin/TCF transcriptional complex will lead to the better understanding of the role of this pathway in human carcinogenesis.

Previously, transcriptome analysis of HepG2 hepatoblastoma cells transfected with β-catenin siRNAs or a dominant negative form of TCF7L2 revealed that the expression of histidine ammonia lyase (*HAL)*, which is involved in histidine catabolism, was negatively regulated by Wnt signaling in liver cancer cells. The analysis of the *HAL* regulatory region revealed that two liver-enriched transcription factors, CEBPA and FOXA1, directly activated *HAL* transcription. Transcriptome and ChIP-seq analyses disclosed that CEBPA and FOXA1 regulated genes involved in cellular metabolism including *ARG1*, which was down-regulated by the Wnt signaling. We also observed that the up-regulation of HAL and ARG1 by the suppression of Wnt signaling resulted in decreased intracellular concentrations of histidine and arginine, respectively. These findings will provide new insights into the understanding between liver cancer metabolism and the Wnt signaling pathway.

3. Discovery of Wnt inhibitors through a screening of large-scale chemical libraries

Kiyoshi Yamaguchi, Yoichi Furukawa, Yoshitaka Ohishi, Satoru Nagatoishi[1], Kouhei Tsumoto[1,2]: [1]Department of Bioengineering, School of Engineering, The University of Tokyo, [2]Medical Proteomics Laboratory, IMSUT

A variety of cell-based assays have contributed to the discovery of small molecules that modulate Wnt signaling. Previously, we developed a sensitive and specific cell-based reporter assay for the detection of the Wnt/β-catenin signaling activity. Using this assay, we established a high-throughput screening system, and performed a screening of small molecule and natural product libraries. As a result, several compounds that inhibit Wnt/β-catenin signaling activity were identified, and their target(s) are currently under investigation. To elucidate the detailed mode of action of these hit compounds, we have adopted an approach based on the estimation of gene network from gene expression data.

4. Establishment of intrahepatic cholangiocarcinoma mouse model by orthotopic transplantation of syngeneic tumor cells

Kiyoko Takane, Yoichi Furukawa

Genetically engineered mice are useful tools for studying human diseases including cancer. In this project, we previously generated a novel mouse model of intrahepatic cholangiocarcinoma (ICC) using liver-specific expression of oncogenic *Kras* and homozygous *Pten* deletion (AKPP: *Alb-Cre*$^{+}$; *LSL-Kras*$^{G12D/+}$; *Pten*$^{flox/flox}$). Subsequently, a cell line was established from the ICC of AKPP mice and named AKPP cells. First, we confirmed that conventional ICC markers such as cytokeratin-19 and pan-cytokeratin were abundantly expressed in AKPP cells. In contrast, the cells expressed low levels of hepatocyte nuclear factor 4 alpha (HNF4α), a marker of hepatocellular carcinoma. To investigate the downstream effects expected from the loss of Pten and activation of Kras, the phosphorylation levels of Akt (Ser473) and Erk1/2 were analyzed by immunoblotting. As a result, increased phosphorylation of these effectors was observed in AKPP cells, compared to mouse fibroblast L-cells. To evaluate the biological properties of AKPP cells, we transplanted the cells into syngeneic immunocompetent mice, and confirmed that the cells retained the characteristics of ICC.

5. Elucidation of the genetic features of rare cancers and the mechanisms of their development

Kiyoko Takane, Saya Nakagawa, Kiyoshi Yamaguchi, Yoichi Furukawa, Seiya Imoto[1], Satoru Miyano[2], Hideaki Yano[3], Atsushi Kaneda[4]: [1]Division of Health Medical Intelligence, Human Genome Center, IMSUT, [2]M&D Data Science Center, Institute of Integrated Research, Institude of Science Tokyo, [3]Department of Surgery, National Center for Global Health and Medicine, [4]Department of Molecular Oncology, Graduate School of Medicine, Chiba University

Pseudomyxoma peritonei (PMP) is a rare disease with an incidence of 1 – 2 cases per million and characterized by the presence of mucin-producing tumors in the abdominal cavity. Although frequent mutations in the *KRAS* and *GNAS* genes have been found in PMP, gene expression profiles of the tumors remain to be fully understood. To elucidate the molecular features of PMP cells, we performed RNA-seq analysis of ten PMPs and their matched non-tumorous colonic epithelium in combination with laser-microdissection. As a result, we identified a total of 32 differently expressed genes between the tumors and non-tumorous colonic epithelium. A cell-of-origin subtype analysis with the nearest template prediction algorithm corroborated that PMP cells belonged to the goblet cell subtype, suggesting that the tumor cells appear to differentiate into goblet cells or originate from goblet cells. Interestingly, functional enrichment analysis uncovered that the tumors were significantly associated with “epithelial mesenchymal transition”, “angiogenesis”, and “inflammatory

response". Comparison of gene expression profiles between disseminated peritoneal adenomucinosis (DPAM) and peritoneal mucinous adenocarcinomas (PMCA) identified a total of 687 differently expressed genes. Additional gene set enrichment analysis revealed that ontology terms "G2M checkpoint" and "E2F targets" were significantly enriched in PMCA, supporting the view that PMCA has more aggressive properties than DPAM. These data may be useful to further understand the molecular characteristics of PMP.

To understand the epigenetic characteristics of PMPs, we further performed genome-wide DNA methylome analysis of 15 appendiceal PMP samples using the Infinium 850K BeadChip. As a result, the 15 PMPs were classified into at least two epigenotypes, unique methylation epigenotype and normal-like methylation epigenotype. We are currently investigating the molecular function of genes whose expression is decreased by the DNA methylation in PMP. In addition, we have successfully established PMP-organoids. Our efforts will contribute to the better understanding of molecular mechanisms underlying PMP, and help in the diagnosis, treatment, and prevention of this life-threatening disease.

6. Clinical sequencing for the implementation of genomic medicine

Kiyoko Takane, Kiyoshi Yamaguchi, Yoichi Furukawa, Eigo Shimizu[1], Rika Kasajima[1], Kotoe Katayama[1], Seiya Imoto[1], Tetsuo Shibuya[2], Kazuaki Yokoyama[3], Yasuhito Nanya[3], Koichiro Yuji[4], Rui Yamaguchi[5], Satoru Miyano[6]: [1]Division of Health Medical Intelligence, [2]Division of Medical Data Informatics, Human Genome Center, [3]Department of Hematology/Oncology, [4]Project Division of International Advanced Medical Research, Advanced Clinical Research Center, IMSUT, [5]Division of Cancer Systems Biology, Aichi Cancer Center Research Institute, [6]M&D Data Science Center, Institute of Integrated Research, Institude of Science Tokyo

The application of Next-Generation Sequencing (NGS) technology in clinical medicine has revolutionized molecular diagnostics by enabling multiple gene testing, or analysis of the entire exon or whole genome with a limited amount of DNA. In collaboration with Human Genome Center and Advanced Clinical Research Center, we have been working on two projects: 1) genetic diagnosis of patients with suspected hereditary cancer predisposition and 2) implementation of precision medicine for patients with rare or intractable cancer.

In the first project, we applied NGS technology for molecular diagnostics of hereditary colon cancer syndromes such as familial adenomatous polyposis (FAP), Lynch syndrome (LS), and polymerase proofreading-associated polyposis (PPAP). Long-read sequencing has technical advantages over short-read sequencing for the detection of structural variants. Therefore, we tested the ability of nanopore sequencing and adaptive sampling for the enrichment of 91 genes involved in hereditary cancer predisposition using DNA from a patient with LS carrying a characterized SV in our previous study. Application of the adaptive sampling using peripheral blood DNA resulted in approximately six times higher target composition than the nonadaptive sampling. Consequently, we successfully identified the breakpoints of a pathogenic SV that was difficult to identify by short-read sequencing technology.

In the second project, we have been working on the implementation of genomic data in clinics. We offered consultation of genetic analysis to patients with rare or intractable cancer as an outpatient clinic service in IMSUT hospital. The study enrolled patients with various types of cancer who provided written informed consent for genetic analysis and treatment prediction using artificial intelligence. Genetic alterations in their tumors were determined by NGS, and the data were subsequently analyzed by QIAGEN Clinical Insights (QCI). Actionable variants were reviewed and discussed in a tumor board to determine recommended therapeutic options. This multidisciplinary board, comprised of physicians, medical oncologists, genetic counselors, geneticists, bioinformaticians, and ethics experts, convened online every two weeks.

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Advanced Clinical Research Center

Division of Innovative Cancer Therapy
先端がん治療分野

Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士(医学)	藤　堂　具　紀
Project Professor	Minoru Tanaka, M.D., Ph.D.	特任教授	博士(医学)	田　中　　実
Assistant Professor	Hirotaka Ito, M.D., Ph.D.	助　教	博士(医学)	伊　藤　博　崇
Assistant Professor	Yoshinori Sakata, M.D., Ph.D.	助　教	博士(医学)	坂　田　義　詞
Assistant Professor	Yuta Takeshima, M.D., Ph.D.	助　教	博士(医学)	竹　島　雄　太
Assistant Professor	Seisaku Kanayama, M.D.	助　教		金　山　政　作

Our laboratory is focused on developing oncolytic virus therapies for various malignant tumors. Oncolytic viruses are engineered to selectively kill tumor cells without affecting normal tissues. G47Δ, a recombinant, triple-mutated oncolytic herpes simplex virus type 1 (HSV-1), exhibits potent anti-tumor efficacy while maintaining safety. G47Δ was approved as the world's first oncolytic virus product for brain tumors in June 2021 and has been in clinical use since November 2021.

Development of novel recombinant oncolytic HSV-1

With a steady increase in cancer mortality, there has been a strong need for novel therapeutics for cancers. Oncolytic virus therapy utilizing genetically engineered viruses not only destroys tumor cells by its direct tumor killing activity but also shows robust antitumor effect by eliciting systemic and specific antitumor immunity. This approach is highly expected as a promising new cancer treatment. Various kinds of virus have been modified and utilized as oncolytic viruses, but genetically engineered HSV-1 is particularly useful because of following favorable characteristics: (1) a highly selective replication in tumor cells while maintaining safety in normal tissues, (2) a high stability of the viral genome, (3) a potent oncolytic activity in a wide range of cancer cells, (4) cell-to-cell spread of the virus minimally affected by serum antiviral antibodies, (5) presence of antiviral drugs that serve as fail safe, (6) a high capacity for incorporating large or multiple transgenes owing to its large genome size (<152kb). We developed G47Δ, a triple-mutated oncolytic HSV-1 with high efficacy and safety. While conventional homologous recombination techniques had required time-consuming processes to create a new recombinant oncolytic HSV-1, our original recombinant HSV-1 construction system, T-BAC, enables quick and accurate generation of a new recombinant HSV-1 with desired transgenes inserted into a specific locus by utilizing two sets of recombinases (Cre/loxP and FLP/FRT). A variety of armed G47Δ with added functions have been developed, including those that express anti-VEGF antibody or an immune checkpoint inhibitor.

Since 2003, translational research of G47Δ was initiated totally by this laboratory, including invention, preclinical studies, clinical lot manufacturing and clinical trials. G47Δ was approved as the world's first oncolytic virus product for malignant glioma in 2021. Besides malignant brain tumors, we have meticulously accumulated pre-clinical data with the intention to expand the application of G47Δ for other cancers, including renal cancer, prostate cancer, bladder cancer, malignant mesothelioma, tongue cancer, esophageal cancer, gastric cancer, colon cancer, lung cancer, breast cancer, nasopharyngeal cancer, cholangiocarcinoma, hepatic cancer, pancreatic cancer, malignant

melanoma, malignant lymphoma and sarcomas.

Preclinical research has revealed that G47Δ is universally effective for all types of solid tumors and is expected to become a standard treatment option for cancer in the near future. The clinical trials of G47Δ for malignant mesothelioma, olfactory neuroblastoma and prostate cancer, as well as trials of human IL-12-expressing G47Δ (T-hIL12) for malignant melanoma, are steadily proceeding.

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Advanced Clinical Research Center

Division of Advanced Medicine Promotion
先端医療開発推進分野

Professor	Fumitaka Nagamura, M.D., D.M.Sc.	教　授　博士(医学)	長　村　文　孝
Associate Professor	Masanori Nojima, M.D., Ph.D., M.P.H.	准教授　博士(医学)	野　島　正　寛

Our mission is to assist the development of translational research. For this purpose, it is critical to discover new "seeds" and to eradicate blockades until the clinical utilization. We also assist the conduct of clinical trials at IMSUT Hospital. At IMSUT Hospital, we work together with the staffs of Center for Translational Research. Concurrently, to concur blockades on translational research, we have been engaging in research on regulatory science and biostatistics.

1. Assistance of Clinical Trials/TRs at Research Hospital

Masanori Nojima, Fumitaka Nagamura

At IMSUT Hospital, we work together with the staffs of Center for Translational Research. The assistance of Translational (Clinical) Research Coordinators is indispensable for the conduct of clinical trials, especially for TR. The activities of Coordinators are the results of the collaboration between Division of Advanced Medicine Promotion and Center for Translational Research. In 2014 we assisted in the preparation and implementation of four investigator-initiated clinical trials.

2. Approach for multi-omics research by methodology of bioinformatics and biostatistics

Masanori Nojima

Multi-omics research using clinical samples in collaborative study or public database of comprehensive omics-analysis, integrating metabolome, DNA methylome, transcriptome, etc.

3. Epidemiological study using NDB (National Database of Health Insurance Claims and Specific Health Checkups of Japan)

Masanori Nojima

We are conducting multiple large-scale epidemiological studies utilizing NDB database, focusing on Specific Health Checkups, long COVID, etc.

4. Statistical consulting for basic research

Masanori Nojima

For basic researchers, we suggest exploratory statistical approach and molecular epidemiological approach.

Publications

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Advanced Clinical Research Center

Division of Advanced Genome Medicine
先端ゲノム医学分野

Associate Professor　Yoshihiro Hirata, M.D., Ph.D.　准教授　博士（医学）　平　田　喜　裕

The goals of our researches are to identify the mechanisms and to establish novel therapies especially for cancers and inflammatory diseases of the digestive system. One of the research fields is the inflammatory diseases, in which we investigated the molecular pathogenesis of gastritis, cholangitis and inflammatory bowel disease. Another research field is the malignancies. We specifically focus on the topics such as, differentiation of stem cells, proliferation and death of epithelium, interactions with immune cells or microbes, inter-organ interactions, and maintenance of tissue homeostasis. Using genetically engineered mice, we try to unveil the pathogenesis of various digestive diseases.

1. Role of IL-33 in the gastrointestinal homeostasis

Yoshihiro Hirata, Nobumi Suzuki[1], Yoku Hayakawa[1]. [1]Department of Gastroenterology, The University of Tokyo

Using several lines of gastric IL-33 overexpression mice (TFF1-cre-LSL-IL33, Mist1creERT-LSL-IL33, TFF1pro-IL33), we found IL33 is involved in the pathogenesis of gastritis, especially recruitment of specific immune cells into the stomach. We have established SigF-cre-EGFP line to visualize SigF+ cells in vivo. Currently, role of acetylcholine signaling in IL-33 triggered gastritis is under investigation.

2. Role of Sox9 in the gastric carcinogenesis

Hu Ke, Kazuya Koyanagi, Nobumi Suzuki[1], Yoku Hayakawa[1], Yoshihiro Hirata. [1]Department of Gastroenterology, The University of Tokyo

Sox9 is a multifunctional transcriptional factor which participates in development, stemness, as well as carcinogenesis of various tissues. To elucidate the role of Sox9 in gastric diseases, we established stomach specific Sox9 knockout mice (TFF1-cre; $Sox9^{f/f}$ mice) and found these mice developed gastritis and gastric tumor in the antrum. We found Sox9 expression in antral UEA1+ mucous gland cells and GS2+ mucus neck cells in WT mice, suggesting the possible origin of SOX9 KO tumor. Now we investigate the tumor development mechanism focusing on RNA expression.

3. Molecular mechanism of the development and the progression of sclerosing cholangitis

Ru Lin, Hisayoshi Natomi, Hayato Nakagawa[3], Yoshihiro Hirata. [3]Department of Gastroenterology, Mie University

Primary sclerosing cholangitis is a rare form of biliary inflammation which can progress to cirrhosis and cancer. We are currently investigating the role of intestinal microflora, on cholangitis using originally developed mouse biliary disease models. We found infiltrated T cells have Th17 signatures, and damaged epithelium express stem cell markers. Antibiotics and UDCA treatment ameliorated immune cell infiltration and fibrosis of bile duct. Currently role of SOX9 in the development of inflammation and fibrosis in PSC model is under investigation using KO mice.

4. Analysis of primary biliary cholangitis mouse model

Jiaqi Zhang[4], Ryo Nakagawa[4], Naoya Kato[4], Hayato Nakagawa[3], Yoshihiro Hirata. [4]Department of Gastroenterology, Chiba University

Primary biliary cholangitis is a rare autoimmune cholestatic liver disease and its cause is not well understood. We have generated transgenic mice which develop immune cell infiltration, bile duct destruction in the liver with elevated serum autoantibody, all of which are characteristics of human PBC. Serum from these mice could induce T-cell dominant cholangitis in normal recipient mice, suggesting the critical role of autoantibody mediated immune reactions in the development of cholangitis in this model.

5. Pathogenesis of squamo-columnar junction cancer of the stomach

Xu Qingpeng, Yoshihiro Hirata

Squamo-columnar junction (SCJ) is one of the transitional zones in body where two different cell types merge. Barrett's adenocarcinoma and squamous cell carcinoma are two major tumors found in human gastric SCJ. The origin of SCJ tumors and the process of tumorigenesis are largely unknown. Using mouse models and lineage tracing, we try to identify cancer initiating cells as well as stem cells specific to gastric SCJ.

6. Pathogenesis of eosinophil in the development and progression of colitis

Daisuke Kajimoto, Yoshihiro Hirata

Eosinophil is an immune cell derived from myeloid cell lineage. Its critical roles in allergy and parasite infection are well established, but the role in the development and progression of colitis is not fully understood. Using eosinophil depletion mouse line, we try to unveil the role of eosinophil in mouse colitis.

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Advanced Clinical Research Center

Division of Frontier Surgery
フロンティア外科学分野

Professor	Dai Shida, M.D., Ph.D.	教　授　博士(医学)	志　田　　大
Associate Professor	Susumu Aikou, M.D., Ph.D.	准教授　博士(医学)	愛　甲　　丞
Assistant Professor	Ai Sadatomo, M.D., Ph.D.	助　教　博士(医学)	佐田友　　藍
Assistant Professor	Yuka Ahiko, M.D.	助　教	阿　彦　友　佳
Assistant Professor	Naoki Sakuyama, M.D., Ph.D.	助　教　博士(医学)	柵　山　尚　紀
Assistant Professor	Satoko Monma, M.D.	助　教	門　間　聡　子
Assistant Professor	Junko Mukohyama, M.D., Ph.D.	助　教　博士(医学)	向　山　順　子

The mission of our division is to generate solid evidence for the surgical treatment of colorectal and gastric cancers by consistently publishing clinical and basic research papers. If our research can help reshape clinical guidelines worldwide, we, as surgeons, will not only be able to cure the patients in front of us but also contribute to the advancement of surgical treatments for gastrointestinal cancer.

1. Introduction

This division was newly established in September 2020 by Professor Shida and Dr. Ahiko. On November 16th, Dr. Aikou joined the division. This year, Dr. Ahiko retired in March 2024, and Dr. Sadatomo joined the division for a six-month period from April to September 2024.

We named our division 'Frontier Surgery' because we aim to boldly explore and develop untapped areas of surgery, contributing to its advancement.

2. Treatment for Diseases of the Colon, Rectum, Anus and Stomach at IMSUT hospital

All of us are also members of the Department of Surgery at IMSUT Hospital. We specialize in the treatment of diseases affecting the colon, rectum, anus, and stomach, with a particular focus on colorectal and gastric cancers. As certified surgeons under the Japan Society for Endoscopic Surgery's Endoscopic Surgical Skill Qualification System (Dr. Shida and Dr. Aikou), we actively perform minimally invasive surgeries, including laparoscopic and robotic surgeries. Additionally, we conduct laparoscopic surgeries for inguinal hernias.

(See NO16-10, Department of Surgery, IMSUT Hospital.)

3. Creating Evidence for Gastrointestinal Malignancies

As gastrointestinal surgeons, we are not only focused on performing surgeries but also planning to conduct translational research as academic surgeons in the near future.

4. Publications

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Advanced Clinical Research Center

Division of Hematopoietic Disease Control
造血病態制御学分野

Professor	Yasuhito Nannya, M.D., Ph.D.	教　授	博士(医学)	南　谷　泰　仁
Associate Professor	Takaaki Konuma, M.D., Ph.D.	准教授	博士(医学)	小　沼　貴　晶
Assisstant Professor	Koji Jimbo, M.D., Ph.D.	助　教	博士(医学)	神　保　光　児

The main goal of our research is to elucidate the pathogenesis of hematopoietic diseases and to study the development of therapeutic strategies for these diseases. We will continue to develop the therapeutic targets that have already been identified and advance them to the next stage for clinical application. In particular, we are collaborating with various research groups in Japan and abroad regarding the elucidation of pathogenesis through genome analysis, and have discovered a novel genetic mutation involved in the progression of myeloproliferative tumors. Further advanced genome analysis has yielded interesting insights into clonal evolution in PNH.
We are also leading a whole genome sequencing project collected from major hematopoietic disease centers in Japan, which has identified numerous novel genomic aberrations in acute lymphocytic leukemia and malignant lymphoma. We are also elucidating the role of specialized neutrophils in MSC therapy by proteomics analysis and the significance of genomic abnormalities specific to a particular type of hairy cell leukemia by generating model mice.

1. Whole-genome sequencing of myeloproliferative neoplasms revealed dynamic clonal changes in the fibrotic or leukemic transformation and novel FOXP1 mutations in the fibrotic transformation

Hiroyuki Takamori[1*], Ying-Jung Huang[2*], Hidehito Fukushima[1], Kazuaki Yokoyama[1], Ting-Yu Huang[2], Ming-Chung Kuo[2,3], Seishi Ogawa[4], Yasuhito Nannya[1†], Lee-Yung Shih[2,3†]
1: Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
2: Division of Hematology-Oncology, Chang Gung Memorial Hospital at Linkou, Taoyuan, Taiwan.
3: School of Medicine, Chang Gung University, Taoyuan, Taiwan.
4: Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Myeloproliferative neoplasms (MPNs) are characterized by clonal proliferation of hematopoietic stem cells, which can lead to secondary myelofibrosis or acute myeloid leukemia. We explored the changes in genomic alterations during MPN transformation using whole-genome sequencing of samples from both the chronic and fibrotic or leukemic phases of 20 patients. We identified *FOXP1* mutations in 3 of 14 (21.4%) patients with secondary myelofibrosis. This novel mutation was identified in another 5 of the 35 patients (14.3%) in an independent cohort. All these 8 patients with *FOXP1* mutations did not experience leukemic transformation after a median follow-up of 5.1 years. The acquisition of non-canonical MPL^{Y591} mutations was detected in the fibrotic or leukemic phase. Clonal expansion, involving both known and unknown driver genes (in 18 and 2 patients, respec-

tively), was observed in all patients. We determined the patterns of clonal evolution based on myeloid driver mutations in 18 patients: linear clonal evolution in 11 patients and branched clonal evolution in 7 patients. Our results suggested that MPN patients carrying *FOXP1* mutations are unlikely to have leukemia transformation and emphasized that the acquisition of specific genetic mutations and dynamic changes in clonal architecture underlie the pathogenesis in patients undergoing MPN transformation.

2. Inferred Trajectories of Clonal Expansion in Paroxysmal Nocturnal Hemoglobinuria.

Hiroyuki Takamori[1,2], Yasutaka Ueda[1], Yoshikazu Matsuoka[3], Tatsuya Fujioka[3], Hideki Makishima[4], Yoshiko Murakami[5], Taroh Kinoshita[5,6,7], Satoru Miyano[8], Yuzuru Kanakura[1,9], Jun-Ichi Nishimura[1], Naoki Hosen[1], Seishi Ogawa[4], Yasuhito Nannya[2,4]

1. Department of Hematology and Oncology, Osaka University Graduate School of Medicine

2. Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo

3. Department of iPS Stem Cell Regenerative Medicine, Kansai Medical University

4. Department of Pathology and Tumor Biology, Kyoto University

5. Laboratory of Immunoglycobiology, Research Institute for Microbial Diseases, Osaka University

6. Immunoglycobiology, WPI Immunology Frontier Research Center, Osaka University

7. Center for Infectious Disease Education and Research, Osaka University

8. M&D Data Science Center, Tokyo Medical and Dental University

9. Department of Hematology Sumitomo Hospital

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare clonal hematological disorder resulting from clonal expansion of PIGA mutated clones. *PIGA* gene is involved in the first step of glycosylphosphatidylinositol (GPI) anchored protein synthesis. CD55 and CD59 are GPI-anchored proteins that function as a central component of complement regulatory factors in red blood cells. Somatic mutations of *PIGA* gene result in the absence of CD55 and CD59, leading to complement-mediated intravascular hemolysis. It is well known that *PIGA* mutations alone are not sufficient for the clonal expansion of *PIGA* mutated clones. T cell mediated immune pressure or additional mutations other than *PIGA* mutations could confer the clonal expansion. Currently, next-generation sequencing and high-sensitivity flow cytometry enable us to observe the clonal dynamics and clonal architecture before the diagnosis of PNH. Here I present our current study in which we attempt to estimate the clonal expansion in PNH before the diagnosis using the whole genome sequence of single cell derived colonies. This study has the potential to clarify the trajectories of PIGA mutated clones before the significant clonal expansion or the diagnosis, thereby gaining insight into the pathogenesis of the clonal expansion in PNH.

3. Investigation of MSC Therapy and Neutrophil Function in Acute GVHD Using Plasma Proteomics

Tomokazu Seki[1], Xinyu Huang[2], Kazuaki Yokoyama[3], Seiya Imoto[4], Tokiko Nagamura-Inoue[2], Yasuhito Nannya[1,3]

1. Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

2. Department of Cell Processing and Transfusion, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

3. Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

4. Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo, Japan.

We analyzed plasma protein profiles in patients with acute graft-versus-host disease (GVHD) following allogeneic hematopoietic stem cell transplantation using targeted proteomics with Olink®. The results revealed that biomarkers and pathways previously reported to be associated with acute GVHD were identified in the GVHD cohort compared to healthy controls. Notably, in the acute GVHD group—particularly those with skin involvement—neutrophil-secreted proteins and pathways related to secretory vesicles and degranulation were downregulated compared to patients without GVHD. These findings suggest potential neutrophil dysfunction in acute GVHD. We further evaluated the plasma protein profiles of patients with steroid-refractory acute GVHD who responded to umbilical cord-derived mesenchymal stromal cell (UC-MSC) therapy. Compared to pre-treatment profiles, post-MSC therapy profiles demonstrated a time-dependent upregulation of neutrophil-secreted proteins and pathways associated with secretory vesicles and degranulation. These results suggest that MSC therapy can enhance neutrophil function, leading to suppress GVHD. We plan to perform T-cell functional analyses using CFSE-MLR assays via flow cytometry to investigate whether MSC-mediated promotion of neutrophils contributes to GVHD suppression.

4. Multiomics Analysis of Drug Sensitivity in AML

Tomokazu Seki[1], Kimihito C Kawabata[2], Chao Li[1,3], Kazuaki Yokoyama[4], Seiya Imoto[5], Yasuhito Nannya[1,4], Satoshi Takahashi[2]

1. Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
2. Division of Clinical Precision Research Platform, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
3. Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan
4. Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
5. Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo, Japan.

We conducted drug sensitivity assays using primary tumor specimens (PTS) from AML patients. Based on the assay results, we performed bulk RNA sequencing (RNA-seq) to identify differentially expressed genes (DEGs) and pathways potentially contributing to the therapeutic responses of specific drugs. We plan to increase the number of bulk RNA-seq samples to enable more robust analyses. Additionally, we aim to integrate PTS data with clinical information, whole-exome sequencing (WES), and whole-genome sequencing (WGS) results from AML patients. This approach will facilitate a comprehensive, multi-faceted evaluation of drug sensitivity.

5. Molecular pathogenesis of hairy leukemia Japanese variant based on whole genome-multi-omics information

Iku Kamitani[1], Kazuaki Yokoyama[2], Keisuke Kidoguchi[3], Shinya Kimura[3], Seiya Imoto[4], Yasuhito Nannya[1]

1. Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan
2. Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
3. Division of Hematology, Respiratory Medicine and Oncology, Department of Internal Medicine, Faculty of Medicine, Saga Unversity, Saga, Japan
4. Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

We have performed whole genome analysis of hairy cell leukemia variants in collaboration with Saga University. Hairy cell leukemia is a rare disease, and hairy cell leukemia without BRAF mutations is extremely rare and has little validation regarding pathogenesis. About 30% of cases showed structural abnormalities such as partial chromosomal deletions and inversions in the TCL1 to IGH region on chromosome 14, which is often observed in other B-cell lymphomas. Therefore, we generated a mouse model in which chromosomal structural abnormalities occur in the IGH region from TCL1A on chromosome 14 using the loxP-Cre system, bred Mb1Cre mice to operate the Cre-loxP system specifically for B cells, and observed the process of disease development.

6. Whole genome sequencing (WGS)-based structural variant detection in Acute lymphoblastic leukemia and other hematological malignancies

Hidehito Fukushima[1], Kaito Mimura[2], Koji Okazaki[3], Hiroyuki Takamori[1], Ryunosuke Saiki[3], Yotaro Ochi[3], Kazuaki Yokoyama[1], Kenichi Yoshida[2], Motohiro Kato[4], Kotoe Katayama[5], Seiya Imoto[5], Seishi Ogawa[3], and Yasuhito Nannya[1]
1) Division of Hematopoietic Disease Control, Institute of Medical Science, The University of Tokyo, Tokyo, Japan
2) Division of Cancer Evolution, National Cancer Center Research Institute, Tokyo, Japan
3) Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan
4) Department of Pediatrics, University of Tokyo, Tokyo, Japan
5) Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, Tokyo, Japan

Our study aimed to i) overcome major obstacles encountered in SV analysis using short-read sequencing data , ii) propose an optimal SV detection pipeline, and iii) to identify novel structural variants. We enrolled 1,453 cases diagnosed with major hematological disorders. For each case, diagnostic samples along with an oral swab were subjected to a range of genomic analyses, including whole-genome sequencing (n=1,453), RNA-seq (n=888), Optical Genome Mapping (n=12), and long-read sequencing (PacBio, n=46). Additionally, we constructed a panel of normals (PoNs) utilizing multiple SV reference panels (ToMMo, gnomAD SVs) and included 53 remission samples from pediatric leukemia patients that underwent whole-genome sequencing. To validate our findings, we compared the outputs from various SV callers (GRIDSS, Manta, Delly, SVABA, GenomonSV) with those obtained through other sequencing modalities. The intersection of the SV callers revealed that only 6% of all ensembled calls overlapped, potentially attributed to tumor cell contamination in normal samples and the presence of caller-specific calls. Furthermore, we assessed the sensitivity of GRIDSS, Manta, and SVABA in detecting fusion events using RNA-seq as a reference standard. Our results indicated that GRIDSS exhibited improved sensitivity (0.75) with our custom filter compared to Manta and SVABA (0.5~0.7). Moreover, we validated the effectiveness

of PoNs, demonstrating its capacity to eliminate approximately 75% of all calls on average, thus maintaining a higher positive predictive value. Finally, our study has identified a substantial number of novel recurrent structural variants, such as ADD3 deletions observed in acute lymphoblastic leukemia samples (15%). Our next steps involve conducting comprehensive functional analyses on these identified variants.

7. Optical Genome Mapping for Hematological Malignancies

Hidehito Fukushima[1], Kaito Mimura[2], Koji Okazaki[3], Hiroyuki Takamori[1], Ryunosuke Saiki[3], Yotaro Ochi[3], Kazuaki Yokoyama[1], Kenichi Yoshida[2], Motohiro Kato[4], Kotoe Katayama[5], Seiya Imoto[5], Seishi Ogawa[3], and Yasuhito Nannya[1]

1) Division of Hematopoietic Disease Control, Institute of Medical Science, The University of Tokyo, Tokyo, Japan

2) Division of Cancer Evolution, National Cancer Center Research Institute, Tokyo, Japan

3) Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

4) Department of Pediatrics, University of Tokyo, Tokyo, Japan

5) Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, Tokyo, Japan

Optical Genome Mapping (OGM), developed by Bionano Genomics, represents a novel cytogenetic technology capable of detecting structural variants (SVs) and copy number variations (CNVs) by fluorescently labeling specific sequences within long DNA fragments (approximately 1 Mb) and mapping them to a reference genome. While promising as an alternative to traditional techniques such as SNP arrays, G-banding, and FISH, its limitations include the inability to detect structural variants smaller than 500 bp or copy number variations below 50,000 bp.

This study analyzed tumor samples from 12 patients diagnosed with acute myeloid leukemia using OGM and evaluated its performance by comparison with G-banding and whole-genome sequencing (WGS). The structural variant analysis with WGS employed GRIDSS, while copy number analysis utilized Battenberg. The sensitivity of OGM for detecting chromosomal abnormalities identified by G-banding was 83%, with five out of six chromosomal abnormalities being successfully detected. Events with clone fractions greater than 80% were detectable by OGM, while a +21 abnormality with a 30% clone fraction was not detected.

When comparing OGM with WGS, GRIDSS identified 93 structural variants, with OGM demonstrating a 100% sensitivity for chromosomal translocations and a 94% sensitivity for events larger than 5,000 bp. However, its sensitivity dropped significantly to 14% for structural variants smaller than 5,000 bp. The average number of structural variant calls per sample by OGM was 1,169, though most lacked supporting evidence. Current efforts to establish optimal thresholds for QUAL and VAF values for filtering have been inconclusive. Some structural variants, such as those near the SPG11 gene, remain undetectable due to mapping challenges posed by pseudogenes and paralogs concentrated in the region. Additionally, positional discrepancies averaging 3,564 bp were observed between structural variants detected by OGM and WGS. OGM's tendency to classify inversions as intrachromosomal translocations underscores the need for expert interpretation.

The CNV detection capabilities of OGM revealed a sensitivity of 0% for events smaller than 50,000 bp and 28% for events exceeding this size threshold. The accuracy of CNV detection is influenced by both clone size and the length of the structural variants. OGM showed particular utility in identifying translocations and large-scale deletions, inversions, and duplications with high sensitivity, making it a viable alternative to G-banding for samples with high clone purity. However, the integration of long-read sequencing technologies is necessary to improve the detection of complex structural variants and to address current limitations.

In conclusion, OGM represents a significant advancement in genomic analysis for hematological malignancies, offering high sensitivity for specific structural variants. Nonetheless, its utility is constrained by limitations in detecting small-scale events and by challenges inherent to the technology. Further development and integration with complementary sequencing modalities are essential to maximize its diagnostic and research potential.

8. Whole genome sequencing (WGS) analysis in Malignant Lymphoma

Hidehito Fukushima[1], Kaito Mimura[2], Koji Okazaki[3], Hiroyuki Takamori[1], Ryunosuke Saiki[3], Yotaro Ochi[3], Kazuaki Yokoyama[1], Kenichi Yoshida[2], Motohiro Kato[4], Kotoe Katayama[5], Seiya Imoto[5], Seishi Ogawa[3], and Yasuhito Nannya[1]

1) Division of Hematopoietic Disease Control, Institute of Medical Science, The University of Tokyo, Tokyo, Japan

2) Division of Cancer Evolution, National Cancer Center Research Institute, Tokyo, Japan

3) Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

4) Department of Pediatrics, University of Tokyo, Tokyo, Japan

5) Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, Tokyo, Japan

In this study, we conducted a comprehensive genomic analysis of 251 cases of malignant lymphoma utilizing whole-genome sequencing (WGS) and RNA sequencing. WGS was applied to identify single nucleotide variants (SNVs), copy number variations (CNVs), and structural variants (SVs), while RNA sequencing enabled detailed profiling of gene expression patterns. This integrative approach allowed us to systematically uncover not only coding-region mutations in genes previously implicated in malignant lymphoma but also non-coding region variants with potential regulatory significance. Importantly, these non-coding mutations exhibited distinct patterns of distribution across subtypes as defined by the LymphGen classification system. Moving forward, we aim to redefine the classification of malignant lymphoma by incorporating these non-coding variants alongside coding-region mutations, with the ultimate goal of refining our understanding of the genomic underpinnings of this disease.

Publications

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2. Wang, Q.S., T. Hasegawa, H. Namkoong, et al., *Statistically and functionally fine-mapped blood eQTLs and pQTLs from 1,405 humans reveal distinct regulation patterns and disease relevance.* Nat Genet, 2024. **56**(10): p. 2054-2067.
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Advanced Clinical Research Center

Division of Advanced Gastroenterology and Endoscopy
先端消化器内視鏡学分野

Professor	Hiroaki Ikematsu, M.D., Ph.D.	教　授　博士(医学)	池　松　弘　朗
Assistant Professor	Tatsunori Minamide, M.D.	助　教	南　出　竜　典

A new field, "Division of Advanced Gastroenterology and Endoscopy," was established at the Institute of Medical Science of the University of Tokyo from October 2023.
Our mission is to conduct cutting-edge endoscopy-related research and develop novel endoscopic equipment, with a focus on gastrointestinal cancer. In addition, we will conduct translational research with other research fields at the institute to create new diagnoses and treatments.

1. Introduction

Advances in endoscopic diagnosis and treatment have been remarkable. Our objective is to continue advancing, spanning from basic research in endoscopy to clinical investigations, and the creation of innovative endoscopic devices for practical clinical applications. In addition, our objective is to create novel diagnostic and therapeutic approaches through translational research in collaboration with various fundamental disciplines.

2. Research Activities

The device development in 2024 was as follows: (1) Development of a device capable of detecting high quantitative fecal occult blood in the toilet, (2) Development of automatic colon insertion endoscope, and (3) Research targeting submucosal tumors and gastric cancer by irradiating with near-infrared light in the wavelength range of 1,000 nm or more, as well as the development of a flexible endoscope for this purpose, (4)Development of a novel hemostatic forceps. All of these are highly novel seeds and systems that have never existed before and can be expected to be clinically useful.

3. Future Prospects

We will continue basic and clinical research to elucidate, detect, and diagnose lesions with the aim of preventing gastrointestinal cancers.

We will continue to develop innovative medical devices desired by the next generation that reflect the needs of clinical practice. In the future, we will continue to develop endoscopic equipment and attempt innovative approaches to produce all-new endoscopy approaches/devices in collaboration with academia and corporations.

In addition, we plan to further promote research and development with the aim of obtaining competitive funding.

4. Publications

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Advanced Clinical Research Center

Division of Anesthesia and Surgical Homeostasis
侵襲防御医学分野

Professor Masahiko Bougaki, M.D., Ph.D.

教 授 博士(医学) 坊 垣 昌 彦

Established in April 2024, this division enhances the Department of Anesthesiology at IMSUT Hospital, focusing on delivering superior perioperative patient care. While clinical duties remain the current priority, future plans include initiating clinical research and contributing to translational research in collaboration with other hospital departments and divisions to improve patient outcomes and advance medical care.

Introduction

This division was newly established in April 2024 to enhance the activities of the Department of Anesthesiology at IMSUT Hospital and to elevate the hospital's ability to deliver superior perioperative patient care. The field of anesthesiology covers a wide range of physiological responses to surgical stimuli and the strategies to protect the body against them. Examples include the management of sedation, respiration, circulation, pain, coagulation, metabolism, and more. Our mission is to establish a robust foundation for delivering optimal anesthetic and intensive care when necessary, not only to general surgical patients but also to those undergoing innovative therapeutic interventions at IMSUT Hospital.

Future Prospects

At present, most of our efforts are focused on clinical duties. In the near future, we plan to initiate clinical research to further enhance our activities and pursue better patient outcomes in collaboration with other surgical departments within the hospital. Additionally, we are preparing to contribute to translational research at IMSUT in partnership with other divisions to advance innovative medical practices.

Publications

Kashiwa K, Kurosawa H, Fujishiro K, Kubo H, Inokuchi R, Bougaki M, Kawamura G, Sato M, Konoeda C, Nakajima J, Doi K. Increased white blood cell count is associated with an increased demand for unfractionated heparin during veno-arterial extracorporeal oxygenation in lung transplantation. J Extra Corpor Technol. 56:108-113, 2024.

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Advanced Clinical Research Center

Division of Hematology and Tumor Biology
血液・腫瘍生物学分野

Associate Professor Ayana Kon, M.D., Ph.D.　准教授　博士（医学）　昆　彩奈

Cancer arises from genetically diverse cells due to repeated clonal selection of driver mutations that confer survival advantages. Advances in next-generation sequencing have expanded our understanding of cancer-associated mutations, but many biological mechanisms remain unclear. Our lab focuses on uncovering unknown genetic abnormalities and molecular mechanisms underlying hematological malignancies. Using patient samples and disease mouse models, we integrate molecular biology techniques with data science approaches to investigate unexplored cancer biology.

1. Functional analysis of germline and somatic *DDX41* Mutations in the pathogenesis of myeloid malignancies

Ayana Kon[1,2], Masahiro M Nakagawa[3], Keisuke Kataoka[4,5], Hideki Makishima[6], Manabu Nakayama[7], Haruhiko Koseki[8], Yasuhito Nannya[9], Seishi Ogawa[3]
1) Division of Hematology and Tumor Biology, Institute of Medical Science, The University of Tokyo
2) Division of Stem Cell and Genome Biology, Institute of Medical Science, The University of Tokyo
3) Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University
4) Division of Molecular Oncology, National Cancer Center Japan Research Institute
5) Department of Hematology, Keio University School of Medicine
6) Department of Hematology, Shinsyu University
7) Chromosome Engineering Team, Department of Technology Development, Kazusa DNA Research Institute
8) Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences
9) Department of Hematology/Oncology, Institute of Medical Science, The University of Tokyo

DDX41 is a newly identified leukemia predisposition gene encoding an RNA helicase, whose germline mutations are tightly associated with late-onset myeloid malignancies. Importantly, germline *DDX41* mutations were also found in as many as ~8 % of sporadic cases with high-risk MDS, conferring the largest germline risk for myeloid malignancies. In typical cases, a germline loss-of-function allele is compounded by a somatic missense mutation affecting the helicase domain in the remaining allele (p.R525H). However, the molecular mechanisms by which *DDX41* mutations lead to myeloid neoplasms have not been fully elucidated.

To clarify the role of these distinct *DDX41* alleles, we generated mice models carrying either or both of conditional/constitutive *Ddx41* knock-out (KO) and conditional R525H knock-in (KI) alleles. By crossing these mice and further breeding with *Rosa26-CreERT2* transgenic mice, we engineered mice that were wild-type for *Ddx41* ($Ddx41^{+/+}$), heterozygous *Ddx41* KO ($Ddx41^{+/-}$), homozygous *Ddx41* KO ($Ddx41^{-/-}$), heterozygous for the *Ddx41* R525H mutation ($Ddx41^{R525H/+}$), or hemizygous for the *Ddx41* R525H mutation ($Ddx41^{R525H/-}$), in which expression of the mutant allele was induced by tamoxifen administration.

First, we assessed cell intrinsic effects of these *Ddx41* alleles, using noncompetitive transplantation experiments. Shortly after tamoxifen administration, most of the recipient mice that were transplanted with BM from $Ddx41^{-/-}$ or $Ddx41^{R525H/-}$ mice died within a month after *CreERT2* induction due to severe BM

failure (BMF), which was not observed in mice transplanted with BM from *Ddx41*$^{+/+}$, *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ mice. By contrast, the mice transplanted with *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ BM showed significantly reduced WBC counts and anemia in long-term observation in both primary and serial transplantations. Some of the *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ BM-transplanted mice exhibited MDS-like phenotypes, showing ineffective hematopoiesis with evidence of erythroid dysplasia.

Transcriptome analysis revealed that stem cells derived from *Ddx41*$^{R525H/-}$ BM-transplanted mice exhibited a significant upregulation of genes involved in innate immunity, including interferon stimulated genes, compared with stem cells derived from *Ddx41*$^{+/+}$ BM-transplanted mice. In addition, ribosomal genes were significantly deregulated in stem cells from *Ddx41*$^{-/-}$ and *Ddx41*$^{R525H/-}$ BM-transplanted mice, which could result in abnormal ribosome biogenesis and protein synthesis in *Ddx41* mutant cells.

Our results revealed that monoallelic *Ddx41* loss-of function led to age-dependent impaired hematopoiesis, 1while biallelic loss-of function and R525 alleles showed a compromised function of hematopoiesis, where activated innate immunity and impaired ribosome functions may play important roles.

Publication

1. 昆 彩奈, 骨髄異形成症候群における動物モデルを用いた病態解明 (Recent advances in experimental animal models of myelodysplastic syndromes) 月刊血液内科(科学評論社), 2024;89(1):8-13. 2024年7月発行

Advanced Clinical Research Center

Division of Bioethics and Medical Law
生命倫理・医事法研究分野

Associate Professor Waki Toya, Ph.D.

准教授 博士(医学) 遠 矢 和 希

Our division took over from the former Division of Bioethics and opened in September 2024. We study ethical, legal, and social issues (ELSI) in cutting-edge medical science research and clinical settings. As experts in research ethics within here IMSUT, we also provide research ethics consultation services to those involved in clinical research who are faced with ethical issues.

1. Project of research ethics consultant training and educational curriculum

Waki Toya

In a research ethics consultant training project (Principal Investigator: Kenji Matsui in the National Cancer Center Japan) based on the Grant by the Japan Agency for Medical Research and Development (AMED) since FY 2016, we have published a text, held many training sessions, and are in the process of launching an association-certified consultant qualification examination. To raise awareness of research ethics consultation and the role of consultants, we made the following presentation at the conference for Clinical Research Coordinator: Waki Toya, Research Ethics Consultant's Practice. In symposium 15, How can I become a CRC who can also be a research ethics consultant? - From the basics of research ethics consultation to the role of a research ethics consultant -, in the 24th Conference on CRC and Clinical Trials 2024 in Sapporo, on 15th Sep. 2024.

2. ELSI on internal medicine research for fetus in the womb

Waki Toya

Advances in fetal diagnostic technology have led to the development of treatments for fetuses in the womb to prevent congenital diseases and miscarriages. These are conditions that would traditionally have been treated after birth. Such technologies must be developed carefully as clinical research. However, to date, there has been insufficient discussion, both globally and in Japan, about how to protect subjects in such interventional research on pregnant women and fetuses. Who are the subjects in fetal treatment research? Who bears the risks of the research, and who directly benefits from the research intervention? At the very least, in many cases there is no direct health benefit to the pregnant woman herself. Our research group made a presentation from a legal perspective, focusing on internal medicine research for fetus in the womb: Waki Toya, Legal Issues in Internal Medicine Research for Fetus. Symposium on ELSI in Internal Medicine Research for Fetus, Japan Association for Bioethics 36th Annual Meeting in Osaka, on 17th Nov. 2024.

3. Future Prospects

Specific themes that we have worked on are as follows:
- Research on ELSI and legal policies in assisted reproductive technology
- ELSI and research ethics regarding life through cutting-edge artificial technology (e.g., ectogenesis).

- Research on ELSI and law regarding biobanks and follow-up research
- Research on medical and clinical research from a gender perspective

We will also investigate ELSI in the areas of infectious disease research. And a textbook on research ethics for medical students will be published next FY.

Center for Stem Cell Biology and Regenerative Medicine

Division of Regenerative Medicine
再生医学分野

Professor	Hideki Taniguchi, M.D., Ph.D.	教　授	博士(医学)	谷　口　英　樹
Associate Professor	Naoki Tanimizu, Ph.D.	准教授	博士(農学)	谷　水　直　樹
Assistant Professor	Yun-Zhong Nie, Ph.D.	助　教	博士(医学)	聶　　運　中
Project Assistant Professor	Yasuharu Ueno, Ph.D.	特任助教	博士(医学)	上　野　康　晴
Project Assistant Professor	Takayoshi Oba, M.D., Ph.D.	特任助教	博士(医学)	大　場　敬　義

Currently, organ transplantation is the only effective treatment for patients with end-stage organ failure. Unfortunately, the limited number of transplantable organs hinders the extensive application of this treatment. On the other hand, recent development of regenerative medicine that aims to generate transplantable organs on a dish has attracted much attention. Regenerative medicine is a challenging scientific field that attempts to convert knowledge from developmental biology and stem cell biology into clinical application. Our established novel organoid culture technologies reconstruct functional human organs derived from human induced pluripotent stem cells (hiPSCs), and finally aim to develop ex vivo human liver disease models and a substitute for organ transplantation therapy. Currently, we are trying to conduct the transplantation of human liver organoids (LOs) generated from hiPSCs to treat liver diseases, such as metabolic disorders and liver fibrosis. Moreover, we expand the application of our technologies to reconstruct artificial cancer tissue (cancer organoid) with a refractory tumor microenvironment for developing a new drug-screening platform to discover candidate compounds that could prevent cancer relapse and metastasis.

1. Development of treatment for metabolic liver disease by transplantation of human iPS cell derived 3D-organoids.

Yasuharu Ueno[1], Naoki Tanimizu[1], Yunzhong Nie[1], Satoshi Okamoto[1], Yu Kamishibahara[1], Takashi Okumura[1], Tomonori Tsuchida[1], Toshiharu Kasai[1], Tatsuya Kobayashi[1], Erika Jinbo[1], Kerrigan Kilpatrick[1], Tomomi Tadokoro[2] and Hideki Taniguchi[1,2]:

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

The liver plays a crucial role in maintaining homeostasis in the living organism by performing various metabolic functions such as glucose metabolism, lipid metabolism, and ammonia metabolism. On the other hand, abnormalities in these metabolic functions can lead to a variety of diseases of the liver. Liver transplantation is the only curative therapy for end-stage liver disease, but the absolute shortage of donor organs is a serious challenge, and alternative treatments are clinically highly demanded. We established a technique to produce and evaluate human pluripotent stem cell (hiPSC) derived liver organoids (hiPSC-LO) by inducing differentiation of hepatic endodermal cells, vascular endothelial cells, and mesenchymal cells from hiPSCs and co-culturing them in a 3D manner (***Nature*** 2013, ***Nature*** 2017, ***Cell Reports*** 2017, ***Sci Rep*** 2020, ***Stem Cell Rev Rep.*** 2022).

Currently, we are developing a novel therapeutic method using hiPSC-LO transplantation for urea cycle disorders, a serious liver disease, and metabolic dysfunction-associated steatohepatitis (MASH), (***Sci Transl Med.*** 2024 Jul 24;16(757):eadg0338.).

Liver cirrhosis is the end stage pathological condition of chronic liver diseases such as MASH. MASH is characterized by reduced liver function and regenerative capacity and is expected to explode in the number of patients worldwide. With the support of AMED, we are currently developing a novel treatment for MASH cirrhosis by transplantation of a newly developed fused-type hiPSC-LO based on hiPSC-LO production technology. To this end, we have established a stable method for creating fused-type hiPSC-LOs and now we are examining its efficacy as MASH treatment by transplanting them into MASH liver cirrhosis model animals. Given that no effective treatment has been developed for MASH cirrhosis, there is great hope for fused-type hiPSC-LOs transplantation.

2. Liver repopulation with hiPSC derived proliferative progenitors

Yun-Zhong Nie[1], Yoshihito Hayashi[1], Qing-Lin LI[1], Xiao-Shan Deng[1], Luo Na[1], Yang Li[1], Xia Yang[1], Riana Plummer[1], Naoki Tanimizu[1], Yasuharu Ueno[1], Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

hiPSCs have shown immense potential for cell replacement therapy for disease treatment. However, hiPSC-derived cells that can effectively repopulate in the damaged tissues such as liver have not been reported. Here, we present the generation of expandable hiPSC-derived hepatoblast (hiPSC-HB) with robust repopulation capacity after transplantation. These hiPSC-HB exhibited an impressive expansion capability and displayed bipotential differentiation abilities both *in vitro* and *in vivo*. Notably, we found that hiPSC-HB transplantation could rescue mice from liver failure, demonstrating a repopulation capacity comparable to that of primary human hepatocytes (PHHs). Further, the engrafted hiPSC-HB matured into functional human hepatocytes with tissue-specific structural features in kinds of disease models. This study marks a breakthrough as the first successful generation of lineages from pluripotent stem cells capable of *in vivo* repopulating and restoring tissue function. Moving forward, we aim to explore the potential clinical applications of hiPSC-HB transplantation in the treatment of liver diseases.

3. Modeling liver diseases with hiPSC-derived organoid

Yun-Zhong Nie[1], Yang Li[1], Xia Yang[1], Riana Plummer[1], Xiao-Shan Deng[1], Yoshihito Hayashi[1,] Qing-Lin LI[1], Luo Na[1], Toshiharu Kasai[1], Takashi Okumura[1], Naoki Tanimizu[1], Yasuharu Ueno[1], Hideki Taniguchi[1,2]

Maximizing the potential of human liver organoids (LOs) for modeling human septic liver requires the integration of innate immune cells, particularly resident macrophage Kupffer cells. In this study, we present a strategy to generate LOs containing Kupffer cells (KuLOs) by recapitulating fetal liver hematopoiesis using hiPSC-derived erythro-myeloid progenitors (EMPs), the origin of tissue-resident macrophages. Remarkably, LOs actively promote EMP hematopoiesis toward myeloid and erythroid lineages. Moreover, supplementing M-CSF proves crucial in sustaining the hematopoietic population during the establishment of KuLOs. Exposing KuLOs to sepsis-like endotoxins leads to significant organoid dysfunction that closely resembles the pathological characteristics of the human septic liver. Furthermore, we observe a notable functional recovery in KuLOs upon endotoxin elimination, which is accelerated by using Toll-like receptor 4-directed endotoxin antagonist. Our study represents a comprehensive framework for integrating hematopoietic cells into organoids, facilitating in-depth investigations into inflammation-mediated liver pathologies. Moving forward, we aim to enhance the complexity of liver organoids by incorporating hepatic stellate cells and sinusoidal endothelial cells, thereby establishing organoids with tissue-specific features for studying disease progression and identifying potential treatments.

4. hiPSC-liver bud *in vitro* growth enhanced by perfusion culture

Yoshiki Kuse[1], Naoki Tanimizu[1], Yasuharu Ueno[1], Megumi Matsuo[2], Nie Yunzhong[1], Shinya Matsumoto[1], Takashi Okumura[1], Erica Carolina[1], Soichiro Yamabe[1], Eriko Kanai[1], Syusaku Tsuzuki[1], Toshiharu Kasai[1], Tomomi Tadokoro[2], Satoshi Okamoto[2], and Hideki Taniguchi[1,2]:

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

To overcome the critical shortage of organ donors, the generation of hiPSC-derived organs with structures and functions is urgently needed. Although hiPSC-organoid is an innovative technology to reconsti-

tute tissue structure and function, an alternative for organ transplantation. Blood perfusion is a critical event for organ growth by supplying nutrients and oxygen. However, blood perfusion is still lacking in the present organoid culture system. We are developing perfusion culture systems using two approaches; hiPSC-liver buds (LBs) connected with pefusable hiPSC-blood vessel, and the decellularized liver tissue infused with hiPSC-LBs.

From our first approach, we generated the perfusable hiPSC-derived blood vessel using collagen gels, hiPSC-derived vascular smooth muscle cells (SMC), and vascular endothelial cells (EC). Although we clarified that hiPSC-derived blood vessel is histologically similar to the vascular structure of *in vivo* blood vessels, EC-seeded blood vessels did not show angiogenesis, resulting no contact/connection with capillaries within hiPSC-LBs under co-culture. Therefore, we established a new culture protocol to differentiate hiPSC into specific EC lineages that exist around the fetal liver. We demonstrated that hiPSC-derived blood vessel containing those specific ECs had higher angiogenic potentials. Under an optimized culture condition, we successfully induced the connection between the hiPSC-derived blood vessels with the capillaries in hiPSC-LBs. Therefore, we recently tried to establish the organoid perfusion system. Our perfusion system enabled us to culture the hiPSC-LBs by 14 days from co-culture and increase the area of hepatic progenitors/hepatocyte within hiPSC-LBs. Optimizing the perfusion culture system to mimic *in vivo* blood perfusion during organogenesis, we are trying to enhance hiPSC-LB growth more efficiently.

As the second approach, we utilize decellularized liver tissue which retains *in vivo* vascular structures. The decellularization technique has been established to prepare the scaffold for organ reconstitution. Decellularized organs potentially retain the architecture of the original tissue, including the extracellular matrix. A recent report shows how the recellularized liver using hepatocytes could exert liver-specific functions after transplantation. However, the vascular structures within this recellularized liver remain unreconstructed, which might explain limited hepatocyte functions in the recellularized liver. Our current study attempts to generate a more functional recellularized liver by adding oxygen supply into our perfusion culture system of the recellularized liver containing hiPSC-LBs.

5. Generation of 3D cancer tissue using patient-derived pancreatic cancer cells

Kenta Takahashi[1], Shunsuke Tabe[1], Yuya Yamomoto[1], Yasuharu Ueno[1], Hideki Taniguchi[1,2], and Naoki Tanimizu[1]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Pancreatic ductal adenocarcinoma (PDAC) has a poor prognosis, with a 5-year survival rate of about 10% due to delayed diagnosis, drug resistance, and recurrence. Organoid technologies have been applied to investigate the properties of PDACs. To recapitulate tumor microenvironment (TME), which is highly correlated with the poor prognosis of PDAC, we generated multicellular spheroids consisting of primary PDAC cells isolated from Japanese pancreatic cancer patients with hiPSC-mesenchymal cells (MCs) and -endothelial cells (ECs), and then fused them to construct fused pancreatic cancer organoid (FPCO). Our FPCO resembles the tissue structure of clinical tissue including PDAC ductal structures, dense deposition of extracellular matrix components compared to conventional organoids, and heterogeneous cancer-associated fibroblasts (CAFs) namely immunological CAF (iCAF), myofibroblastic (myCAF), and antigen presenting ones (apCAFs). In addition to CAFs, we recently developed FPCO containing tumor associated macrophages (TAMs) to recapitulate immunosuppressive TME. Since the PDAC organoid showed strong resistance to anti-cancer drugs, we apply this new cancer organoid in drug screening and biological analysis to develop effective therapies against PDAC.

6. Space Organogenesis (Development of advanced 3D organ culture system utilizing microgravity environment)

Tomomi Tadokoro[2], Tatsuya Kobayashi[1,2], Yoshiki Kuse[1], Yasuharu Ueno[1], Yoshiharu Kasai[1], Takashi Okumura[1], and Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Microgravity in orbit does not cause subsidence or convection and is considered advantageous in expanding cells in three dimensions. Utilizing this microgravity environment, we aim to develop a novel method for generating human iPSCs-derived liver tissue in collaboration with Japan Aerospace Exploration Agency (JAXA). In particular, we attempt to establish a new technique for generating three-dimensional organs containing large blood vessels. The second space experiment was conducted in March 2024 to uncover the effects of microgravity on cell growth and differentiation of hiPSC-derived liver tissue. After we prepared hiPSC-liver buds (LBs) and

hiPSC-derived blood vessels (BVs) on the earth, we placed those organoids into the culture container and launched them to the International Space Station "KIBO". We confirmed that hiPSC-LBs were successfully assembled around the hiPSC-BVs under microgravity, as how *in silico* simulation suggested. After the perfusion culture of BV-equipped hiPSC-liver organoids (LOs) for a predetermined period in the incubator installed in "KIBO", the samples were transported back to the earth. Adherence of hiPSC-LBs to the BVs and the formation of hiPSC-LOs by fusion of hiPSC-LBs were observed in the post-flight samples. Moreover, endothelial cells formed more vascular networks within the hiPSC-LOs in the post-flight samples than the ground controls. Gene ontology analysis of RNA-seq data revealed that genes related to hepatic functions such as complement and coagulation cascades, fat digestion and absorption, bile secretion, steroid hormone biosynthesis, and retinol metabolism were enriched in the post-flight samples, indicating how the space environment could provide an optimal condition for tissue reconstruction. We hope these results from space experiments will contribute to the subsequent development and understanding of (1) The development of a new technique in human three-dimensional tissue preservation and transportation, which is crucial to the practical use of regenerative medicine products. (2) The establishment of a novel technique for generating human organs equipped with large blood vessels. (3) The development of a new three-dimensional culture device simulating the microgravity environment on earth.

7. Generation of bile duct tubules in hiPSC-liver buds

Ayumu Okumura[1], Erica Carolina[1], Kenji Aoshima[1], Taichi Tsuyuki[1], Minjia Zhong[1], Li Zoushuyang[1], Kazuki Yanagisawa[1], Yoshiki Kuse[1], Takashi Okumura[1], Toshiharu Kasai[1], Tomomi Tadokoro[2], Hideki Taniguchi[1,2], and Naoki Tanimizu[1]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

The biliary system consisting of intrahepatic bile duct (IHBD), extrahepatic bile ducts (EHBDs), and gallbladder, is a crucial tissue structure for maintaining liver homeostasis by providing the excretion route for the bile secreted from hepatocytes. Although various types of liver organoids have been established, the generation of hiPSC-liver organoids associated with the bile drainage system consisting of IHBD and EHBD has not been reported so far.

Previous works demonstrated that IHBD formation depends on JAGGED1(JAG1)-NOTCH signal activated by vascular smooth muscle cells of the portal vein. Therefore, to generate liver organoid containing IHBD-like structures, we developed a new co-culture system in which the hiPSC-liver progenitors are located next to the hiPSC-blood vessel (BV) to recapitulate the fetal portal vein-IHBD tissue interaction. In this condition, hiPSC-liver progenitors differentiated into cholangiocytes and formed duct structures. We named this organoid as blood vessel incorporated liver organoid (BVLO). hiPSC-cholangiocytes in BVLO showed secretory functions in vitro and formed duct structures within the recipient liver after organoid transplantation to immunodeficient mice. Furthermore, when BVLO was transplanted to bile duct ligated mice, it temporally attenuated cholestatic symptoms and extended the survival period of injured mice. Finally, we introduced the artificial BV containing mesenchymal cells derived from JAG1 KO hiPSCs and found that bile duct formation was attenuated. This could be an in vitro model for human Alagille syndrome, a human congenital biliary disease caused by JAG1 mutation for understating underlying mechanisms.

Now, we focus on the establishment of EHBD organoids. We induced EHBD progenitor cells from hiPSC-definitive endoderm cells and generated 3D cystic structures. Currently, we are further optimizing culture condition to confer EHBD characteristics on those cystic structures. Our final goal is to eventually connect these two tubular structures with hiPSC-derived hepatocytes on a dish to generate Hepatobiliary Tubular Organoids (HBTO) that possess a long-term hepatic function *in vitro* as well as *in vivo*.

8. Generation of a novel treatment for pediatric craniofacial deformity using human auricular perichondrium-derived elastic cartilage devices

Takayoshi Oba[1,2], Satoshi Okamoto[1], Yasuharu Ueno[1], Chie Ikezuki[1,2], Takuya Ohkuma[1], Yuriko Yamakawa[1,2], Konka Boku[1] and Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Department of Regenerative Medicine, Graduate School of Medical Science, Yokohama City University

Auto-transplantation of rib cartilage segments is the current most popular treatment for patients with craniofacial deformity. However, major disadvantages such as limited harvestable amounts and post-operative pain of the donor site remain to be solved. To this end, a none-invasive, morphologically stable scaffold-free elastic cartilage implantation treatment for patients with craniofacial deformity is essential.

Our previous study showed the world's first technology of separating and identifying chondroprogenitor cells from the human auricular perichondrium (Kobayashi S et al. PNAS 2011, Patent registration no. 4748222; PCT/JP2008/051327). We succeeded in developing non-scaffold elastic cartilage, which is obtainable in vitro, by using three-dimensional rotation culture and U–bottomed micropatterned plate culture (Enomura M et al. Int J Mol Sci 2020, Oba T et al. J Tissue Eng 2022, Patent application no. 2021-141210; PCT/JP2022/25582). Furthermore, the size and elasticity of the tissue were maintained after craniofacial transplantation in immunodeficient mice, indicating the tissue to be morphologically stable.

Our major goal is to establish a non-invasive novel promising treatment for pediatric patients with nasal deformity by transplanting morphologically stable non-scaffold elastic cartilage. To obtain the clinical POC of the novel treatment, we established the manufacturing system, quality control methods, product specification, evaluation of nonclinical safeness and determination of clinical protocol with Japan Tissue Engineering and JTEC. Currently we are discussing with the Pharmaceuticals and Medical Devices Agency (PMDA) to obtain an approval of the clinical trial which is planned to be carried out next summer.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell and Molecular Medicine
幹細胞分子医学分野

Professor	Atsushi Iwama, M.D., Ph.D.		教　授	博士(医学)	岩　間　厚　志
Senior Assistant Professor	Motohiko Oshima, Ph.D.		講　師	博士(医学)	大　島　基　彦
Assistant Professor	Yaeko Nakajima, Ph.D.		助　教	博士(医学)	中　島　やえ子
Assistant Professor	Masayuki Yamashita, M.D., Ph.D.		助　教	博士(医学)	山　下　真　幸
Project Assistant Professor	Takako Yokomizo, Ph.D.		特任助教	博士(医学)	横　溝　貴　子
Project Assistant Professor	Shuhei Koide, Ph.D.		特任助教	博士(医学)	小　出　周　平
Project Assistant Professor	Ola Rizq, M.D., Ph.D.		特任助教	博士(医学)	オ　ラ　リ　ズ　ク

Stem cells have the remarkable capacity to both self-renew and give rise to many types of more specialized cells in the body, which explains their great therapeutic potential in regenerative medicine. But that's not the only reason stem cells have become such a hotbed of scientific inquiry. These cellular transformers also offer an invaluable research tool for probing the disease mechanisms that underpin cancer, aging and a host of other health problems. Our major interest is to elucidate the mechanisms of self-renewal and multi-lineage differentiation of hematopoietic stem cells (HSCs). We are also interested in how the deregulated HSC functions are associated with aging of our body and the development of age-related hematological malignancies. We approach these issues mainly from the view point of epigenetics.

1. Inhibition of TOPORS ubiquitin ligase augments the efficacy of DNA hypomethylating agents through DNMT1 stabilization

Satoshi Kaito[1,2], Kazumasa Aoyama[1,3], Motohiko Oshima[1], Akiho Tsuchiya[1], Makiko Miyota[1], Masayuki Yamashita[1,4], Shuhei Koide[1], Yaeko Nakajima-Takagi[1], Hiroko Kozuka-Hata[5], Masaaki Oyama[5], Takao Yogo[6], Tomohiro Yabushita[7], Ryoji Ito[8], Masaya Ueno[9], Atsushi Hirao[9], Kaoru Tohyama[10], Chao Li[11], Kimihito Cojin Kawabata[12], Kiyoshi Yamaguchi[13], Yoichi Furukawa[13], Hidetaka Kosako[14], Akihide Yoshimi[2], Susumu Goyama[15], Yasuhito Nannya[16], Seishi Ogawa[17,18], Karl Agger[19], Kristian Helin[19,20], Satoshi Yamazaki[6,21], Haruhiko Koseki[22,23], Noriko Doki[24], Yuka Harada[25], Hironori Harada[24,26], Atsuya Nishiyama[27], Makoto Nakanishi[27], Atsushi Iwama[1,28]

[1]Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan. [2]Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. [3]Division of Immunobiology, Research Institute for Biomedical Sciences, Tokyo University of Science, Chiba 278-0022, Japan [4]Department of Cellular and Molecular Medicine, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan [5]Department of Stem Cell Biology and Medicine, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan [6]Division of Clinical Genome Research, Advanced Clinical Research Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [7]Laboratoty of Cellular and Molecular Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan

[1] Division of Stem Cell and Molecular Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [2] Division of Cancer RNA Research, National Cancer Center Research Institute, Tokyo, Japan. [3] Division of Hygienic Chemistry, Faculty of Pharmacy, Keio University, Tokyo, Japan. [4] Division of Experimental Hematology, Department of Hematology, St. Jude Children's Research Hospital, Memphis TN, USA. [5] Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [6] Division of Cell Regulation, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [7] Division of Cellular Therapy, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [8] Central Institute for Experimental Animals, Kanagawa, Japan. [9] Cancer Research Institute, Kanazawa University, Kanazawa, Japan. [10] Department of Laboratory Medicine, Kawasaki Medical School, Okayama, Japan. [11] Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan. [12] Division of Clinical Precision Research, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [13] Division of Clinical Genome Research, Advanced Clinical Research Center, Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [14] Division of Cell Signaling, Fujii Memorial Institute of Medical Sciences, Institute of Advanced Medical Sciences, Tokushima University, Tokushima, Japan. [15] Division of Molecular Oncology, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan. [16] Division of Hematopoietic Disease Control, Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [17] Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan. [18] Institute for the Advanced Study of Human Biology (WPI-ASHBi), Kyoto University, Kyoto, Japan. [19] BRIC, University of Copenhagen, Denmark. [20] The Institute of Cancer Research (ICR), London, UK. [21] Division of Cell Engineering, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan. [22] Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences, Yokohama, Japan. [23] Department of Molecular and Cellular Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan. [24] Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan. [25] Clinical Research Support Center, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan. [26] Laboratory of Oncology, School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan. [27] Division of Cancer Cell Biology, Institute of Medical Science, University of Tokyo, Tokyo, Japan. [28] Laboratory of Cellular and Molecular Chemistry, Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan.

DNA hypomethylating agents (HMAs) are used for the treatment of myeloid malignancies, although their therapeutic effects have been unsatisfactory. Here we show that CRISPR-Cas9 screening reveals that knockout of topoisomerase 1-binding arginine/serine-rich protein (*TOPORS*), which encodes a ubiquitin/SUMO E3 ligase, augments the efficacy of HMAs on myeloid leukemic cells with little effect on normal hematopoiesis, suggesting that TOPORS is involved in resistance to HMAs. HMAs are incorporated into the DNA and trap DNA methyltransferase-1 (DNMT1) to form DNA-DNMT1 crosslinks, which undergo SUMOylation, followed by proteasomal degradation. Persistent crosslinking is cytotoxic. The TOPORS RING finger domain, which mediates ubiquitination, is responsible for HMA resistance. In *TOPORS* knockout cells, DNMT1 is stabilized by HMA treatment due to inefficient ubiquitination, resulting in the accumulation of unresolved SUMOylated DNMT1. This indicates that TOPORS ubiquitinates SUMOylated DNMT1, thereby promoting the resolution of DNA-DNMT1 crosslinks. Consistently, the ubiquitination inhibitor, TAK-243, and the SUMOylation inhibitor, TAK-981, show synergistic effects with HMAs through DNMT1 stabilization. Our study provides a novel HMA-based therapeutic strategy that interferes with the resolution of DNA-DNMT1 crosslinks.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Transplantation
幹細胞移植分野

Professor	Yasuhito Nannya, M.D., D.M.Sc.		教　授	博士(医学)	南　谷　泰　仁
Project Professor	Satoshi Takahashi, M.D., D.M.Sc.		特任教授	博士(医学)	高　橋　　聡

We are studying the clinical promotion and medical development of hematopoietic stem cell transplantation with a focus on cord blood transplantation. We conducted 23 allogeneic transplantation in 2024 in the Department of Hematology & Oncology. In addition to data on hematopoietic stem cell transplantation, we are analyzing various issues related to clinical transplantation using the Japanese transplant database. Basic studies include the development of efficient in vitro amplification of patient-derived primary cells and preclinical studies on the use of virus-specific CTLs in post-transplant and other immunocompromised patients. Our goal is to make allogeneic transplantation a safer treatment option and extend it to older patients.

1 Levels of C-Reactive Protein and Body Temperature Elevation During Neutropenia Predict Engraftment and Non-Relapse Mortality for Unrelated Single-Unit Cord Blood Transplantation in Adults.

Konuma T1, Monna-Oiwa M1, Kato S1, Andoh S1, Isobe M1, Nannya Y1, Takahashi S2.
1 Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
2 Division of Clinical Precision Research Platform, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Cord blood transplantation (CBT) presents unique challenges related to inflammation during neutropenia, such as mucosal damage, infections, and the potential development of pre-engraftment syndrome or pre-engraftment immune reaction. These factors can contribute to significant inflammation and infection shortly after CBT. However, the effect of severe inflammation during neutropenia, specifically elevated C-reactive protein (CRP) level and body temperature, on post-transplant outcomes after CBT remains unclear. This retrospective study aimed to investigate the association between maximum CRP level, maximum body temperature during neutropenia, and post-transplantation outcomes in adult patients undergoing single-unit CBT. We retrospectively evaluated the impact of maximum CRP level and maximum body temperature during neutropenia on post-transplantation outcomes in adults who underwent single-unit unrelated CBT between 1998 and 2023 at our institution. A total of 336 adult patients were included in this study. The median maximum CRP level before neutrophil recovery was 7.75 mg/dL (interquartile range [IQR], 4.70 to 12.05 mg/dL) at a median of 14 d (IQR, 8 to 16 d). The median maximum body temperature before neutrophil recovery was 39.5°C (IQR, 39.0 to 40.0°C) at a median of 15 d (IQR, 12 to 17 d). In the multivariate analysis, a maximum CRP level≥20 mg/dL was significantly associated with lower neutrophil recovery (hazard ratio [HR], 0.37; 95% confidence interval [CI], 0.23 to 0.59; P < .001), lower platelet recovery (HR, 0.28; 95% CI, 0.16 to 0.48; P < .001), and a higher incidence of veno-occlusive disease/sinusoidal obstruction syndrome (HR, 16.42; 95% CI, 4.11 to 65.54; P < .001), which resulted in higher non-relapse mortality (NRM) (HR, 5.16; 95% CI, 2.62 to 10.15; P < .001) and worse overall survival (HR,

2.81; 95% CI, 1.66 to 4.78; P < .001). Similarly, a maximum body temperature≥40.5°C was significantly associated with lower neutrophil recovery (HR, 0.51; 95% CI, 0.33 to 0.79; P = .002), lower platelet recovery (HR, 0.55; 95% CI, 0.38 to 0.79; P = .001), higher incidence of grades III to IV acute GVHD (HR, 2.93; 95% CI, 1.24 to 6.88; P = .013), and extensive chronic GVHD (HR, 2.47; 95% CI, 1.22 to 4.97; P = .011), which resulted in higher NRM (HR, 3.43; 95% CI, 1.53 to 7.67; P = .002). Maximum CRP level and maximum body temperature during neutropenia were significantly associated with lower hematopoietic recovery and higher NRM following single-unit CBT in adults. Further studies are warranted to explore early intervention strategies aimed at preventing severe inflammation and improving post-transplant outcomes in single-unit CBT.

2 Feasibility and safety of the discontinuation of systemic immunosuppressive treatment after single-unit cord blood transplantation in adults.

Konuma T1, Monna-Oiwa M1, Kato S1, Isobe M1, Nannya Y1, Takahashi S2.
1 Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
2 Division of Clinical Precision Research Platform, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

We retrospectively evaluated the incidence, factors, and clinical outcomes of the discontinuation of immunosuppressive treatment (IST) after single-unit unrelated cord blood transplantation (CBT) in adults receiving cyclosporine-based graft-versus-host disease (GVHD) prophylaxis at our institute. Among the 309 patients who achieved engraftment, 247 were able to discontinue IST with a median follow-up of 121 months for survivors. The cumulative incidence of the discontinuation of IST was 46.2% at 180 days, 72.8% at 2 years, and 79.3% at 5 years post-CBT. In the multivariate analysis, discontinuation of IST after CBT was significantly associated with the requirement for steroid therapy (hazard ratio [HR]: 0.46; P < 0.001) and the recent calendar year of CBT (HR: 1.79; P < 0.001). In the conditional landmark analysis at 180 days, discontinuation of IST was not associated with the development of extensive chronic GVHD (HR: 1.00; P = 0.989), non-relapse mortality (HR: 0.49; P = 0.122), relapse (HR: 1.46; P = 0.388), or overall survival (HR: 1.91; P = 0.065). Our data showed that successful discontinuation of IST is common after single-unit CBT in adults. Discontinuation of IST did not affect subsequent outcomes, suggesting that discontinuation of IST is both feasible and safe in adults undergoing single-unit CBT.

3 Association of individual comorbidities with outcomes in allogeneic hematopoietic cell transplantation from unrelated adult donors versus unrelated cord blood: A study on behalf of the Donor/Source and Transplant Complications Working Groups of the Japanese Society for Transplantation and Cellular Therapy.

Konuma T1, Harada K2, Shinohara A3, Uchida N4, Shingai N5, Ito A6, Ozawa Y7, Tanaka M8, Sawa M9, Onizuka M10, Katayama Y11, Hiramoto N12, Nakano N13, Kimura T14, Kanda Y15, Fukuda T16, Atsuta Y17, Nakasone H18, Kanda J19.
1 Department of Hematology and Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
2 Department of Hematology and Oncology, Tokai University School of Medicine, Isehara, Japan.
3 Department of Hematology, Tokyo Women's Medical University, Tokyo, Japan.
4 Department of Hematology, Toranomon Hospital, Tokyo, Japan.
5 Hematology Division, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan.
6 Department of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan.
7 Department of Hematology, Japanese Red Cross Aichi Medical Center Nagoya Daiichi Hospital, Nagoya, Japan.
8 Department of Hematology, Kanagawa Cancer Center, Yokohama, Japan.
9 Department of Hematology and Oncology, Anjo Kosei Hospital, Anjo, Japan.
10 Department of Hematology, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, Hiroshima, Japan.
11 Department of Hematology, Kobe City Medical Centre General Hospital, Kobe, Japan.
12 Department of Hematology, Imamura General Hospital, Kagoshima, Japan.
13 Preparation Department, Japanese Red Cross Kinki Block Blood Center, Osaka, Japan.
14 Division of Hematology, Jichi Medical University, Shimotsuke, Japan.
15 Division of Hematology, Jichi Medical University Saitama Medical Center, Saitama, Japan.
16 Japanese Data Center for Hematopoietic Cell Transplantation, Nagakute, Japan.
17 Department of Registry Science for Transplant and Cellular Therapy, Aichi Medical University School of Medicine, Nagakute, Japan.
18 Division of Stem Cell Regulation, Center for Molecular Medicine, Jichi Medical University, Shimotsuke, Japan.
19 Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan.

We retrospectively evaluated the effect of 17 individual comorbidities, defined by the hematopoietic cell transplantation (HCT)-specific comorbidity index, on non-relapse mortality (NRM) and overall survival (OS) in 9531 patients aged between 16 and 70 years who underwent their first allogeneic HCT from 8/8 and 7/8 allele-matched unrelated donors (8/8 and 7/8 MUDs) or single-unit unrelated cord blood (UCB) between 2011 and 2020 using data from a Japanese registry database. In the multivariate analysis, infection (adjusted hazard ratio [HR], 1.62, 95% confidence interval [CI], 1.33-1.99 for 8/8 and 7/8 MUDs; adjusted HR, 1.33, 95%CI, 1.12-1.58 for UCB) and moderate/severe hepatic comorbidity (adjusted HR, 1.57, 95%CI, 1.04-2.38 for 8/8 and 7/8 MUDs; adjusted HR, 1.53, 95%CI, 1.09-2.15 for UCB) had a significant impact on NRM in both donor groups. Cardiac comorbidity (adjusted HR, 1.40, 95%CI, 1.08-1.80), mild hepatic comorbidity (adjusted HR, 1.22, 95%CI, 1.01-1.48), rheumatologic comorbidity (adjusted HR, 1.67, 95%CI, 1.11-2.51), renal comorbidity (adjusted HR, 2.44, 95%CI, 1.46-4.09), and severe pulmonary comorbidity (adjusted HR, 1.40, 95%CI, 1.11-1.77) were significantly associated with an increased risk of NRM but only in UCB recipients. Renal comorbidity had the strongest impact on poor OS in both donor groups (adjusted HR, 1.73, 95%CI, 1.10-2.72 for 8/8 and 7/8 MUDs; adjusted HR, 2.24, 95%CI, 1.54-3.24 for UCB). Therefore, unrelated donor selection should be taken into consideration along with the presence of specific comorbidities, such as cardiac, rheumatologic, renal, mild hepatic, and severe pulmonary comorbidities.

4 Development of ex vivo amplification system for patient-derived hematopoietic malignant cells

Chao Li[1, 3], Kimihito C Kawabata[2], Yasuhito Nannya[1, 4], Tomokazu Seki[1], Satoshi Yamazaki[5]

1. Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
2. Division of Clinical Precision Research Platform, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
3. Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan
4. Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
5. Division of Cell Regulation, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

We have developed an optimized serum-free and OP9-free culture system for primary tumor specimens (PTS) from AML patients, focusing on the long-term maintenance and expansion of CD45 dim CD34+ leukemia stem-like cells. By reducing cytokine complexity, incorporating serum substitutes such as Soluplus, and introducing small molecules including Pomalidomide, UM729, and SR-1, we achieved significant improvements in PTS culture efficiency. Our system supports the long-term expansion of leukemia stem-like cells while maintaining their functional properties, as demonstrated by clonogenic assays and CRISPR-Cas9 gene-editing experiments.

This optimized culture system provides a promising alternative to traditional serum- or feeder-dependent methods, offering a robust platform for leukemia research and therapeutic development. The ability to expand PTS *ex vivo* opens new opportunities for studying leukemia stem cell biology, drug resistance mechanisms, and personalized medicine approaches.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Processing
幹細胞プロセシング分野

Professor　Hideki Taniguchi, M.D., Ph.D.

教　授　博士(医学)　　谷　口　英　樹

Stem cells represent a valuable cell source in the field of regenerative medicine. Human induced pluripotent stem cells (hiPSCs) have emerged as a promising tool, being utilized both in basic research and in the development of curative treatments for various diseases. Our focus has been specifically on precise control of the hiPSC differentiation process, thereby developing safe and effective cell replacement therapy for patients suffering from a wide range of currently incurable conditions.

Highly efficient generation of hiPSC derived proliferative hepatic progenitor for disease treatment

Yun-Zhong Nie[1], Yoshihito Hayashi[1], Qing-Lin LI[1], Xiao-Shan Deng[1], Luo Na[1], Yang Li[1], Xia Yang[1], Riana Plummer[1], Tomonori Tsuchida[1], Naoki Tanimizu[1], Yasuharu Ueno[1], Hideki Taniguchi[1,2]

[1] Division of Regenerative Medicine, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

[2] Division of Stem Cell Processing, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo

Although hiPSC holds immense potential for cell replacement therapy for disease treatment, challenges such as cellular heterogeneity and potential tumorigenicity have significantly limited their clinical applications. To address these challenges, we propose a transplantation therapy strategy based on hiPSC-derived proliferative progenitors that effectively mitigates issues of cellular heterogeneity and residual undifferentiated hiPSC. In this study, we developed a protocol that accurately directs hiPSC to differentiate into hepatic progenitors with a purity exceeding 99%. Notably, these hiPSC-derived hepatic progenitors maintained their purity and characteristic properties under optimized culture conditions, ensuring sufficient cell quantities to meet the demands of clinical applications. Long-term transplantation experiments further confirmed the absence of tumor formation risk in these cells. Moreover, we demonstrated that hiPSC-derived hepatic progenitors exhibit robust *in vivo* repopulation capacity, leading to significant improvements in liver diseases. These findings highlight the enhanced safety and flexibility of hiPSC-derived proliferative progenitor-based transplantation therapy for disease treatment. Moving forward, we aim to establish a GMP-grade manufacturing process for the generation and expansion of hiPSC-derived hepatic progenitors, thereby accelerating the clinical translation of hiPSC-based cell replacement therapies.

Center for Stem Cell Biology and Regenerative Medicine

Division of Mammalian Embryology
再生発生学分野

Project Associate Professor Toshihiro Kobayashi, Ph.D. 特任准教授 博士(生命科学) 小 林 俊 寛

Our lab aims to understand mechanisms underlying the cell fate decisions in early mammalian embryos and to apply their principle for future reproductive and regenerative medicine. In particular, we use pluripotent stem cells and early embryos from various mammals, which will enable us to investigate conserved mechanisms among the mammals and to develop novel technology by the use of species-specific features.

1. Induction of primordial germ cell-like cells from rat pluripotent stem cells

Mami Oikawa[1,2], Masumi Hirabayashi[3,4], Toshihiro Kobayashi[1,3]

[1] Division of Mammalian Embryology, Center for Stem Cell Biology and Regenerative Medicine, The Institute of Medical Science, The University of Tokyo, Tokyo, 108-8639, Japan.

[2] Laboratory of Regenerative Medicine, Tokyo University of Pharmacy and Life Sciences, Tokyo, 192-0392, Japan

[3] Center for Genetic Analysis of Behavior, National Institute for Physiological Sciences, Aichi, 444-8787, Japan.

[4] The Graduate University of Advanced Studies, Aichi, 444-8787, Japan.

In vitro induction of primordial germ cell like-cells (PGCLCs) from pluripotent stem cells (PSCs) is a robust method that will contribute to understanding the fundamentals of cell fate decisions, animal breeding, and future reproductive medicine. We develop a stepwise protocol to induce epiblast-like cells and subsequent PGCLCs via the formation of spherical aggregates from rat PSCs. We also develop a protocol to mature these PGCLCs from specified/migratory- to the gonadal stage by aggregation with female gonadal somatic cells. We summarize the detailed protocols above in a book chapter.

2. Transcription factor-mediated germ cell induction in rats

Mami Oikawa[1,2], Hiroki Kojima[1,2], Hisato Kobayashi[5], Kenyu Iwatsuki[1], Hijiri Saito[1], Makoto Sanbo[3], Kazumi Nishioka[3], Tomoyuki Yamaguchi[2], Takuya Yamamoto[6,7,8], Kazuki Kurimoto[5], Masumi Hirabayashi[3,4], Toshihiro Kobayashi[1,3]

[5] Department of Embryology, Nara Medical University; Kashihara, Nara, 634-0813, Japan

[6] Center for iPS Cell Research and Application, Kyoto University; Sakyo-ku, Kyoto, 606-8507, Japan.

[7] Institute for the Advanced Study of Human Biology, Kyoto University; Sakyo-ku, Kyoto, 606-8501, Japan.

[8] Medical-risk Avoidance based on iPS Cells Team, RIKEN Center for Advanced Intelligence Project; Sakyo-ku, Kyoto, 606-8507, Japan.

The specification of primordial germ cells (PGCs) marks a crucial branch point in early embryonic development. Studying the molecular mechanisms underlying this process is key to gaining insights into reproduction and evolution. Here, we identify transcription factors essential for PGC specification in rats using an *in vitro* system to induce PGC-like cells (PG-

CLCs) from pluripotent cells. Overexpression of a key mesodermal factor activating the germ cell program in epiblast-like cells induces functional rat PGCLCs, similar to mice. However, unlike in mice, overexpression of the PGC specifiers alone is not sufficient in rats, additional signals are necessary for PGCLC induction. Through a candidate screen, we identified a transcription factor acting cooperatively with the PGC specifiers. Our study provides insight into the mechanism behind germline segregation in mammals and underscores the importance of using the rat model in addition to mice.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell Aging Medicine
幹細胞加齢医学分野

Professor　Emi K. Nishimura, M.D., Ph.D.

教　授　博士(医学)　　西　村　栄　美

Stem cell systems play fundamental roles in sustaining tissue turnover and homeostasis. Our goal is to understand the mechanisms of tissue aging and cancer development in mammals and to apply that knowledge to develop strategies to resist against tissue/organ aging, cancer development and other relevant diseases associated with aging. We further aim to apply this knowledge to drug discovery and the prevention and treatment of age-associated diseases.

1. Stem cell fate governs hair graying and melanoma development

Yasuaki Mohri[1], Jialiang Nie[1], Hironobu Morinaga[2], Tomoki Kato[2], Takahiro Aoto[2], Takashi Yamanashi[3,4], Daisuke Nanba[1], Hiroyuki Matsumura[1], Sakura Okamoto[2], Kouji Kobiyama[5], Ken J Ishii[5], Masahiro Hayashi[6], Tamio Suzuki[6], Takeshi Namiki[7], Jun Seita[3,4], and Emi K Nishimura[1]

[1] Division of Aging and Regeneration, The Institute of Medical Science, The University ofTokyo, Japan
[2]Department of Stem Cell Biology, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan.
[3]Advanced Data Science Project, RIKEN Information R&D and Strategy Headquarters, Tokyo, Japan.
[4] Center for Integrative Medical Sciences, RIKEN, Kanagawa, Japan.
[5]Division of Vaccine Science, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; International Vaccine Design Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
[6]Department of Dermatology, Faculty of Medicine, Yamagata University, Yamagata, Japan.
[7]Department of Dermatology, Tokyo Medical and Dental University Graduate School and Faculty of Medicine, Tokyo, Japan.

The accumulation of an individual's lifelong environmental exposure, known as the "exposome", has a significant impact on health. Somatic tissues undergo functional decline with age, exhibiting characteristic ageing phenotypes such as hair graying and cancer. However, specific genotoxins and signals driving each phenotype and their underlying cellular mechanisms remain largely unknown. Importantly, DNA damage foci are relatively frequently found in somatic stem cells in the skin during physiological aging. Using a DNA damage inducing model, we previously found that the induction of DNA double strand breaks (DSBs) advances the expression of aging phenotypes including hair graying. To study the fate and dynamics of DNA-damaged stem cells in tissues and the resultant impact in the expression of aging phenotypes, we first focused on the melanocyte lineage and traced the fate of melanocyte stem cells (McSCs) which acquired DNA DSBs and demonstrated that those cells disappear from the niche, causing the loss of mature melanocytes for hair pigmentation.

We studied the impact of DSBs in McSCs and found that McSCs and their niche coordinately determine individual stem cell fate through antagonistic, stress-responsive pathways, depending on the type of genotoxic damage incurred. Chronological stem cell fate-tracking in mice revealed that McSCs undergo

cellular senescence-associated differentiation (seno-differentiation) in response to DSBs and downstream signaling, resulting in their selective depletion and hair graying, effectively acting as a protective mechanism against melanoma development. Conversely, carcinogens can suppress McSC seno-differentiation, even in DSB-harboring cells, by activating KITL (KIT ligand), a master niche factor for McSC self-renewal. Collectively, our data demonstrate that the fate of individual stem cell clones - expansion versus exhaustion - cumulatively and antagonistically governs a degenerative ageing phenotype and/or cancer development through the stem cell niche, depending on the exposome. We are currently testing whether DNA DSBs in other stem cells similarly promotes degenerative tissue aging.

2. Fate tracing of hair follicle stem cells and their seno-differentiation clearance out of the niche

Miranda-Salmeron M[1], Higa M[1], Matsumura H[1], Muroyama Y[1], Kato T[2], Tan L[1], Kawamura Y[1], Nanba D[1], Mohri Y[1], and Nishimura EK[1],.

Hair follicles, mammalian mini-organs that grow hair, miniaturize during aging, leading to hair thinning and loss. In the event of severe genotoxicity such as DNA double-strand breaks (DSBs), stem cells are largely believed to choose between cell death (apoptosis) or irreversible cell cycle arrest (senescence) to prevent further damage to neighboring healthy cells and tissues. Accumulation of these senescent cells across organs has been implicated in disease and aging-related morbidities such as cancer and frailty. However, the exact fate and dynamics of sublethally damaged cells in tissues during aging/chemotherapy and the de- velopment of alopecia and where exactly senescent cells exist in tissues are still largely unknown because of the lack of any single perfect marker of senescent cells. Previous work from our group demonstrated that various stem cells in the skin will aberrantly commit to differentiation in response to DNA damage by abrogating their self-renewal capabilities to discard unfit/stressed/aged stem cells. We are testing the unique hypothesis that the tissue youth is achieved through rapid, dynamic clearance of DNA-damaged cells out of the epithelia as a robust genomic quality control mechanism. We are evaluating a combination of recently devised mouse lines that can induce DSBs in a small number of stem cells to visualize and trace the exact fate, senescent state, and dynamics of those individual cells in epithelial tissue such as the hair follicle. We are in the process of characterizing the identity of those DNA-damaged HFSCs and their fate switching in the HFSC niche that leads to hair follicle miniaturization and hair loss. Taken together, our findings demonstrate a tissue-autonomous mechanism within the hair follicle niche that can effectively discard DNA-damaged cells.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Somatic Stem Cell Research
体性幹細胞研究分野

Associate Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.		准教授	博士(医学)	長 村 登紀子
Project Assistant Professor	Kazuhiro Sudo, Ph.D.		特任助教	博士(医学)	須 藤 和 寛

Somatic stem cells, which are derived from mesoderm, include mesenchymal stromal cells (MSCs), blood cells, and other mesenchymal tissues. MSCs exist in the interstitium of systemic organs; they have self-renewal ability, migrate to the sites of inflammation and tissue damage, and exert anti-inflammatory effects and tissue-repair ability. Among various somatic stem cells, we focused on umbilical cord blood (CB) and umbilical cord-derived MSCs (UC-MSCs) and we explored new immune and regenerative gene/cell therapies using CB and UC-MSCs. Another mission is to manage the IMSUT-HLC cell processing facility (IMSUT-HLC-CPF) for translational research. To achieve the high-quality processing and tests for UC-MSCs therapy, IMSUT-HLC-CPF obtained manufacturing license as the first national University in 2023.

Cord blood and umbilical cord–derived cells for immune-cell therapy and regenerative medicine

Sudo K, Takahashi A, Hori A, Miharu Y, Nagaya N, Mori Y, Ogami K, Nagamura-Inoue T

We explored new immune and regenerative gene/cell therapies using umbilical cord blood (CB) and umbilical cord-derived MSCs (UC-MSCs) with high quality and safety standards. For the high quality and safety standards

In addition, it is our mission to keep the IMSUT-HLC cell processing facility clean and functional to enable high-quality manufacturing for translation al gene and cell therapy. To achieve this mission IMSUT-HLC-CPF obtained manufacturing license for UC-MSCs therapy as the first national University in 2023.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Cell Engineering
幹細胞基盤技術研究分野

Professor Satoshi Yamazaki, Ph.D.

教 授 博士(生命科学) 山 﨑 聡

Our studies focus mainly on investigation of stem cell biology using the hematopoietic stem cell (HSC) as a research model. Recent identification of a variety of stem cell sources including embryonic and somatic (tissue-specific) stem cells has brought about substantial progress in the field of stem cell research.

1. Purging myeloma cell contaminants and simultaneous expansion of peripheral blood-mobilized stem cells

Kantaro Ishitsuka, Hidekazu Nishikii , Takaharu Kimura , Ayano Sugiyama-Finnis , Satoshi Yamazaki

Human hematopoietic stem cells (HSCs) are widely used as a cellular source for hematopoietic stem cell transplantation (HSCT) in the clinical treatment of hematological malignancies. After transplantation therapy, delays in hematopoietic recovery due to insufficient donor-derived HSCs can lead to increased risks of life-threatening infections and bleeding. Our previous studies developed an efficient ex vivo expansion culture medium (3a medium) for umbilical cord blood-derived HSCs (CBSCs), offering a potential solution to this problem. Nevertheless, the broader applicability of our culture method to alternative cell sources and, of greater significance, its efficacy in eliminating potentially disease-associated contaminated tumor cells, especially in autologous transplantation, raise critical clinical questions. In this study, we modified the 3a medium by incorporating UM729 to replace UM171, adding FMS-like tyrosine kinase 3 (Flt3) ligand, and adjusting the concentrations of butyzamide, 740Y-P, polyvinyl caprolactam-polyvinyl acetate-polyethylene glycol graft copolymer (PCL-PVAc-PEG, Soluplus) to create the modified-3a medium. This sophistication allowed the efficient expansion of not only CBSCs but also peripheral blood-mobilized HSCs (PBSCs). Additionally, we successfully removed contaminated myeloma cells by adding bortezomib and tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) at appropriate concentrations, although we maintained HSCs through the addition of lenalidomide. Our research findings present the potential for widespread clinical application of the modified-3a medium and suggest a safe ex vivo culture technique for expanding human HSCs within peripheral blood-derived donor grafts used for autologous HSCT.

2. Activated mesenchymal stem/stromal cells promote myeloid cell differentiation via CCL2/CCR2 signaling

Satoshi Yamazaki, Yo Mabuchi, Takaharu Kimura, Eriko Grace Suto, Daisuke Hisamatsu, Yuna Naraoka, Ayako Kondo, Yuzuki Azuma, Riko Kikuchi, Hidekazu Nishikii, Soji Morishita, Marito Araki, Norio Komatsu, Chihiro Akazawa

Myeloid cells, which originate from hematopoietic stem/progenitor cells (HSPCs), play a crucial role in mitigating infections. This study aimed to explore the impact of mesenchymal stem/stromal cells (MSCs) on

the differentiation of HSPCs and progenitors through the C-C motif chemokine CCL2/CCR2 signaling pathway. Murine MSCs, identified as PDGFRα^{+}Sca-1^{+} cells (PαS cells), were found to secrete CCL2, particularly in response to lipopolysaccharide stimulation. MSC-secreted CCL2 promoted the differentiation of granulocyte/macrophage progenitors into the myeloid lineage. MSC-derived CCL2 plays an important role in the early phase of myeloid cell differentiation in vivo. Single-cell RNA sequencing analysis confirmed that CCL2-mediated cell fate determination was also observed in human bone marrow cells. These findings provide valuable insights for investigating the in vivo effects of MSC transplantation.

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Center for Stem Cell Biology and Regenerative Medicine

Division of Stem Cell and Genome Biology
幹細胞ゲノム生物学分野

Associate Professor　Ayana Kon, M.D., Ph.D.

准教授　博士（医学）　昆　　彩　奈

Hematologic malignancies result from genomic abnormalities in hematopoietic stem and progenitor cells, leading to genetically diverse populations through clonal selection. Advances in next-generation sequencing have expanded our understanding of cancer mutations, but many mechanisms remain elusive. Our lab focuses on identifying unknown genetic abnormalities and molecular mechanisms in hematologic malignancies. Using patient samples and disease models, we combine molecular biology with data science to uncover the biology of hematological stem cells and cancers.

1. Functional analysis of germline and somatic *DDX41* Mutations in the pathogenesis of myeloid malignancies

Ayana Kon[1,2], Masahiro M Nakagawa[3], Keisuke Kataoka[4,5], Hideki Makishima[6], Manabu Nakayama[7], Haruhiko Koseki[8], Yasuhito Nannya[9], Seishi Ogawa[3]

1) Division of Hematology and Tumor Biology, Institute of Medical Science, The University of Tokyo
2) Division of Stem Cell and Genome Biology, Institute of Medical Science, The University of Tokyo
3) Department of Pathology and Tumor Biology, Graduate School of Medicine, Kyoto University
4) Division of Molecular Oncology, National Cancer Center Japan Research Institute
5) Department of Hematology, Keio University School of Medicine
6) Department of Hematology, Shinsyu University
7) Chromosome Engineering Team, Department of Technology Development, Kazusa DNA Research Institute
8) Laboratory for Developmental Genetics, RIKEN Center for Integrative Medical Sciences
9) Department of Hematology/Oncology, Institute of Medical Science, The University of Tokyo

DDX41 is a newly identified leukemia predisposition gene encoding an RNA helicase, whose germline mutations are tightly associated with late-onset myeloid malignancies. Importantly, germline *DDX41* mutations were also found in as many as ~8 % of sporadic cases with high-risk MDS, conferring the largest germline risk for myeloid malignancies. In typical cases, a germline loss-of-function allele is compounded by a somatic missense mutation affecting the helicase domain in the remaining allele (p.R525H). However, the molecular mechanisms by which *DDX41* mutations lead to myeloid neoplasms have not been fully elucidated.

To clarify the role of these distinct *DDX41* alleles, we generated mice models carrying either or both of conditional/constitutive *Ddx41* knock-out (KO) and conditional R525H knock-in (KI) alleles. By crossing these mice and further breeding with *Rosa26-CreERT2* transgenic mice, we engineered mice that were wild-type for *Ddx41* ($Ddx41^{+/+}$), heterozygous *Ddx41* KO ($Ddx41^{+/-}$), homozygous *Ddx41* KO ($Ddx41^{-/-}$), heterozygous for the *Ddx41* R525H mutation ($Ddx41^{R525H/+}$), or hemizygous for the *Ddx41* R525H mutation ($Ddx41^{R525H/-}$), in which expression of the mutant allele was induced by tamoxifen administration.

First, we assessed cell intrinsic effects of these *Ddx41* alleles, using noncompetitive transplantation experiments. Shortly after tamoxifen administration, most of the recipient mice that were transplanted with BM from $Ddx41^{-/-}$ or $Ddx41^{R525H/-}$ mice died within a month after *CreERT2* induction due to severe BM

failure (BMF), which was not observed in mice transplanted with BM from *Ddx41*$^{+/+}$, *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ mice. By contrast, the mice transplanted with *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ BM showed significantly reduced WBC counts and anemia in long-term observation in both primary and serial transplantations. Some of the *Ddx41*$^{+/-}$ or *Ddx41*$^{R525H/+}$ BM-transplanted mice exhibited MDS-like phenotypes, showing ineffective hematopoiesis with evidence of erythroid dysplasia.

Transcriptome analysis revealed that stem cells derived from *Ddx41*$^{R525H/-}$ BM-transplanted mice exhibited a significant upregulation of genes involved in innate immunity, including interferon stimulated genes, compared with stem cells derived from *Ddx41*$^{+/+}$ BM-transplanted mice. In addition, ribosomal genes were significantly deregulated in stem cells from *Ddx41*$^{-/-}$ and *Ddx41*$^{R525H/-}$ BM-transplanted mice, which could result in abnormal ribosome biogenesis and protein synthesis in *Ddx41* mutant cells.

Our results revealed that monoallelic *Ddx41* loss-of function led to age-dependent impaired hematopoiesis, 1while biallelic loss-of function and R525 alleles showed a compromised function of hematopoiesis, where activated innate immunity and impaired ribosome functions may play important roles.

Publication

1.昆 彩奈, 骨髄異形成症候群における動物モデルを用いた病態解明 (Recent advances in experimental animal models of myelodysplastic syndromes) 月刊血液内科（科学評論社）, 2024;89(1):8-13. 2024年7月発行

Center for Stem Cell Biology and Regenerative Medicine

FACS Core Laboratory
FACS コアラボラトリー

Professor　Atsushi Iwama, M.D., Ph.D.

教　授　博士(医学)　　岩　間　厚　志

The FACS Core Laboratory provides high quality, cost-effective and state-of-the-art flow cytometry (FCM) services for internal and external researchers. We offer assistance in the following areas: (1) initial project planning (2) antibody panel design and optimization (3) instrument operation and maintenance (4) data analysis.

Instruments at the FACS Core Laboratory

For cell sorting, the FACS Core is equipped with four Cell sorters (SORPAria and 2 AriaIII from BD Biosciences and CytoFLEX SRT from Beckman Coulter. For cell analysis, the FACS Core Laboratory is equipped with four benchtop analyzers (Verse and CantoII from BD Biosciences and CytoFLEX LX and CytoFLEX S from Beckman Coulter).

FCM usage performance in 2024

FCM analysis and sorting is performed either by the FACS Core staff or by trained users. There were about 3,000 cases of FCM use in 2024.

Seminar and Training

The FACS Core provided training and technical seminars about the theory and practical use of the FCM technology to students, fellows, and principal investigators at IMSUT.

International Research Center for Infectious Diseases

Department of Special Pathogens
高病原性感染症系

Professor	Kei Sato, Ph.D.	教　授	博士(医学)	佐　藤　　佳
Visiting Professor	Masaki Imai, D.V.M., Ph.D.	客員教授	博士(獣医学)	今　井　正　樹
Visiting Professor	Seiya Yamayoshi, D.V.M., Ph.D.	客員教授	博士(医学)	山　吉　誠　也
Associate Professor	Takeshi Ichinohe, Ph.D.	准教授	博士(工学)	一　戸　猛　志
Associate Professor	Jumpei Ito, Ph.D., D.V.M.	准教授	博士(理学)	伊　東　潤　平

The aim of this laboratory is to launch an interdisciplinary research platform to comprehensively understand the behavior of viruses from macroscale to microscale. COVID pandemic alarmed the importance of understanding viral transmissibility and spreading pathway. These knowledges are brought from epidemiology and public health (science at macroscale). Viral surveillance, molecular phylogenetic and bioinformatics provide information of the variant currently spreading (science at macroscale). "Science at mesoscale", the use of animal models and cell cultures, performing experiments, and assessing clinical data, provide the knowledge of viral pathogenicity, features and drug efficacy. When certain variants that are resistant to antivirals or vaccines emerged, the molecular mechanisms of actions should be understood. For that, the understanding based on structural biology is essential (science at micro scale). Our study will launch the platform to perform multiscale investigation of viruses.

1. Emergence of SARS-CoV-2 Variant JN.1 Raises Concerns with Increased Transmissibility and Immune Evasion

Yu Kaku [1], Kaho Okumura [1,2], Miguel Padilla-Blanco [3,7], Yusuke Kosugi [1,4], Keiya Uriu [1,4], Alfredo A Hinay Jr [1], Luo Chen [1,5], Arnon Plianchaisuk [1], Kouji Kobiyama [6,9], Ken J Ishii [6,9]; Genotype to Phenotype Japan (G2P-Japan) Consortium; Jiri Zahradnik [3], Jumpei Ito [1,4,8], Kei Sato [1,4,5,8-11]:

[1]Division of Systems Virology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Japan; [2]Faculty of Liberal Arts, Sophia University, Japan; [3]First Medical Faculty at Biocev, Charles University, Czechia; [4]Graduate School of Medicine, The Institute of Medical Science, The University of Tokyo, Japan; [5]Graduate School of Frontier Sciences, The University of Tokyo, Japan; [6]Division of Vaccine Science, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Japan; [7]Departamento de Farmacia, Facultad de Ciencias de la Salud, Universidad Cardenal Herrera-CEU, CEU Universities, Spain; [8]International Research Center for Infectious Diseases, The Institute of Medical Science, The University of Tokyo, Japan; [9]International Vaccine Design Center, The Institute of Medical Science, The University of Tokyo, Japan; [10]Collaboration Unit for Infection, Joint Research Center for Human Retrovirus infection, Kumamoto University, Japan; [11]Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Agency, Japan.

Over time, the SARS-CoV-2 variant BA.2.86 underwent alterations, giving rise to a new variant named JN.1 (BA.2.86.1.1) by the end of 2023. JN.1 is characterized by a specific mutation in its spike pro-

tein—Leu455Ser—along with mutations in other parts of the virus. This mutation, similar to Leu455Phe previously identified in variant like HK.3, has been associated with increased transmissibility and the ability to escape immune responses. Due to its distinctive mutation profile, indicating a high potential for immune evasion and transmissibility, the study of the virological properties of JN.1 has become imperative.

By the end of November 2023, JN.1 had already surpassed HK.3 in both France and Spain in terms of the reproductive number, marking a significant shift in the landscape of SARS-CoV-2 variants.

Of concern to public health is that JN.1 not only spreads easily, but also seems to resist immunity. Initial experiments using the blood of rodents infected or vaccinated against BA.2.86 showed that those rodents demonstrated an effective neutralization of both BA.2.86 and JN.1, which is called a cross-reactive immune response. However, on comparing breakthrough infections in people where the virus overcomes immunity, JN.1 proved more challenging to neutralize than BA.2.86. Particularly notable was the finding that JN.1 strongly resisted the XBB.1.5 vaccine, making it one of the most immune-evading variants discovered so far.

Our findings will help people understand the risk of the SARS-CoV-2 JN.1 variant including its potential to cause epidemic surges around the world.

Publications

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International Research Center for Infectious Diseases

Department of Infectious Disease Control
感染制御系

Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	教　授	博士(獣医学)	川　口　　寧
Associate Professor	Akihisa Kato, Ph.D.	准教授	博士(医学)	加　藤　哲　久
Assistant Professor	Naoto Koyanagi, Ph.D.	助　教	博士(生命科学)	小　柳　直　人
Assistant Professor	Yuhei Maruzuru, Ph.D.	助　教	博士(生命科学)	丸　鶴　雄　平

Our special interest is focused upon searching for effective methods to protect or control viral infection by using accumulated knowledge based on molecular pathogenicity, and developing novel anti-viral drugs and attenuated strains for novel vaccines. The works have been conducted by close collaboration with Division of Molecular Virology, Department of Microbiology and Immunology.

1. Impact of the Interaction between Herpes Simplex Virus 1 ICP22 and FACT on Viral Gene Expression and Pathogenesis.

Shaocong Liu, Yuhei Maruzuru, Kosuke Takeshima, Naoto Koyanagi, Akihisa Kato, and Yasushi Kawaguchi

Facilitates chromatin transcription (FACT) interacts with nucleosomes to promote gene transcription by regulating the dissociation and reassembly of nucleosomes downstream and upstream of RNA polymerase II (Pol II). A previous study reported that herpes simplex virus 1 (HSV-1) regulatory protein ICP22 interacted with FACT and was required for its recruitment to the viral DNA genome in HSV-1 infected cells. However, the biological importance of interactions between ICP22 and FACT in relation to HSV-1 infection is unclear. Here, we mapped the minimal domain of ICP22 required for its efficient interaction with FACT to a cluster of five basic amino acids in ICP22. A recombinant virus harboring alanine substitutions in this identified cluster led to the decreased accumulation of viral mRNAs from UL54, UL38, and UL44 genes, reduced Pol II occupancy of these genes in MRC-5 cells, and impaired HSV-1 virulence in mice following ocular or intracranial infection. Furthermore, the treatment of mice infected with wild-type HSV-1 with CBL0137, a FACT inhibitor currently being investigated in clinical trials, significantly improved the survival rate of mice. These results suggested that the interaction between ICP22 and FACT was required for efficient HSV-1 gene expression and pathogenicity. Therefore, FACT might be a potential therapeutic target for HSV-1 infection.

2. Identification of a novel neurovirulence factor encoded by the cryptic orphan gene UL31.6 of herpes simplex virus 1

Akihisa Kato, Ryoji Iwasaki, Kousuke Takeshima, Yuhei Maruzuru, Naoto Koyanagi, Tohru Natsume[1], Hideo Kusano[1,2], Shungo Adachi[1,2], Shuichi Kawano[3], and Yasushi Kawaguchi. [1]Molecular Profiling Research Center for Drug Discovery (molprof), National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, [2]Department of Proteomics, National Cancer Center Research institute, Tokyo, [3]Faculty of Mathematics, Kyushu University, Fukuoka

Although the herpes simplex virus type 1 (HSV-1)

genome was thought to contain approximately 80 different protein coding sequences (CDSs), recent multi-omics analyses reported HSV-1 encodes more than 200 potential CDSs. However, few of the newly identified CDSs were confirmed to be expressed at the peptide or protein level in HSV-1-infected cells. Furthermore, the impact of the proteins they encode on HSV-1 infection is largely unknown. This study focused on a newly identified CDS, UL31.6. Re-analyzation of our previous chemical proteomics data verified that UL31.6 was expressed at the peptide level in HSV-1-infected cells. Antisera raised against a viral protein encoded by UL31.6 (pUL31.6) reacted with a protein with an approximate molecular mass of 37 kDa in lysates of Vero cells infected with each of three HSV-1 strains. pUL31.6 was efficiently dissociated from virions in high salt solution. A UL31.6-null mutation had a minimal effect on HSV-1 gene expression, replication, cell-to-cell spread, and morphogenesis in Vero cells; in contrast, it significantly reduced HSV-1 cell-to-cell spread in three neural cells but not in four non-neural cells including Vero cells. The UL31.6-null mutation also significantly reduced the mortality and viral replication in the brains of mice after intracranial infection, but had minimal effects on pathogenic manifestations in and around the eyes, and viral replication detected in the tear films of mice after ocular infection. These results indicated that pUL31.6 was a tegument protein and specifically acted as a neurovirulence factor by potentially promoting viral transmission between neuronal cells in the central nervous system.

3. MYBBP1A is required for efficient replication and gene expression of herpes simplex virus 1

Moeka Nobe, Yuhei Maruzuru, Kosuke Takeshima, Naoto Koyanagi, Akihisa Kato and Yasushi Kawaguchi

More than 100 different herpes simplex virus 1 (HSV-1) genes belong to three major classes, and their expression is coordinately regulated and sequentially ordered in a cascade. This complex HSV-1 gene expression is thought to be regulated by various viral and host cellular proteins. A host cellular protein, Myb-binding protein 1A (MYBBP1A), has been reported to be associated with HSV-1 viral genomes in conjunction with viral and cellular proteins critical for DNA replication, repair, and transcription within infected cells. However, the role(s) of MYBBP1A in HSV-1 infections remains unclear. In this study, we examined the effects of MYBBP1A depletion on HSV-1 infection and found that MYBBP1A depletion significantly reduced HSV-1 replication, as well as the accumulation of several viral proteins. These results suggest that MYBBP1A is an important host cellular factor that contributes to HSV-1 replication, plausibly by promoting viral gene expression.

Publications

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International Research Center for Infectious Diseases

Department of Infectious Disease Control Division of Viral Infection

感染制御系・ウイルス学分野

Associate Professor　Takeshi Ichinohe, Ph.D.

准教授　博士（工学）　一　戸　猛　志

We focus on understanding how viruses are recognized by NLRP3 inflammasome and how the innate recognition receptor controls antigen-specific adaptive immune responses. We study immune responses to influenza viruses in the lung. Our recent focus also includes the study of how microbiota regulates adaptive immune responses to these pathogens. Our ultimate goal is to utilize the knowledge we gain through these areas of research in the rational design of effective vaccines for the prevention of infectious diseases.

1. TNF-α exacerbates SARS-CoV-2 infection by stimulating CXCL1 production from macrophages.

Kobayashi M, Kobayashi N, Deguchi K, Omori S, Nagai M, Fukui R, Song I, Fukuda S, Miyake K, and Ichinohe T.

Since most genetically modified mice are C57BL/6 background, a mouse-adapted SARS-CoV-2 that causes lethal infection in young C57BL/6 mice is useful for studying innate immune protection against SARS-CoV-2 infection. Here, we established two mouse-adapted SARS-CoV-2, ancestral and Delta variants, by serial passaging 80 times in C57BL/6 mice. Although young C57BL/6 mice were resistant to infection with the mouse-adapted ancestral SARS-CoV-2, the mouse-adapted SARS-CoV-2 Delta variant caused lethal infection in young C57BL/6 mice. In contrast, MyD88 and IFNAR1 KO mice exhibited resistance to lethal infection with the mouse-adapted SARS-CoV-2 Delta variant. Treatment with recombinant IFN-α/β at the time of infection protected mice from lethal infection with the mouse-adapted SARS-CoV-2 Delta variant, but intranasal administration of recombinant IFN-α/β at 2 days post infection exacerbated the disease severity following the mouse-adapted ancestral SARS-CoV-2 infection. Moreover, we showed that TNF-α amplified by type I IFN signals exacerbated the SARS-CoV-2 infection by stimulating CXCL1 production from macrophages and neutrophil recruitment into the lung tissue. Finally, we showed that intravenous administration to mice or hamsters with TNF protease inhibitor 2 alleviated the severity of SARS-CoV-2 and influenza virus infection. Our results uncover an unexpected mechanism by which type I interferon-mediated TNF-α signaling exacerbates the disease severity and will aid in the development of novel therapeutic strategies to treat respiratory virus infection and associated diseases such as influenza and COVID-19.

2. Prebiotic inulin ameliorates SARS-CoV-2 infection in hamsters by modulating the gut microbiome.

Song I, Yang J, Saito M, Hartanto T, Nakayama Y, Ichinohe T, Fukuda S.

Current treatment options for COVID-19 are limited, with many antivirals and immunomodulators restricted to the most severe cases and preventative care

limited to vaccination. As the SARS-CoV-2 virus and its increasing variants threaten to become a permanent fixture of our lives, this new reality necessitates the development of cost-effective and accessible treatment options for COVID-19. Studies have shown that there are correlations between the gut microbiome and severity of COVID-19, especially with regards to production of physiologically beneficial short-chain fatty acids (SCFAs) by gut microbes. In this study, we used a Syrian hamster model to study how dietary consumption of the prebiotic inulin affected morbidity and mortality resulting from SARS-CoV-2 infection. After two weeks of observation, we discovered that inulin supplementation attenuated morbid weight loss and increased survival rate in hamster subjects. An analysis of microbiome community structure showed significant alterations in 15 genera. Notably, there were also small increases in fecal DCA and a significant increase in serum DCA, perhaps highlighting a role for this secondary bile acid in conferring protection against SARS-CoV-2. In light of these results, inulin and other prebiotics are promising targets for future investigation as preventative treatment options for COVID-19.

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Kobayashi M, Kobayashi N, Deguchi K, Omori S, Nagai M, Fukui R, Song I, Fukuda S, Miyake K, Ichinohe T. TNF-α exacerbates SARS-CoV-2 infection by stimulating CXCL1 production from macrophages. ***PLoS Pathog.*** 20(12):e1012776. 2024

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International Vaccine Design Center

Division of Systems Immunology (Human Immune-Profiling Team)

ヒト免疫プロファイリング系・数理免疫学分野

Professor Kei Sato, Ph.D.

教 授 博士(医学) 佐 藤 佳

The aim of this laboratory is to launch an interdisciplinary research network to "quantitatively" understand the behaviors of pathogens and the immune reaction against pathogen infection. Our study will connect microbiology and immunology, which will lead to the development of novel vaccines in the future.

1. Understanding the evolution of SARS-CoV-2

Yu Kaku, Arnon Plianchaisuk, Ziyi Guo, Alfredo Amolong Hinay, Jr., Kaoru Usui, Wilaiporn Saikruang, Spyridon Lytras, Keiya Uriu, Shusuke Kawakubo, Luca Nishimura, Yusuke Kosugi, Shigeru Fujita, Luo Chen, Jarel Elgin Tolentino, Lin Pan, Wenye Li, Maximilian Stanley Yo, Yukun Zhu, Yueying Zhang, Ruojin Tian, Mai Suganami, Adam Patrick Strange, Naomi Ohsumi, Shiho Tanaka, Eiko Ogawa, Mika Chiba, Kyoko Yasuda, Keiko Iida, Kaho Okumura, Tsuki Fukuda, Tamaki Yoshihara, Keiko Koizumi, Hiroaki Unno, Jumpei Ito, Kei Sato.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a causative agent of coronavirus disease 2019 (COVID-19). SARS-CoV-2 emerged at the end of 2019 and has spread all over the world. Since then, more than 770 million people have been infected with this virus and more than 7 million people have died of COVID-19, meaning that COVID-19 is ongoing pandemic and a most urgent and crucial problem in the current human society. To proceed and accelerate COVID-19-releated researches in Japan, we launched a consortium, called "The Genotype to Phenotype Japan (G2P-Japan) Consortium" in January 2021. As of December 2024, more than 10 principal investigators participate in this consortium and proceed fruitful collaboration. We aim to elucidate the virological characteristics of the SARS-CoV-2 variants continuously emerging in the world.

Publications

Yu Kaku, Kaho Okumura, Miguel Padilla-Blanco, Yusuke Kosugi, Keiya Uriu, Alfredo A. Hinay, Luo Chen, Arnon Plianchaisuk, Kouji Kobiyama, Ken J. Ishii, Genotype to Phenotype Japan (G2P-Japan) Consortium, Jiri Zahradnik, Jumpei Ito, Kei Sato. Virological Characteristics of the SARS-CoV-2 JN.1 Variant. **Lancet Infectious Diseases** 24(2):e82 (2024).

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International Vaccine Design Center

Division of Human Immunology (Human Immne-Profiling Team)

ヒト免疫プロファイリング系・ヒト免疫学分野

Professor Ken Ishii, M.D., Ph.D.

教 授 博士(医学) 石 井 健

The laboratory is consisted of two groups working on vaccine and immunometabolism lead by Ken Ishii and Noriko Toyama-Sorimachi, respectively to conduct novel research on vaccine immunology and immunometabolism towards human immune-profiling to understand why and how our immune system responds to infection and other immunological disorder.

1. Supporting research in response to Public Health Emergency Declaration for Mpox

Based on the sequence information of Mpox clade I, we requested support from AMED BINDS for the preparation of research tools including viral proteins and antibodies. Prof. Junichi Takagi of the Institute for Protein Research, Osaka University, a BINDS core researcher, was able to rapidly express and purify several recombinant Mpox membrane proteins and provided them to us. Using these recombinant proteins, we confirmed that antibodies against Mpox could be induced in mice immunized with LC16m8, a smallpox vaccine strain owned by Japan, and also in human serum vaccinated with LC16m8. This provided a POC on the efficacy of the LC16m8 smallpox vaccine strain against Mpox, helping to accelerate the supply of this smallpox vaccine to African countries.

In addition, as a research tool for Mpox, we asked Dr. Yukinari Kato of Tohoku University, a BINDS core researcher, to develop monoclonal antibodies for virus detection and observed a significant increase in antibody titer after immunization. Multiple clones of the Mpox monoclonal antibodies are now being established.

2. Out-licensed to a global mega-pharmaceutical company and signed a research collaboration agreement for a lead compound for a novel therapeutic target in autoimmune disease

We successfully out-licensed a lead compound for a novel therapeutic target for autoimmune diseases that we independently identified to a global mega-pharmaceutical company. We also entered into a collaboration agreement.

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International Vaccine Design Center

Division of Infection Immunology (Human Immune-Profiling Team)

ヒト免疫プロファイリング系・感染免疫学分野

Professor	Cevayir Coban, M.D., Ph.D. (Clinical Microbiology)	教　授	博士(医学)(臨床微生物学位)	チョバン　ジェヴァイア
Associate Professor	Niloufar Kavian-Tessler, Pharm.D., Ph.D.	准教授	博士(医学)	カビアン･テスラー　ニルファー
Visiting Professor	Anavaj Sakuntabhai, M.D., Ph.D.	客員教授	博士(医学)	サクンタバイ　アナヴァジ
Assistant Professor	Jalal Alshaweesh, Ph.D.	助　教	博士(医学)	アルシャウィシュ　ジャラル
Assistant Professor	Michelle S.J. Lee, Ph.D.	特任助教	博士(医学)	リー　ミシェル

As part of the International Vaccine Design Center at IMSUT, our team investigate how pathogens interact with human immune system. Initially specializing in malaria immunology, we have expanded our research to include emerging infectious diseases like dengue and COVID-19, respiratory viral diseases, and neglected parasitic infections such as leishmaniasis. By elucidating the virulence factors of these pathogens, our goal is to advance vaccine and drug development.

1. Elucidation of host-pathogen interactions

Chronic bone loss is an under-recognized complication of malaria, with mechanisms still not fully understood. Persistent accumulation of *Plasmodium* products in the bone marrow triggers chronic inflammation in osteoblast (OB) and osteoclast (OC) precursors via MyD88, an adaptor molecule for inflammatory signals (Lee et al., *Science Immunology*, 2017). Following our previous studies, we recently investigated the cell-intrinsic role of MyD88 in bone metabolism under physiological and malaria-infected conditions using Lox-Cre-based depletion of MyD88 in OB or OC lineages. We found that mice lacking MyD88 in maturing OBs exhibited significant trabecular bone loss during *Plasmodium yoelii* infection, comparable to controls. In contrast, mice with MyD88 deficiency in OC precursors showed less bone loss during malaria, indicating that inflammatory mediators are predominantly regulated by MyD88 in the OC lineage. However, under normal conditions, MyD88 depletion in OBs led to reduced bone mass and formation rates due to lower systemic and local levels of insulin-like growth factor-1, crucial for OB differentiation. These results demonstrate MyD88's dual role: as indispensable for OB differentiation and bone formation under normal conditions and as a partial mediator of malaria-induced inflammatory bone pathology in the OC lineage. These findings could inform therapeutic strategies for bone pathologies, especially in malaria-endemic regions and beyond in rheumatological diseases ***(Alshaweesh et al., International Immunology, 2024)***.

Bone marrow (BM) is essential for hematopoiesis and immune cell generation, governed by signals from stromal and hematopoietic cells. Acute malaria alters the BM microenvironment, particularly the CXCL12-abundant reticular (CAR) cell population critical for hematopoietic stem cell (HSC) niches. We recently identified a significant reduction in CXCL12 and interleukin-7 signals during acute malaria, leading to the depletion of common lymphoid progenitors, B cell progenitors, and mature B cells, including plasma cells. Particularly, IFNγ upregulated Sca1 ex-

pression on CAR cells but was not responsible for the CAR cell and B cell population decline. A simultaneous increase in HSCs and multipotent progenitors, along with myelopoiesis and erythropoiesis, indicated a bias in multipotent progenitor differentiation during infection.

These findings emhasize malaria's capacity to modulate host immunity by disrupting the BM lymphopoietic niche, with implications for host-pathogen interactions and immune recovery ***(Lee et al., International Immunology, 2024).***

Leishmaniasis, driven by human migration and environmental changes, is increasingly reported in non-endemic regions like Türkiye and Europe. Rising cases of cutaneous leishmaniasis (CL), particularly those caused by atypical *Leishmania infantum*, present diagnostic challenges. A retrospective study of 12 CL cases in Türkiye (2013–2022) revealed that only 58.3% of cases included CL in the initial clinical pre-diagnosis, while 41.7% were misdiagnosed, often as skin tumors. Misdiagnosed cases led to invasive procedures such as wide surgical excision. Histopathological examinations revealed chronic or mixed inflammation rich in histiocytes. Molecular diagnostics identified *L. infantum* in 10 cases and *L. major* in two. These findings show the need for increased clinical awareness and molecular diagnostics to prevent misdiagnosis and unnecessary interventions, especially in re-emerging non-endemic regions ***(Ekemen et al., Frontiers in Medicine, 2024).***

Taken together, these studies collectively advance our understanding of the intersection between infectious diseases and host pathophysiology, from malaria-induced bone remodeling and immune niche disruption to diagnostic challenges posed by re-emerging cutaneous leishmaniasis.

2. Adjuvant discovery and development platform

Stimulator of interferon genes (STING) is one of the key molecules at the intersection of various cytosolic nucleic acid-sensing pathways, including cyclic GMP-AMP synthase (cGAS), DEAD-box helicase family, and interferon gamma inducible protein. DMXAA is a mouse-selective stimulator of interferon gene (STING) agonist exerting STING-dependent anti-tumor activity. Although DMXAA cannot fully activate human STING, DMXAA reached phase III in lung cancer clinical trials. How DMXAA is effective against human lung cancer is completely unknown. Here, we show that DMXAA is a partial STING agonist interfering with agonistic STING activation, which may explain its partial anti-tumor effect observed in humans, as STING was reported to be pro-tumorigenic for lung cancer cells with low antigenicity. Furthermore, we developed a DMXAA derivative—3-hydroxy-5-(4-hydroxybenzyl)-4-methyl-9H-xanthen-9-one (HHMX)—that can potently antagonize STING-mediated immune responses both in humans and mice. Notably, HHMX suppressed aberrant responses induced by STING gain-of-function mutations causing STING-associated vasculopathy with onset in infancy (SAVI) in in vitro experiments. Furthermore, HHMX treatment suppressed aberrant STING pathway activity in peripheral blood mononuclear cells from SAVI patients. Lastly, HHMX showed a potent therapeutic effect in SAVI mouse model by mitigating disease progression. Thus, HHMX offers therapeutic potential for STING-associated autoinflammatory diseases ***(Temizoz et al., Frontiers Immunology, 2024).***

3. Infection and beyond

Our previous research demonstrated that Lipocalin 2 (LCN2), also known as siderocalin or neutrophil gelatinase-associated lipocalin (NGAL), enhances innate and adaptive immune responses in malaria by modulating iron metabolism (*Zhao et al., Cell Host Microbe, 2012*). Interestingly, LCN2 expression is also elevated in cancer, highlighting its broader role beyond infection. In tumorigenesis, alongside somatic mutations, stroma-associated immunity significantly influences tumor progression. Tumor cells create a supportive microenvironment by releasing mediators, attracting monocytes and leukocytes, and disrupting iron balance through excessive consumption, potentially upregulating LCN2 as an intracellular iron transporter. Recently, we investigated the expression of LCN2 and the immune checkpoint molecule programmed cell death ligand-1 (PD-L1) in breast cancers across molecular subtypes. This retrospective analysis of 89 primary breast cancer cases revealed that LCN2 expression correlates with poor prognostic factors, including high histological grade, elevated Ki-67 proliferation index, and ER/PR negativity. Elevated LCN2 and PD-L1 expressions were significantly associated with triple-negative and HER2-positive breast cancers. These findings demonstrate the prognostic potential of LCN2 and its relevance in immune modulation within the tumor microenvironment. Furthermore, this research suggests the potential for immunotherapeutic applications of LCN2, advancing breast cancer management ***(Ekemen et al., Breast Cancer: Targets and Therapy, 2024).*** By bridging infection and cancer research, our work demonstrates the versatile roles of LCN2 in regulating immunity and iron metabolism, offering insights into its therapeutic potential in diverse pathological contexts.

4. Infections and associated risk factors

Dr. Sakuntabhai's group together with Pasteur Network recently evaluated risk factors for the Crimean-Congo haemorrhagic fever (CCHF) outbreak happened in 2022 in Northern Senegal. CCHF is a severe zoonotic arboviral disease that occurs widely in Eastern and Western Europe, Asia and Africa. The disease

is becoming of growing public health importance in Senegal. However, analysis of tick infestation, CCHF virus (CCHFV) circulation extent and risk factors during ongoing outbreak are scarce. A thorough outbreak investigation was carried out during a CCHF outbreak in Podor (Northern Senegal) in August 2022. Ticks and blood samples were collected from animals (cattle, goats and sheep) randomly selected from confirmed CCHF human cases houses, neighbourhoods and surrounding villages. Blood samples were tested for CCHFV antibodies using a commercial enzyme-linked immunosorbent assay (ELISA) test. Tick samples were screened for CCHFV RNA by RT-PCR. Overall, tick infestation rate (TIR) and CCHFV seroprevalence of livestock were 52.12% (95% confidence interval (CI): 45.54%-58.64%) and 43.28% (95% CI: 36.33%-50.44%), respectively. The TIRs were 87.7% in cattle, 57.6% in sheep and 20.0% in goats. These rates were significantly associated with location, host species and tick control ($p < 0.001$) but not with animal age and sex ($p > 0.7$). CCHFV seroprevalence was 80.4% (95% CI: 67.57%-89.77%) in cattle, 35.4% (95% CI: 25.00%-47.01%) in sheep and 21.2% (95% CI: 12.11%-33.02%) in goats. Age, sex, location, animal host and presence of ticks were significantly associated to the presence of antibodies. The 950 ticks collected included among other species, Hyalomma impeltatum (48.84%) and H. rufipes (10.21%). Five pools of Hyalomma ssp. were found CCHFV RT-PCR positive. These infected ticks included 0.86% (4/464) of H. impeltatum collected on cattle and sheep and 1.03% (1/97) of H. rufipes collected on a sheep. This is possibly the first report on the extend of tick infestation and CCHFV infection in livestock during an outbreak in Senegal. The results highlight the risk of human infections and the importance of strengthening vector, animal and human surveillance as well as tick control measures in this area to prevent CCHF infections in humans ***(Ngom et al., Zoonoses Public Health, 2024).***

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International Vaccine Design Center

Division of Vaccine Engineering (New Dimensional Vaccine Design Team)

新次元ワクチンデザイン系・ワクチン工学分野

Project Professor　Kouhei Tsumoto, Ph.D.

特任教授　博士(工学)　津　本　浩　平

Biomolecular recognition is based on collective and specific non-covalent interactions between discrete biological molecules. Our laboratory studies various protein systems, for instance, antibody-antigen and protein-ligand complexes, to understand quantitatively how these coordinated non-covalent interactions contribute to their specific recognition in biological and artificial systems. We seek to elucidate the molecular mechanisms by which biological molecules obtain high-specificity and affinity from multiple angles using advanced instrumentation. To produce functional molecules with higher performance and better properties, we aim to build a solid foundation from which to develop drugs that modulate specific interactions between biomolecules and ultimately to understand the principles of molecular interactions in our lives.

1. Experimental modification in thermal stability of oligomers by alanine substitution and site saturation mutagenesis of interfacial residues

Hoya M, Matsunaga R, Nagatoishi S, Tsumoto K.

For certain industrial applications, the stability of protein oligomers is important. In this study, we demonstrated an efficient method to improve the thermal stability of oligomers using the trimeric protein chloramphenicol acetyltransferase (CAT) as the model. We substituted all interfacial residues of CAT with alanine to detect residues critical for oligomer stability. Mutation of six of the forty-nine interfacial residues enhanced oligomer thermal stability. Site saturation mutagenesis was performed on these six residues to optimize the side chains. About 15% of mutations enhanced thermal stability by more than 0.5 °C and most did not disrupt activity of CAT. Certain combinations of mutations further improved thermal stability and resistance against heat treatment. The quadruple mutant, H17V/N34S/F134A/D157C, retained the same activity as the wild-type after heat treatment at 9 °C higher temperature than the wild-type CAT. Furthermore, combinations with only alanine substitutions also improved thermal stability, suggesting the method we developed can be used for rapid modification of industrially important proteins.

2. IL-6 Reduces Spheroid Sizes of Osteophytic Cells Derived from Osteoarthritis Knee Joint via Induction of Apoptosis

Negishi Y, Adili Arepati, Susana de Vega, Momoeda M, Kaneko H, Mehmet Zeynel Cilek, Yoshinaga C, Takafuji K, Otsuka Y, Shimoda M, Negishi-Koga T, Ishijima M, Okada Y

Osteophytes in osteoarthritis (OA) joints contribute to restriction of joint movement, joint pain, and OA progression, but little is known about osteophyte regulators. Examination of gene expression related to cartilage extracellular matrix, endochondral ossifica-

tion, and growth factor signaling in articular cartilage and osteophytes obtained from OA knee joints showed that several genes such as COL1A1, VCAN, BGLAP, BMP8B, RUNX2, and SOST were overexpressed in osteophytes compared with articular cartilage. Ratios of mesenchymal stem/progenitor cells, which were characterized by co-expression of CD105 and CD166, were significantly higher in osteophytic cells than articular cells. A three-dimensional culture method for cartilage and osteophyte cells was developed by modification of cultures of self-assembled spheroid cell organoids (spheroids). These spheroids cultured in the media for mesenchymal stem cells containing transforming growth factor-β3 showed characteristic morphologies and gene expression profiles of articular cartilage and osteophytes, respectively. The effects of IL-1β, tumor necrosis factor-α, and IL-6 on the spheroids of articular and osteophytic cells were studied. To the best of our knowledge, they provide the first evidence that IL-6 suppresses the spheroid size of osteophytic cells by inducing apoptosis and reducing extracellular matrix molecules. These data show that IL-6 is the suppressor of osteophyte growth and suggest that IL-6 expression and/or activity are implicated in the regulation of osteophyte formation in pathologic joints.

3. Generation of antibodies to an extracellular region of the transporters Glut1/Glut4 by immunization with a designed antigen

Sumikawa T, Nakakido M, Matsunaga R, Kuroda D, Nagatoishi S, Tsumoto K.

Monoclonal antibodies are one of the fastest growing class of drugs. Nevertheless, relatively few biologics target multispanning membrane proteins because of technical challenges. To target relatively small extracellular regions of multiple membrane-spanning proteins, synthetic peptides, which are composed of amino acids corresponding to an extracellular region of a membrane protein, are often utilized in antibody discovery. However, antibodies to these peptides often do not recognize parental membrane proteins. In this study, we designed fusion proteins in which an extracellular helix of the membrane protein glucose transporter 1 (Glut1) was grafted onto the scaffold protein Adhiron. In the initial design, the grafted fragment did not form a helical conformation. Molecular dynamics simulations of full-length Glut1 suggested the importance of intramolecular interactions formed by surrounding residues in the formation of the helical conformation. A fusion protein designed to maintain such intramolecular interactions did form the desired helical conformation in the grafted region. We then immunized an alpaca with the designed fusion protein and obtained VHH (variable region of heavy-chain antibodies) using the phage display method. The binding of these VHH antibodies to the recombinant Glut1 protein was evaluated by surface plasmon resonance, and their binding to Glut1 on the cell membrane was further validated by flow cytometry. Furthermore, we also succeeded in the generation of a VHH against another integral membrane protein, glucose transporter 4 (Glut4) with the same strategy. These illustrates that our combined biochemical and computational approach can be applied to designing other novel fusion proteins for generating site-specific antibodies.

4. Unveiling the affinity-stability relationship in anti-measles virus antibodies: a computational approach for hotspots prediction

Paul R, Kasahara K, Sasaki J, Pérez JF, Matsunaga R, Hashiguchi T, Kuroda D, Tsumoto K.

Recent years have seen an uptick in the use of computational applications in antibody engineering. These tools have enhanced our ability to predict interactions with antigens and immunogenicity, facilitate humanization, and serve other critical functions. However, several studies highlight the concern of potential trade-offs between antibody affinity and stability in antibody engineering. In this study, we analyzed anti-measles virus antibodies as a case study, to examine the relationship between binding affinity and stability, upon identifying the binding hotspots. We leverage in silico tools like Rosetta and FoldX, along with molecular dynamics (MD) simulations, offering a cost-effective alternative to traditional in vitro mutagenesis. We introduced a pattern in identifying key residues in pairs, shedding light on hotspots identification. Experimental physicochemical analysis validated the predicted key residues by confirming significant decrease in binding affinity for the high-affinity antibodies to measles virus hemagglutinin. Through the nature of the identified pairs, which represented the relative hydropathy of amino acid side chain, a connection was proposed between affinity and stability. The findings of the study enhance our understanding of the interactions between antibody and measles virus hemagglutinin. Moreover, the implications of the observed correlation between binding affinity and stability extend beyond the field of anti-measles virus antibodies, thereby opening doors for advancements in antibody research.

5. THOUSAND-GRAIN WEIGHT 6, which is an IAA-glucose hydrolase, preferentially recognizes the structure of the indole ring

Akabane T, Suzuki N, Ikeda K, Yonezawa T, Nagatoishi S, Matsumura H, Yoshizawa T, Tsuchiya W, Kamino S, Tsumoto K, Ishimaru K, Katoh E, Hirotsu N.

An indole-3-acetic acid (IAA)-glucose hydrolase,

THOUSAND-GRAIN WEIGHT 6 (TGW6), negatively regulates the grain weight in rice. TGW6 has been used as a target for breeding increased rice yield. Moreover, the activity of TGW6 has been thought to involve auxin homeostasis, yet the details of this putative TGW6 activity remain unclear. Here, we show the three-dimensional structure and substrate preference of TGW6 using X-ray crystallography, thermal shift assays and fluorine nuclear magnetic resonance (19F NMR). The crystal structure of TGW6 was determined at 2.6 Å resolution and exhibited a six-bladed β-propeller structure. Thermal shift assays revealed that TGW6 preferably interacted with indole compounds among the tested substrates, enzyme products and their analogs. Further analysis using 19F NMR with 1,134 fluorinated fragments emphasized the importance of indole fragments in recognition by TGW6. Finally, docking simulation analyses of the substrate and related fragments in the presence of TGW6 supported the interaction specificity for indole compounds. Herein, we describe the structure and substrate preference of TGW6 for interacting with indole fragments during substrate recognition. Uncovering the molecular details of TGW6 activity will stimulate the use of this enzyme for increasing crop yields and contributes to functional studies of IAA glycoconjugate hydrolases in auxin homeostasis.

6. Structural basis for the recognition of human hemoglobin by the heme-acquisition protein Shr from Streptococcus pyogenes

Senoo A, Hoshino M, Shiomi T, Nakakido M, Nagatoishi S, Kuroda D, Nakagawa I, Tame JRH, Caaveiro JMM, Tsumoto K.

In Gram-positive bacteria, sophisticated machineries to acquire the heme group of hemoglobin (Hb) have evolved to extract the precious iron atom contained in it. In the human pathogen Streptococcus pyogenes, the Shr protein is a key component of this machinery. Herein we present the crystal structure of hemoglobin-interacting domain 2 (HID2) of Shr bound to Hb. HID2 interacts with both, the protein and heme portions of Hb, explaining the specificity of HID2 for the heme-bound form of Hb, but not its heme-depleted form. Further mutational analysis shows little tolerance of HID2 to interfacial mutations, suggesting that its interaction surface with Hb could be a suitable candidate to develop efficient inhibitors abrogating the binding of Shr to Hb.

7. A high-resolution structural characterization and physicochemical study of how a peptoid binds to an oncoprotein MDM2

Yokomine M, Morimoto J, Fukuda Y, Ueda T, Takeuchi K, Umezawa K, Ago H, Matsuura H, Ueno G, Senoo A, Nagatoishi S, Tsumoto K, Sando S.

Peptoids are a promising drug modality targeting disease-related proteins, but how a peptoid engages in protein binding is poorly understood. This is primarily due to a lack of high-resolution peptoid-protein complex structures and systematic physicochemical studies. Here, we present the first crystal structure of a peptoid bound to a protein, providing high-resolution structural information about how a peptoid binds to a protein. We previously reported a rigid peptoid, oligo(N-substituted alanine) (oligo-NSA), and developed an oligo-NSA-type peptoid that binds to MDM2. X-ray crystallographic analysis of the peptoid bound to MDM2 showed that the peptoid recognizes the MDM2 surface predominantly through the interaction of the N-substituents, while the main chain acts as a scaffold. Additionally, conformational, thermodynamic, and kinetic analysis of the peptoid and its derivatives with a less rigid main chain revealed that rigidification of the peptoid main chain contributes to improving the protein binding affinity. This improvement is thermodynamically attributed to an increased magnitude of the binding enthalpy change, and kinetically to an increased association rate and decreased dissociation rate. This study provides invaluable insights into the design of protein-targeting peptoids.

8. Thermodynamic and molecular dynamic insights into how fusion influences peptide-tag recognition of an antibody

Miyanabe K, Yamashita T, Tsumoto K.

To understand the effect of protein fusion on the recognition of a peptide-tag by an antibody, we fused a CCR5-derived peptide-tag (pep1) to GFP and investigated its recognition by an anti-pep1 antibody, 4B08. First, to characterize the thermodynamic properties associated with the pep1-4B08 binding, isothermal titration calorimetry experiments were conducted. It was found that pep1 fused to the C-terminus of GFP (GFP-CT) enhanced the enthalpic gain by 2.1 kcal mol-1 and the entropic loss only by 0.9 kcal mol-1, resulting in an 8-fold increase in the binding affinity compared to the unfused pep1. On the other hand, pep1 fused to the N-terminus of GFP (GFP-NT) enhanced the enthalpic gain by 3.0 kcal mol-1 and the entropic loss by 3.2 kcal mol-1, leading to no significant enhancement of the binding affinity. To gain deeper insights, molecular dynamics simulations of GFP-NT, GFP-CT, and pep1 were performed. The results showed that the location of the fusion point sensitively affects the interaction energy, the solvent accessible surface area, and the fluctuation of pep1 in the unbound state, which explains the difference in the experimental thermodynamic properties.

9. Functional insights of Tyr37 in framework region 2 directly contributing to the binding affinities and dissociation kinetics in single-domain VHH antibodies

Yamamoto K, Nagatoishi S, Nakakido M, Kuroda D, Tsumoto K.

Single-domain VHH antibody is regarded as one of the promising antibody classes for therapeutic and diagnostic applications. VHH antibodies have amino acids in framework region 2 that are distinct from those in conventional antibodies, such as the Val-37Phe/Tyr (V37F/Y) substitution. Correlations between the residue type at position 37 and the conformation of the CDR3 in VHH antigen recognition have been previously reported. However, few studies focused on the meaning of harboring two residue types in position 37 of VHH antibodies, and the concrete roles of Y37 have been little to be elucidated. Here, we investigated the functional states of position 37 in co-crystal structures and performed analyses of three model antibodies with either F or Y at position 37. Our analysis indicates that Y at position 37 enhances the dissociation rate, which is highly correlated with drug efficacy. Our findings help to explain the molecular mechanisms that distinguish VHH antibodies from conventional antibodies.

10. Cryo-EM structures elucidate the multiligand receptor nature of megalin

Goto S, Tsutsumi A, Lee Y, Hosojima M, Kabasawa H, Komochi K, Nagatoishi S, Takemoto K, Tsumoto K, Nishizawa T, Kikkawa M, Saito A.

Megalin (low-density lipoprotein receptor-related protein 2) is a giant glycoprotein of about 600 kDa, mediating the endocytosis of more than 60 ligands, including those of proteins, peptides, and drug compounds [S. Goto, M. Hosojima, H. Kabasawa, A. Saito, Int. J. Biochem. Cell Biol. 157, 106393 (2023)]. It is expressed predominantly in renal proximal tubule epithelial cells, as well as in the brain, lungs, eyes, inner ear, thyroid gland, and placenta. Megalin is also known to mediate the endocytosis of toxic compounds, particularly those that cause renal and hearing disorders [Y. Hori et al., J. Am. Soc. Nephrol. 28, 1783-1791 (2017)]. Genetic megalin deficiency causes Donnai-Barrow syndrome/facio-oculo-acoustico-renal syndrome in humans. However, it is not known how megalin interacts with such a wide variety of ligands and plays pathological roles in various organs. In this study, we elucidated the dimeric architecture of megalin, purified from rat kidneys, using cryoelectron microscopy. The maps revealed the densities of endogenous ligands bound to various regions throughout the dimer, elucidating the multiligand receptor nature of megalin. We also determined the structure of megalin in complex with receptor-associated protein, a molecular chaperone for megalin. The results will facilitate further studies on the pathophysiology of megalin-dependent multiligand endocytic pathways in multiple organs and will also be useful for the development of megalin-targeted drugs for renal and hearing disorders, Alzheimer's disease [B. V. Zlokovic et al., Proc. Natl. Acad. Sci. U.S.A. 93, 4229-4234 (1996)], and other illnesses.

11. Next-Generation Anti-TNFα Agents: The Example of Ozoralizumab

Tsumoto K, Takeuchi T.

Biologic therapy involving anti-tumor necrosis factor-α (anti-TNFα) agents has fundamentally changed the management of patients with immune-mediated inflammatory diseases, including rheumatoid arthritis, thus benefiting many patients. Nevertheless, the inability of some patients to achieve low disease activity or clinical remission remains a major concern. To address such concerns, next-generation anti-TNFα agents that differ from the immunoglobulin G-format anti-TNFα agents that have been used to date are being developed using antibody-engineering technology. Their unique design employing novel molecular characteristics affords several advantages, such as early improvement of clinical symptoms, optimization of drug bioavailability, enhancement of tissue penetration, and a reduction in side effects. This holds promise for a new paradigm shift in biologic therapy via the use of next-generation anti-TNFα agents. Ozoralizumab, a next-generation anti-TNFα agent that was recently approved in Japan, comprises a variable region heavy-chain format. It has a completely different structure from conventional therapeutic antibodies, such as a small molecular size, an albumin-binding module, and a unique format that produces an avidity effect. Ozoralizumab exhibited rapid biodistribution into joints, provided attenuation of Fcγ receptor-mediated inflammatory responses, and had a high binding affinity to TNFα in non-clinical studies. In clinical trials, ozoralizumab yielded an early improvement in clinical symptoms, a sustained efficacy for up to 52 weeks, and an acceptable tolerability in patients with rheumatoid arthritis. This review focuses on the results of pre-clinical and clinical trials for ozoralizumab and outlines the progress in next-generation antibody development.

12. Characterization of a novel format scFv×VHH single-chain biparatopic antibody against metal binding protein MtsA

Asano R, Takeuchi M, Nakakido M, Ito S, Aikawa C, Yokoyama T, Senoo A, Ueno G, Nagatoishi S, Tanaka Y, Nakagawa I, Tsumoto K.

Biparatopic antibodies (bpAbs) are engineered antibodies that bind to multiple different epitopes within the same antigens. bpAbs comprise diverse formats, including fragment-based formats, and choosing the appropriate molecular format for a desired function against a target molecule is a challenging task. Moreover, optimizing the design of constructs requires selecting appropriate antibody modalities and adjusting linker length for individual bpAbs. Therefore, it is crucial to understand the characteristics of bpAbs at the molecular level. In this study, we first obtained single-chain variable fragments and camelid heavy-chain variable domains targeting distinct epitopes of the metal binding protein MtsA and then developed a novel format single-chain bpAb connecting these fragment antibodies with various linkers. The physicochemical properties, binding activities, complex formation states with antigen, and functions of the bpAb were analyzed using multiple approaches. Notably, we found that the assembly state of the complexes was controlled by a linker and that longer linkers tended to form more compact complexes. These observations provide detailed molecular information that should be considered in the design of bpAbs.

13. Crystal structures of human CD40 in complex with monoclonal antibodies dacetuzumab and bleselumab

Asano R, Nakakido M, Pérez JF, Ise T, Caaveiro JMM, Nagata S, Tsumoto K.

CD40 is a member of the tumor necrosis factor receptor superfamily, and it is widely expressed on immune and non-immune cell types. The interaction between CD40 and the CD40 ligand (CD40L) plays an essential function in signaling, and the CD40/CD40L complex works as an immune checkpoint molecule. CD40 has become a therapeutic target, and a variety of agonistic/antagonistic anti-CD40 monoclonal antibodies (mAbs) have been developed. To better understand the mode of action of anti-CD40 mAbs, we determined the X-ray crystal structures of dacetuzumab (agonist) and bleselumab (antagonist) in complex with the extracellular domain of human CD40, respectively. The structure reveals that dacetuzumab binds to CD40 on the top of cysteine-rich domain 1 (CRD1), which is the domain most distant from the cell surface, and it does not compete with CD40L binding. The binding interface of bleselumab spread between CRD2 and CRD1, overlapping with the binding surface of the ligand. Our results offer important insights for future structural and functional studies of CD40 and provide clues to understanding the mechanism of biological response. These data can be applied to developing new strategies for designing antibodies with more therapeutic efficacy.

14. High-throughput system for the thermostability analysis of proteins

Ito S, Matsunaga R, Nakakido M, Komura D, Katoh H, Ishikawa S, Tsumoto K.

Thermal stability of proteins is a primary metric for evaluating their physical properties. Although researchers attempted to predict it using machine learning frameworks, their performance has been dependent on the quality and quantity of published data. This is due to the technical limitation that thermodynamic characterization of protein denaturation by fluorescence or calorimetry in a high-throughput manner has been challenging. Obtaining a melting curve that derives solely from the target protein requires laborious purification, making it far from practical to prepare a hundred or more samples in a single workflow. Here, we aimed to overcome this throughput limitation by leveraging the high protein secretion efficacy of Brevibacillus and consecutive treatment with plate-scale purification methodologies. By handling the entire process of expression, purification, and analysis on a per-plate basis, we enabled the direct observation of protein denaturation in 384 samples within 4 days. To demonstrate a practical application of the system, we conducted a comprehensive analysis of 186 single mutants of a single-chain variable fragment of nivolumab, harvesting the melting temperature (Tm) ranging from -9.3 up to +10.8°C compared to the wild-type sequence. Our findings will allow for data-driven stabilization in protein design and streamlining the rational approaches.

15. Impact of single-residue mutations on protein thermal stability: The case of threonine 83 of BC2L-CN lectin

Hoya M, Matsunaga R, Nagatoishi S, Ide T, Kuroda D, Tsumoto K.

The thermal stability of trimeric lectin BC2L-CN was investigated and found to be considerably altered when mutating residue 83, originally a threonine, located at the fucose-binding loop. Mutants were analyzed using differential scanning calorimetry and isothermal microcalorimetry. Although most mutations decreased the affinity of the protein for oligosaccharide H type 1, six mutations increased the melting temperature (Tm) by >5 °C; one mutation, T83P, increased the Tm value by 18.2 °C(T83P, Tm = 96.3 °C). In molecular dynamic simulations, the investigated thermostable mutants, T83P, T83A, and T83S, had decreased fluctuations in the loop containing residue 83. In the T83S mutation, the side-chain hydroxyl group of serine formed a hydrogen bond with a nearby residue, suggesting that the restricted movement of the side-chain resulted in fewer fluctuations and enhanced thermal stability. Residue 83 is located

at the interface and near the upstream end of the equivalent loop in a different protomer; therefore, fluctuations by this residue likely propagate throughout the loop. Our study of the dramatic change in thermal stability by a single amino acid mutation provides useful insights into the rational design of protein structures, especially the structures of oligomeric proteins.

16. PDZD8-FKBP8 tethering complex at ER-mitochondria contact sites regulates mitochondrial complexity

Nakamura K, Aoyama-Ishiwatari S, Nagao T, Paaran M, Obara CJ, Sakurai-Saito Y, Johnston J, Du Y, Suga S, Tsuboi M, Nakakido M, Tsumoto K, Kishi Y, Gotoh Y, Kwak C, Rhee HW, Seo JK, Kosako H, Potter C, Carragher B, Lippincott-Schwartz J, Polleux F, Hirabayashi Y.

Mitochondria-ER membrane contact sites (MERCS) represent a fundamental ultrastructural feature underlying unique biochemistry and physiology in eukaryotic cells. The ER protein PDZD8 is required for the formation of MERCS in many cell types, however, its tethering partner on the outer mitochondrial membrane (OMM) is currently unknown. Here we identified the OMM protein FKBP8 as the tethering partner of PDZD8 using a combination of unbiased proximity proteomics, CRISPR-Cas9 endogenous protein tagging, Cryo-Electron Microscopy (Cryo-EM) tomography, and correlative light-EM (CLEM). Single molecule tracking revealed highly dynamic diffusion properties of PDZD8 along the ER membrane with significant pauses and capture at MERCS. Overexpression of FKBP8 was sufficient to narrow the ER-OMM distance, whereas independent versus combined deletions of these two proteins demonstrated their interdependence for MERCS formation. Furthermore, PDZD8 enhances mitochondrial complexity in a FKBP8-dependent manner. Our results identify a novel ER-mitochondria tethering complex that regulates mitochondrial morphology in mammalian cells.

17. Development of novel humanized VHH synthetic libraries based on physicochemical analyses

Nakakido M, Kinoshita S, Tsumoto K.

Due to the high affinity and specificity of antibodies toward antigens, various antibody-based applications have been developed. Recently, variable antigen-binding domains of heavy-chain antibodies (VHH) have become an attractive alternative to conventional fragment antibodies due to their unique molecular characteristics. As an antibody-generating strategy, synthetic VHH libraries (including humanized VHH libraries) have been developed using distinct strategies to constrain the diversity of amino acid sequences. In this study, we designed and constructed several novel synthetic humanized VHH libraries based on biophysical analyses conducted using the complementarity determining region-grafting method and comprehensive sequence analyses of VHHs deposited in the protein data bank. We obtained VHHs from the libraries, and hit clones exhibited considerable thermal stability. We also found that VHHs from distinct libraries tended to have different epitopes. Based on our results, we propose a strategy for generating humanized VHHs with distinct epitopes toward various antigens by utilizing our library combinations.

18. Systematic Preparation of a 66-IgG Library with Symmetric and Asymmetric Homogeneous Glycans and Their Functional Evaluation

Manabe S, Iwamoto S, Nagatoishi S, Hoshinoo A, Mitani A, Sumiyoshi W, Kinoshita T, Yamaguchi Y, Tsumoto K.

Immunoglobulin G (IgG) antibodies possess a conserved N-glycosylation site in the Fc domain. In FcγRIIIa affinity column chromatography, unglycosylated, hemiglycosylated, and fully glycosylated IgG retention times differ considerably. Using retention-time differences, 66 different trastuzumab antibodies with symmetric and asymmetric homogeneous glycans were prepared systematically, substantially expanding the scope of IgGs with homogeneous glycans. Using the prepared trastuzumab with homogeneous glycans, thermal stability and antibody-dependent cellular cytotoxicity were investigated. In some glycan series, a directly proportional relationship was observed between the thermal unfolding temperature (Tm) and the calorimetric unfolding heat (ΔHcal). Antibody function could be deduced from the combination of a pair of glycans in an intact form. Controlling glycan structure through the combination of a pair of glycans permits the precise tuning of stability and effector functions of IgG. Overall, our technology can be used to investigate the effects of glycans on antibody functions.

19. Triphenylphosphonium-modified cationomers enhance in vivo mRNA delivery through stabilized polyion complexation

Norimatsu J, Mizuno HL, Watanabe T, Obara T, Nakakido M, Tsumoto K, Cabral H, Kuroda D, Anraku Y.

Nanocarriers based on cationic materials play a central role in the success of mRNA-based therapies. Traditionally, amine-bearing lipids and polymers have been successfully employed for creating mR-

NA-loaded nanocarriers, though they still present challenges, such as physical and biological instability, limiting both delivery efficiency and therapeutic potential. Non-amine cations could be a promising avenue in addressing these limitations. However, such alternatives remain notably underexplored. Herein, we introduced triphenylphosphonium (TPP) as an alternative cationic moiety for mRNA delivery, leveraging its advantageous properties for nucleic acid complexation. Through the modification of amine-bearing catiomers, we replaced traditional amine-based counterparts with TPP to create innovative polymeric micelles as mRNA nanocarriers. A comprehensive analysis, encompassing physicochemical, thermodynamic, and computational approaches, revealed that the TPP substitution significantly influenced polymer self-assembly, mRNA binding, and the overall stability of mRNA-loaded polymeric micelles. Upon intravenous injection, TPP-bearing micelles demonstrated a remarkable increase in mRNA bioavailability, facilitating efficient protein production in solid tumors. These findings provide a compelling rationale for substituting amines with TPP, emphasizing their potential for advancing mRNA therapeutics.

20. Unveiling the structural mechanisms behind high affinity and selectivity in phosphorylated epitope-specific rabbit antibodies

Kasahara K, Kawade R, Nakakido M, Matsunaga R, Akiba H, Entzminger KC, Maruyama T, Okumura SCJ, Caaveiro JMM, Kuroda D, Tsumoto K.

Protein phosphorylation is a crucial process in various cellular functions, and its irregularities have been implicated in several diseases, including cancer. Antibodies are commonly employed to detect protein phosphorylation in research. However, unlike the extensive studies on recognition mechanisms of the phosphate group by proteins such as kinases and phosphatases, only a few studies have explored antibody mechanisms. In this study, we produced and characterized two rabbit monoclonal antibodies that recognize a monophosphorylated Akt peptide. Through crystallography, thermodynamic mutational analyses, and molecular dynamics simulations, we investigated the unique recognition mechanism that enables higher binding affinity and selectivity of the antibodies compared to other generic proteins with lower binding affinity to phosphorylated epitopes. Our results demonstrate that molecular dynamics simulations provide novel insights into the dynamic aspects of molecular recognition of posttranslational modifications by proteins beyond static crystal structures, highlighting how specific atomic level interactions drive the exceptional affinity and selectivity of antibodies.

21. Structural basis for the ligand promiscuity of the hydroxamate siderophore binding protein FtsB from Streptococcus pyogenes

Fernandez-Perez J, Senoo A, Caaveiro JMM, Nakakido M, de Vega S, Nakagawa I, Tsumoto K.

Pathogenic bacteria must secure the uptake of nutritional metals such as iron for their growth, making their import systems attractive targets for the development of new antimicrobial modalities. In the pathogenic bacterium Streptococcus pyogenes, the iron uptake system FtsABCD transports iron encapsulated by siderophores of the hydroxamate class. However, the inability of S. pyogenes to produce these metabolites makes the biological and clinical relevance of this route unresolved. Herein, we demonstrated that the periplasmic binding protein FtsB recognizes not only the hydroxamate siderophore ferrichrome, as previously documented, but also ferrioxamine E (FOE), ferrioxamine B (FOB), and bisucaberin (BIS), each of them with high affinity (nM level). Up to seven aromatic residues in the binding pocket accommodate the variable backbones of the different siderophores through CH-π interactions, explaining ligand promiscuity. Collectively, our observations revealed how S. pyogenes exploits the diverse xenosiderophores produced by other microorganisms as iron sources to secure this precious nutrient.

22. Malaria parasites require a divergent heme oxygenase for apicoplast gene expression and biogenesis

Blackwell AM, Jami-Alahmadi Y, Nasamu AS, Kudo S, Senoo A, Slam C, Tsumoto K, Wohlschlegel JA, Manuel Martinez Caaveiro J, Goldberg DE, Sigala PA.

Malaria parasites have evolved unusual metabolic adaptations that specialize them for growth within heme-rich human erythrocytes. During blood-stage infection, Plasmodium falciparum parasites internalize and digest abundant host hemoglobin within the digestive vacuole. This massive catabolic process generates copious free heme, most of which is biomineralized into inert hemozoin. Parasites also express a divergent heme oxygenase (HO)-like protein (PfHO) that lacks key active-site residues and has lost canonical HO activity. The cellular role of this unusual protein that underpins its retention by parasites has been unknown. To unravel PfHO function, we first determined a 2.8 Å-resolution X-ray structure that revealed a highly α-helical fold indicative of distant HO homology. Localization studies unveiled PfHO targeting to the apicoplast organelle, where it is imported and undergoes N-terminal processing but retains most of the electropositive transit peptide. We observed that conditional knockdown of PfHO was le-

thal to parasites, which died from defective apicoplast biogenesis and impaired isoprenoid-precursor synthesis. Complementation and molecular-interaction studies revealed an essential role for the electropositive N-terminus of PfHO, which selectively associates with the apicoplast genome and enzymes involved in nucleic acid metabolism and gene expression. PfHO knockdown resulted in a specific deficiency in levels of apicoplast-encoded RNA but not DNA. These studies reveal an essential function for PfHO in apicoplast maintenance and suggest that Plasmodium repurposed the conserved HO scaffold from its canonical heme-degrading function in the ancestral chloroplast to fulfill a critical adaptive role in organelle gene expression.

23. Specific recognition mechanism of an antibody to sulfated tyrosine and its potential use in biological research

Ujiie K, Nakakido M, Kinoshita S, Jose Caaveiro M M, Entzminger, C J Okumura, Maruyama, Miyauchi k, Matano T, Tsumoto K

Post-translational modification of proteins is a crucial biological reaction that regulates protein functions by altering molecular properties. The specific detection of such modifications in proteins has made significant contributions to molecular biology research and holds potential for future drug development applications. In HIV research, for example, tyrosine sulfation at the N-terminus of C-C chemokine receptor type 5 (CCR5) is considered to significantly enhance HIV infection efficiency. However, antibodies specific to sulfated CCR5 still need to be developed. In this study, we successfully generated an antibody that specifically recognized the sulfated N-terminal peptide of CCR5 through rabbit immunization and panning via phage display using a CCR5 N-terminal peptide containing sulfate modification. We used various physicochemical methods in combination with molecular dynamics simulation to screen for residues that could be involved in recognition of the sulfated peptide by this antibody. We also confirmed that this antibody recognized the sulfated full-length CCR5 on the cell surface, which suggested it should be useful as a research tool that could lead to the development of novel therapeutics. Although the antibody binding did not inhibit HIV infection, it could be also described as sulfation site-specific binding, beyond sulfation-specific binding.

24. The pericellular function of Fibulin-7 in the adhesion of oligodendrocyte lineage cells to neuronal axons during CNS myelination

Yamada M, Sasaki B,Yamada N, Hayashi C, Tsumoto K, de Vega S, Suzuki N

Myelin is an electrical insulator that enables saltatory nerve conduction and is essential for proper functioning of the central nervous system (CNS). It is formed by oligodendrocytes (OLs) in the CNS, and during OL development various molecules, including extracellular matrix (ECM) proteins, regulate OL differentiation and myelination; however, the role of ECM proteins in these processes is not well understood. Our present work is centered on the analyses of the expression and function of fibulin-7 (Fbln7), an ECM protein of the fibulin family, in OL differentiation. In the expression analysis of Fbln7 in the CNS, we found that it was expressed at early postnatal stage and localized in the processes of OL precursor cells (OPCs), in the inner region of myelin, and in axons. The functional analysis using recombinant Fbln7 protein (rFbln7) revealed that rFbln7 promoted OPC attachment activity via β1 integrin and heparan sulfate receptors. Further, rFbln7 induced the adhesion to neurites and the differentiation of OLs. Altogether, our results show that Fbln7 promotes the adhesion between OLs and axons and OL differentiation.

<Group III>

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<Group II>

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<Group III>

International Vaccine Design Center

Division of Adjuvant Innovation (New Dimensional Vaccine Design Team)

新次元ワクチンデザイン系・アジュバント開発分野

Professor	Ken Ishii, M.D., Ph.D.		教　授	博士(医学)	石　井　　健
Associate Professor	Kouji Kobiyama, Ph.D.		准教授	博士(医学)	小檜山　康　司
Visiting Professor	Jun Kunisawa, Ph.D.		客員教授	博士(薬学)	國　澤　　純

The laboratory led by Ken Ishii and Jun Kunisawa, advances rational vaccine design. In FY 2024, we reported various papers related to vaccine immunology, focusing on vaccines, mucosal adjuvant, and autoimmunity, contributing to safer, more effective immunotherapies and elucidating the mechanism of vaccine adjuvant.

1. 5,6-dimethylxanthenone-4-acetic acid (DMXAA), a partial stimulator of interferon gene (STING) agonist, competes for human STING activation.

We previously demonstrated that DMXAA acts as Th2 type adjuvant through IRF3-mediated innate immune activation. In addition to the adjuvant effect, DMXAA acts as anti-cancer drug through STING activation, but that effect was found in only mouse. Although DMXAA cannot fully activate human STING, DMXAA reached phase III in lung cancer clinical trials. However, the effect of DMXAA against human lung cancer is completely unknown. Here, we show that DMXAA is a partial STING agonist interfering with agonistic STING activation, which may explain its partial anti-tumor effect observed in humans, as STING was reported to be pro-tumorigenic for lung cancer cells with low antigenicity. Furthermore, we developed a DMXAA derivative-3-hydroxy-5-(4-hydroxybenzyl)-4-methyl-9*H*-xanthen-9-one (HHMX)-that can potently antagonize STING-mediated immune responses both in humans and mice. Notably, HHMX suppressed aberrant responses induced by *STING* gain-of-function mutations causing STING-associated vasculopathy with onset in infancy (SAVI) in *in vitro* experiments. HHMX treatment suppressed aberrant STING pathway activity in peripheral blood mononuclear cells from SAVI patients, and showed a potent therapeutic effect in SAVI mouse model by mitigating disease progression.

2. Tridecylcyclohexane in incomplete Freund's adjuvant is a critical component in inducing experimental autoimmune diseases.

Incomplete Freund's adjuvant (IFA) has been used for many years to induce autoimmune diseases in animal models, including experimental autoimmune encephalitis (EAE) and collagen-induced arthritis. However, it remains unclear why it is necessary to emulsify autoantigen and heat-killed *Mycobacterium tuberculosis* (HKMtb) with IFA to induce experimental autoimmune diseases. Here, we found that immunization with self-antigen and HKMtb was insufficient to induce autoimmune diseases in mice. Furthermore, IFA or one of its components, mineral oil, but not mannide monooleate, was required for the develop-

ment of experimental autoimmune disease. Immunization with autoantigen and HKMtb emulsified in mineral oil facilitated innate immune activation and promoted the differentiation of pathogenic $CD4^+$ T cells, followed by their accumulation in neuronal tissues. Several water-soluble hydrocarbon compounds were identified in mineral oil. Of these, immunization with HKMtb and autoantigen emulsified with the same amount of hexadecane or tridecylcyclohexane as mineral oil induced the development of EAE. In contrast, immunization with HKMtb and autoantigen emulsified with tridecylcyclohexane, but not hexadecane, at doses equivalent to those found in mineral oil, resulted in neuronal dysfunction. These data indicate that tridecylcyclohexane in mineral oil is a critical component in the induction of experimental autoimmune disease.

3. Alcaligenes lipid A functions as a superior mucosal adjuvant to monophosphoryl lipid A via the recruitment and activation of $CD11b^+$ dendritic cells in nasal tissue.

We previously demonstrated that Alcaligenes-derived lipid A (ALA), which is produced from an intestinal lymphoid tissue-resident commensal bacterium, is an effective adjuvant for inducing antigen-specific immune responses. To understand the immunologic characteristics of ALA as a vaccine adjuvant, we here compared the adjuvant activity of ALA with that of a licensed adjuvant (monophosphoryl lipid A, MPLA) in mice. Although the adjuvant activity of ALA was only slightly greater than that of MPLA for subcutaneous immunization, ALA induced significantly greater IgA antibody production than did MPLA during nasal immunization. Regarding the underlying mechanism, ALA increased and activated $CD11b^+$ $CD103^-$ $CD11c^+$ dendritic cells in the nasal tissue by stimulating chemokine responses. These findings revealed the superiority of ALA as a mucosal adjuvant due to the unique immunologic functions of ALA in nasal tissue.

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International Vaccine Design Center

Division of Mucosal Vaccines (New Dimensional Vaccine Design Team)

新次元ワクチンデザイン系・粘膜ワクチン分野

Project Professor	Kohtaro Fujihashi, D.D.S., Ph.D.	特任教授	博士(歯学)	藤 橋 浩太郎
Visiting Professor	Koji Hase, Ph.D.	客員教授	博士(薬学)	長 谷 耕 二
Visiting Professor	Tomonori Nochi, Ph.D.	客員教授	博士(農学)	野 地 智 法

To explore new avenues for mucosal vaccine development and immune-regulation, investigators have begun to employ novel adjuvants and targeting mucosal tissues and immune cells for vaccine delivery and elucidate the mechanisms of immune-regulation in the mucosal tissues. Despite recent advanced sciences, it remains to develop effective mucosal vaccines for human use. To this end, our main task is to define the effectiveness and safety of novel mucosal vaccines including adjuvant- and delivery system-development, and bring them from bench-top to practical applications.

1. Novel mucosal vaccine development for the induction of mucosal immunity in the aero-, digestive- and reproductive mucosa.

Koichiro Suzuki[1], Rika Nakahashi[2-4], Yoshikazu Yuki[2], Koji Hase[1], Hiroshi Kiyono[2-7] and Kohtaro Fujihashi[2-4,8,9]

[1]Division of Biochemistry, Keiko University Faculty of Pharmacy, Tokyo 105-8512 Japan, [2]Department of Human Mucosal Vaccinology, Chiba University Hospital, Chiba 260-8670, Japan, [3] Research Institute of Disaster Medicine, Chiba University, Chiba 260-8670, Japan, [4]Chiba University Synergy Institute for Futuristic Mucosal Vaccine Research and Development Synergy Institute (cSIMVa), Chiba University, Chiba 260-8670, Japan, [5]Institute for Advanced Academic Research, Chiba University, Chiba 260-8670, Japan, [6]CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines (cMAV), Division of Gastroenterology, Department of Medicine, University of California, San Diego 92093-0956, CA, USA [7] Future Medicine Education and Research Organization, Mucosal Immunology and Allergy Therapeutics, Institute for Global Prominent Research, Chiba University, Chiba 260-8670, Japan, [8]Division of Mucosal Vaccines, International Vaccine Design Center, The Institute of Medical Science, The University of Tokyo, Minato-ku, Tokyo 108-8639, JAPAN, [9]Department of Pediatric Dentistry, The University of Alabama at Birmingham, Birmingham, AL 35294-0007 USA

It has been shown that oral antigen (Ag) plus adjuvant delivery for induction of immunity, as opposed to nasal delivery, is an effective non-invasive route. Further, it is well-tolerated and avoids the possibility of Ag and /or adjuvant uptake into the olfactory tissues with subsequent entry into the central nervous system (CNS). However, oral vaccines require relatively large amounts of Ag and adjuvant and are exposed to the proteolytic enzymes and lower pH of the stomach. Considerably, their efficacy limits the mainly gastrointestinal mucosa. In this regard, it is essential to develop a new generation of oral adju-

vants which elicit mucosal immunity in the entire mucosal surfaces including respiratory and reproductive tracts. In order to accomplish this goal, we planned to discover novel molecules which could use potential oral adjuvant for inducing global protective mucosal immunity by using a single-cell mRNA sequencing approach. We have successfully established several DNA libraries from nasopharyngeal-associated lymphoid tissues and Peyer's patches of naïve mice as well as mice given either oral or nasal vaccine. The sequence data have been analyzed using SHIROKANE supercomputer system and we have identified several unique molecules which preferentially upregulated in the NALT of mice given nasal vaccine when compared with those in Peyer's patches of mice given an oral vaccine. Our results showed that one of these molecules, X is indeed up-regulated in NALT and the reproductive tract. We showed that mice deficient with this molecule X resulted in reduced levels of antigen-specific IgA antibody responses in the vaginal washes despite intact levels of serum IgG titers. Further, mononuclear cells isolated from the uterus of molecule X-deficient mice contained reduced numbers of IgA antibody-producing cells when compared with those of wild-type and heterogygous littermates. When we have assessed chemokine receptor expression which involved for the regulation of antigen-specific IgA responses, none of these receptors are essential for the regulation of mucosal IgA antibody responses.

2. MucoRice-CTB line 19A, a new marker-free transgenic rice-based cholera vaccine produced in an LED-based hydroponic system

Yoshikazu Yuki [1-3], Shiho Kurokawa[1,3], Kotomi Sugiura[1,3], Koji Kashima[4], Shinichi Maruyama[4], Tomoyuki Yamanoue[1,3], Ayaka Honma[1], Mio Mejima[1], Natsumi Takeyama[1,5], Masaharu Kuroda[6], Hiroko Kozuka-Hata[7], Masaaki Oyama[7], Takehiro Masumura[8], Rika Nakahashi-Ouchida[1,3,9], Kohtaro Fujihashi[1,3,9,10], Takeshi Hiraizumi[4], Eiji Goto[11], and Hiroshi Kiyono[1-3,7,12,13]

[1]Division of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [2]R&D department, HanaVax Inc., Chiba, Japan, [3]Department of Human Mucosal Vaccinology, Chiba University Hospital, Chiba, Japan, [4]Technical Research Institute, Asahi Kogyosha Co., Ltd., Tokyo, Japan, [5]Research Department, Nisseiken Co., Ltd., Tokyo, Japan, [6]Division of Genome Editing Research, National Agriculture and Food Research Organization, Tsukuba, Japan, [7]Medical Proteomics Laboratory, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan, [8]Laboratory of Genetic Engineering, Graduate School of Agriculture, Kyoto Prefectural University, Kyoto, Japan, [9]Future Mucosal Vaccine Research and Development Synergy Institute, Chiba University, Chiba, Japan, [10]Department of Pediatric Dentistry, The University of Alabama at Birmingham, Birmingham, AL, United States, [11]Graduate School of Horticulture, Chiba University, Chiba, Japan,[12]Mucosal Immunology and Allergy Therapeutics, Institute for Global Prominent Research, Research Institute of Disaster Medicine, Chiba University Future Medicine Education and Research Organization, Chiba University, Chiba, Japan,[13]CU-UCSD Center for Mucosal Immunology, Allergy, and Vaccine (cMAV), Division of Gastroenterology, Department of Medicine, University of California, San Diego, San Diego, CA, United States.

We previously established the selection-marker-free rice-based oral cholera vaccine (MucoRice-CTB) line 51A for human use by *Agrobacterium*-mediated co-transformation and conducted a double-blind, randomized, placebo-controlled phase I trial in Japan and the United States. Although MucoRice-CTB 51A was acceptably safe and well tolerated by healthy Japanese and U.S. subjects and induced CTB-specific antibodies neutralizing cholera toxin secreted by *Vibrio cholerae*, we were limited to a 6-g cohort in the U.S. trial because of insufficient production of MucoRice-CTB. Since MucoRice-CTB 51A did not grow in sunlight, we re-examined the previously established marker-free lines and selected MucoRice-CTB line 19A. Southern blot analysis of line 19A showed a single copy of the *CTB* gene. We resequenced the whole genome and detected the transgene in an intergenic region in chromosome 1. After establishing a master seed bank of MucoRice-CTB line 19A, we established a hydroponic production facility with LED lighting to reduce electricity consumption and to increase production capacity for clinical trials. Shotgun MS/MS proteomics analysis of MucoRice-CTB 19A showed low levels of α-amylase/trypsin inhibitor-like proteins (major rice allergens), which was consistent with the data for line 51A. We also demonstrated that MucoRice-CTB 19A had high oral immunogenicity and induced protective immunity against cholera toxin challenge in mice. These results indicate that MucoRice-CTB 19A is a suitable oral cholera vaccine candidate for Phase I and II clinical trials in humans, including a *V. cholerae* challenge study.

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International Vaccine Design Center

Division of Immunology and Genomics (New Dimensional Vaccine Design Team)

新次元ワクチンデザイン系・ゲノム免疫学分野

Professor	Ken Ishii, M.D., Ph.D.		教授	博士（医学）	石　井　　健
Visiting professor	Anavaj Sakuntabhai, M.D., Ph.D.		客員教授	博士（医学）	サクンタバイ　アナヴァジ

This division has been collaborating with Pasteur Institute in Paris, France via Prof. James DiSanto and Prf. Anavaj Sakuntabhai based on MOU between IMSUT and Pasteur Institute. In FY 2024, Pasteur International Unit on Mucosal Immunomics led by Ken Ishii and James Di Santo together with Prof Sakuntabhai conducted the research and development on immunology and genomics on infectious diseases and cancer. The team also contributed to the establishment of Institute Pasteur Japon (IPJ)

Pasteur Japan SCIENTIFIC WORKSHOP were held June 24 2024 at The French Embassy, Tokyo. Objective was to discuss scientific direction/strategy of Institut Pasteur du Japon with scientists in Japan. Scientific discussion was conducted by Yasmine Belkaid, President, Institut Pasteur and moderated by James Di-Santo

Immunology and vaccine/drug design was chaired by James Di Santo and Ken Ishii

James Di Santo, Institut Pasteur described PIU at IMSUT, AMED-SCARDA – the UTOPIA project. Followingly Dr. Cevayir Coban, U of Tokyo presented "Interactions between host and pathogens at the barriers", Dr. Niloufar Kavian, U of Tokyo presented "Beyond the Spike: SARS-CoV-2 antibody landscape in adults and children, and its relevance for sero-surveillance in a pandemic era", Prof. Noriko Sorimachi, U of Tokyo presented " Developing a research platform for the next pandemic and promoting drug discovery in academia", and Prof. Ken J. Ishii presented "Science and design of vaccine/adjuvant as nanoparticles". In the session for Emerging Infectious Diseases

Chaired by Prof. Anavaj Sakuntabhai and Prof. Fumihiko Matsuda, Prof. Anavaj Sakuntabhai presented "PICREID and AMED-ASPIRE", Prof. Kei Sato, U of Tokyo presented "SARS-CoV-2". These discussion led to the establishment of various projects between U Tokyo and PI.

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Center for Gene & Cell Therapy

Division of Molecular and Medical Genetics
分子遺伝医学分野

Professor	Takashi Okada, M.D., Ph.D.	教　授	博士(医学)	岡　田　尚　巳
Project Associate Professor	Yasushi Soda, M.D., Ph.D.	特任准教授	博士(医学)	曽　田　　泰
Project Senior Assistant Professor	Yasunari Matsuzaka, Ph.D.	特任講師	博士(医学)	松　坂　恭　成
Project Senior Assistant Professor	Yuko Nitahara-Kasahara, Ph.D.	特任講師	博士(工学)	笠　原　優　子
Assistant Professor	Yuji Tsunekawa, Ph.D.	助　教	博士(医学)	恒　川　雄　二
Project Assistant Professor	Hiromi Hayashita-Kinoh, Ph.D.	特任助教	博士(医学)	喜　納　裕　美
Project Assistant Professor	Ken Sugo, Ph.D.	特任助教	博士(工学)	菅　生　　健

To promote the clinical development of gene therapy in Japan, we have been developing facilities and fundamental technologies for the manufacture of viral vectors such as adeno-associated virus (AAV) vectors and lentivirus vectors under Good Manufacturing Practices (GMP) grade. We are also developing treatments for intractable rare diseases using AAV, next-generation AAV vaccines, new cancer gene therapies, and treatments for Duchenne muscular dystrophy (DMD) using mesenchymal stromal cells (MSC).

Virus vector-related technology development

Viral vector technologies have made significant advances, particularly in the development of AAV and lentiviral vectors for gene therapy applications. AAV vectors have emerged as a leading choice for gene therapy due to their safety profile and ability to target various tissues. Novel AAV variants through improved capsid engineering have shown enhanced transduction efficiency. A nanosensor-based approach has been developed to differentiate between functional and faulty AAV vectors at the single-particle level, addressing manufacturing challenges. Significant progress has been made in AAV vector technology, particularly in manufacturing processes and applications. As for purification techniques, the combination of ultracentrifugation and ion exchange chromatography has enhanced vector purity for clinical applications. These advances in AAV vector technology are paving the way for more efficient and targeted gene therapies, with applications ranging from neurological disorders to infectious diseases.

Development of next-generation AAV vaccines

Next-generation vaccine development focuses on combining AAV and extracellular vesicle (EV) technologies to create hybrid vectors called vexosomes. These innovative vaccines aim to overcome the limitations of traditional approaches and offer improved efficacy against SARS-CoV-2 infection, as well as various other diseases, including cancer and emerging infectious diseases. Vexosomes are hybrid vectors that combine the advantages of both AAVs and EVs. AAVs provide long-term gene expression and minimal pathogenicity. EVs offer natural infection-mimicking properties and enhanced immune system interaction. This combination results in a more effective and targeted vaccine delivery system. Vexosomes can efficiently encapsulate and deliver AAV, improving antigen presentation. The hybrid nature of vexosomes can elicit stronger humoral and cellular immune responses. EV-based vaccines carrying the SARS-CoV-2 spike protein have demonstrated robust neutralizing antibody production and T cell responses in animal models. While vexosomes have great potential, sever-

al challenges need to be addressed. We are exploring methods to increase the efficiency of EV production and AAV encapsulation. We are also developing consistent and cost-effective manufacturing processes for clinical applications.

Novel treatment for DMD with MSCs

MSCs have emerged as a promising novel treatment for DMD. MSCs offer several potential benefits for DMD patients, including muscle regeneration, anti-inflammatory effects, and paracrine signaling. MSCs can differentiate into muscle cells, potentially replacing damaged dystrophin-expressing cells and improving muscle function. In addition, MSCs secrete anti-inflammatory cytokines that can modulate the immune response, reducing inflammation and protecting muscle tissue from further damage. MSCs also secrete bioactive molecules that promote tissue repair and regeneration, enhancing the survival and function of existing muscle cells. Several clinical trials are evaluating the safety and efficacy of MSC-based therapies for DMD. Early-phase trials have shown promising results, with patients exhibiting improved muscle strength, reduced fibrosis, and improved quality of life. To date, we have developed a human amnion-derived mesenchymal stromal cell (hAMSC) therapy for the treatment of DMD in collaboration with Kaneka Corporation, which has recently started a clinical trial. While MSC therapy shows significant promise for DMD treatment, challenges still remain, including ensuring consistent cell production, preventing immune rejection, and maximizing cell engraftment and survival in dystrophic muscle. Ongoing research aims to address these hurdles and enhance the potential of MSCs to effectively treat DMD.

Development of gene therapy using lentiviral vectors

In recent years, *ex vivo* gene therapy, chimeric antigen receptor (CAR) T-cell therapy has been approved for the treatment of hematological malignancies, including leukemia and malignant lymphoma, and its use is increasing due to its high efficacy. CAR-T cells are genetically modified T cells that bind and kill leukemia cells via the transduced CAR and mainly produced using lentiviral vectors. We are working to develop next-generation CAR immune cell therapy by overcoming the problems of current autologous CAR-T cell therapy, such as insufficient persistence in the body, long production time, and low efficacy against solid tumors. In the context of i*n vivo* gene therapy for genetic diseases, lentiviral vectors that enable long-term stable gene expression are also attracting attention. Our laboratory is utilizing our expertise in optimizing AAV vector production to develop a method for producing lentiviral vectors that can be administered *in vivo*.

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Center for Gene & Cell Therapy
遺伝子・細胞治療センター

Director/Professor	Takashi Okada, M.D., Ph.D.	センター長／教授	博士(医学)	岡 田 尚 巳
Professor	Tomoki Todo, M.D., Ph.D.	教 授	博士(医学)	藤 堂 具 紀
Professor	Fumitaka Nagamura, M.D., D.M.Sc.	教 授	博士(医学)	長 村 文 孝
Invited Professor	Koji Tamada, M.D., Ph.D.	教授(委嘱)	博士(医学)	玉 田 耕 治
Project Professor	Satoshi Takahashi, M.D., D.M.Sc.	特任教授	博士(医学)	高 橋 聡
Associate Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.	准教授	博士(医学)	長 村 登紀子

To improve the safety and effectiveness of gene and cell therapies, we are developing Good Manufacturing Practices (GMP)-based production systems for high-quality viral vectors and cell products. Our main focus is on oncolytic virotherapy, gene therapy and vaccine development with Adeno-associated virus vectors, genetically modified T cell therapy, T cell therapy for viral infections after hematopoietic stem cell transplantation, and mesenchymal stromal cell (MSC) therapy.

Construction of large-scale viral vector purification systems

Large-scale purification systems for viral vectors, such as adeno-associated virus and lentivirus, typically require multiple steps to ensure the high purity and yield of functional viral particles that are crucial for gene therapy applications. Advances in these purification systems are crucial to meet the increasing demand for gene therapies, enabling more efficient and cost-effective production of high-quality viral vectors for clinical applications. Of particular importance is the scalability and efficiency of large-scale viral vector purification systems. This demand-supply gap necessitates the development of more efficient and scalable purification processes. Thus, selecting the proper manufacturing platform is crucial to ensure consistent quality and compliance with GMP standards.

Development of an oncolytic virus therapy using third-generation herpesvirus G47D

G47Δ is a third-generation oncolytic herpes simplex virus type 1 (HSV-1) that has been developed for cancer therapy. It is a triple-mutant virus with enhanced replication capability and highly selective to tumor cells. The development of G47Δ represents a significant advancement in oncolytic virus therapy. G47Δ has three genetically engineered mutations in the HSV-1 genome, that significantly improve its safety profile compared to existing cancer treatment viruses. These modifications allow G47Δ to selectively replicate in cancer cells while leaving healthy cells unharmed, and to induce both innate and adaptive immune responses against the tumor. In addition, G47Δ has shown promising results in various cancer types, such as glioblastoma, esophageal cancer, and gastric cancer. In a Phase II trial, G47Δ demonstrated a 1-year survival rate of 84.2% and a median overall survival of 20.2 months in patients with residual or recurrent glioblastoma. Preclinical studies have shown significant inhibition of tumor growth in both subcutaneous and orthotopic models. G47Δ exhibited efficacy in advanced gastric cancer models, including scirrhous gastric cancer with peritoneal dissemination. In addition, research has shown that combining G47Δ with other cancer treatments can enhance its efficacy. When used in combination with checkpoint inhibitors, G47Δ led to tumor disappearance in more than two-thirds of mice in a melanoma model. The development of G47Δ represents a successful academic-led approach to creating a novel cancer therapy, from invention to practical application. The effica-

cy and safety of this oncolytic virus were confirmed in an investigator-initiated clinical trial conducted at IMSUT Hospital for malignant glioma patients. An application for manufacturing and marketing approval was submitted in 2020, and it was approved in 2021 as a regenerative medical product (teselpatulev, Delytact) for the treatment of malignant glioma. As research continues, G47Δ and other oncolytic viruses may become integral components of multimodal cancer treatment strategies, potentially revolutionizing cancer therapy.

Development of a treatment for Duchenne muscular dystrophy (DMD) using MSCs

Another promising new therapy method for Duchenne muscular dystrophy (DMD) involves cell therapy using mesenchymal stromal cells (MSCs), which can improve pulmonary and cardiac functions, thereby enhancing survival in DMD patients. MSCs have several advantages, including immunomodulatory properties, non-tumorigenicity, enhanced regeneration capabilities, in vitro expansion ability, and anti-senescence properties. While MSC therapy shows promise for the treatment of DMD, further research is needed to fully understand its characteristics and potential long-term effects. As the field of regenerative medicine advances, MSC therapy may offer new hope for improving the quality of life and extending the lifespan of DMD patients.

Improvement of CAR-T cell therapy for solid cancers by using interleukin (IL)-7 and chemokine (C-C motif) ligand 19 (CCL19) production

IL7/CCL19-expressing CAR-T cells (7×19 CAR-T) have shown promising therapeutic efficacy in intractable solid tumor models of glioblastoma and pancreatic cancer. These next-generation CAR-T cells have demonstrated superior antitumor activity compared to conventional CAR-T cells in preclinical studies. This study is the first to demonstrate the therapeutic efficacy of next-generation CAR-T cells in an autologous model using patient-derived tumor organoids and CAR-T cells generated from the same patient's PBMCs. This approach eliminates unwanted allogeneic immune responses, providing a more accurate representation of potential clinical outcomes. The promising results of 7×19 CAR-T cells in these intractable solid tumor models suggest that this technology could become a viable therapeutic option for glioblastoma and pancreatic cancer, which have shown resistance to conventional immunotherapies and have poor prognoses.

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Laboratory Animal Research Center

Division of Animal Genetics
先進動物ゲノム研究分野

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　士
Associate Professor	Kazuto Yoshimi, Ph.D.	准教授	博士(医科学)	吉　見　一　人
Assistant Professor	Saeko Ishida, D.V.M., Ph.D.	助　教	博士(医学)	石　田　紗恵子

Genome engineering technologies have revolutionized life science and medical research. These techniques enable precise manipulation of target genes for various purposes. Using these technologies, we have developed numerous valuable mouse and rat strains. Our current focus is on generating "humanized animals" and "immunodeficient animals." These specialized models allow xenotransplantation of human cells and tissues, including hematopoietic stem cells (HSCs) and induced pluripotent stem cells (iPSCs). Additionally, we are developing therapeutic strategies that combine genome editing tools with cell and gene therapy approaches.

A novel Kit mutant rat enables hematopoietic stem cell engraftment without irradiation.

Ryuya Iida [1], Saeko Ishida [2], Jinxi Wang [1], Kosuke Hattori [1], Kazuto Yoshimi [3], Satoshi Yamazaki [4], Tomoji Mashimo [3]

1, Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, the University of Tokyo

2, Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, the University of Tokyo

3, Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, the University of Tokyo, Tokyo, Japan; Division of Genome Engineering, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo

4, Division of Cell Regulation, Center of Experimental Medicine and Systems Biology, the Institute of Medical Science, the University of Tokyo, Tokyo, Japan; Laboratory of Stem Cell Therapy, Faculty of Medicine, University of Tsukuba

Hematopoietic stem cell (HSC) transplantation is extensively studied in mouse models, but their limited scale presents challenges for effective engraftment and comprehensive evaluations. Rats, owing to their larger size and anatomical similarity to humans, offer a promising alternative. In this study, we establish a rat model with the Kit^{V834M} mutation, mirroring Kit^{W41} mice often used in KIT signaling and HSC research. Kit^{V834M} rats are viable and fertile, displaying anemia and mast cell depletion similar to Kit^{W41} mice. The colony-forming unit assay revealed that the Kit^{V834M} mutation leads to reduced proliferation and loss of or decreased pluripotency of hematopoietic stem and progenitor cells (HSPCs), resulting in diminished competitive repopulating capacity of Kit^{V834M} HSPCs in competitive transplantation assays. Importantly, Kit^{V834M} rats support donor rat-HSC engraftment without irradiation. Leveraging the larger scale of this rat model enhances our understanding of HSC biology and transplantation dynamics, potentially advancing our knowledge in this field.

CRISPR-Cas3 mediated gene therapy for Transthyretin Amyloidosis

Saeko Ishida[1], Yusuke Sato[2], Keisuke Chosa[1,3], Eri

Ezawa[1], Yuko Yamauchi[1], Masaaki Oyama[4], Hiroko Kozuka-Hata[4], Rina Ito[2], Rikako Sato[2], Masatoshi Maeki[5], Kenichi Yamamura[6], Yoshiki Sekijima[7], Kazuto Yoshimi[1,8], Tomoji Mashimo[1,8]

1, Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo
2, Laboratory for Molecular Design of Pharmaceutics, Faculty of Pharmaceutical Sciences, Hokkaido University
3, C4U Corporation
4, Medical Proteomics Laboratory, Institute of Medical Science, The University of Tokyo
5, Division of Applied Chemistry, Faculty of Engineering, Hokkaido University
6, Transgenic, Inc.,
7, Department of Medicine, Shinshu University
8, Division of Genome Engineering, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, University of Tokyo

Gene therapy using genome editing holds promise as a fundamental treatment for hereditary diseases, and its efficacy has been confirmed in several recent clinical trials. However, concerns regarding safety remain. We have been developing a CRISPR/Cas3 system, which has a longer target recognition sequence compared to Cas9, thereby reducing the risk of off-target effects. Additionally, Cas3 exhibits the unique capability to induce large deletions in the genome, making it a promising tool for achieving precise and safe genetic modifications. Our research focuses on developing safer in vivo gene therapy approaches using the CRISPR/Cas3 system.

Transthyretin amyloidosis (ATTR) is a systemic disorder caused by the deposition of misfolded transthyretin (TTR) proteins in various organs, leading to organ dysfunction. ATTR can be classified into two types: hereditary ATTR (ATTRv), which is caused by mutations in the TTR gene, and wild-type ATTR (ATTRwt), which develops with aging in the absence of TTR mutations. Current treatments primarily include siRNA-based therapies to suppress TTR production and TTR stabilizers to prevent fibril formation; however, these treatments require continuous administration. Therefore, the development of genome-editing-based therapies offers a promising alternative for a more permanent solution.

In our study, we successfully achieved approximately 75% reduction in serum TTR levels — a threshold associated with clinical efficacy — by delivering modified mRNA encoding CRISPR/Cas3 via lipid nanoparticles to the liver, which produces about 90% of TTR. Unlike CRISPR/Cas9, which has been reported to introduce new mutations and off-target effects, our CRISPR/Cas3-based approach did not result in the generation of mutated TTR variants, demonstrating its superior safety profile.

This study provides a novel genome-editing strategy using CRISPR/Cas3, offering a safer and more effective therapeutic option for the treatment of various hereditary diseases, including ATTR.

Resource and Model Animal Production Support - NBRP-Rat and AdAMS to promote Rat Research

Kosuke Hattori [1] , Saeko Ishida [1] ,Hiroaki Taketsuru [1] , Yuko Yamauchi [1] ,Ryuya Iida [1] , Kazuto Yoshimi [1,2] , Tomoji Mashimo [1,2]

1, Division of Animal Genetics, Laboratory Animal Research Center, The Institute of Medical Science, The University of Tokyo
2, Division of Genome Engineering, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo

The rat has a history of more than 100 years as a laboratory animal, accumulating physiological and pharmacological data. As the development of genome editing technology progresses, modifying genes in rats has become much easier, making laboratory rats more valuable in contemporary research. Various genome editing technologies have been developed and utilized to create useful rat models.

Participation in two platform projects is actively promoting rat research. The National Bio Resource Project-Rat, initiated in 2002 with Kyoto University as the core institution, stands as a world-class resource center. It has collected and conserved more than 800 rat strains to date. Researchers are provided with three immunodeficient rat strains, namely *Il2rg* knockout, *Rag2* knockout, and *Il2rg*/*Rag2* double knockout, under genetically and microbiologically controlled conditions. So far, 152 MTAs have been signed , resulting in the provision of 855 immunodeficient rats.

As a member of the Advanced Animal Model Support Platform, support is extended for the generation of genetically engineered rats. 113 rat strains have been generated to date, including those intended for domestic researchers to aid in their life science research.

Diverse Cre recombinase expression pattern in Albumin-Cre driver rats

Saeko ISHIDA[1], Keiko TAGUCHI[2,3], Ryuya IIDA[1], Kosuke HATTORI[1], Hiroaki TAKETSURU[1], Kazuto YOSHIMI[1,4], Masayuki YAMAMOTO[2], Tomoji MASHIMO[1,4]

[1]Division of Animal Genetics, Laboratory Animal Research Center, Institute of Medical Science, The University of Tokyo
[2]Department of Biochemistry and Molecular Biology, Tohoku Medical Megabank Organization, Tohoku University
[3]Graduate School of Agricultural and Life Sciences,

The University of Tokyo
[4]Division of Genome Engineering, Center for Experimental Medicine and Systems Biology, Institute of Medical Science, The University of Tokyo

Rats (*Rattus norvegicus*) have been widely utilized as model animals due to their physiological characteristics, making them suitable for surgical and long-term studies. They have played a crucial role in biomedical research, complementing studies conducted in mice. The advent of genome editing technologies has facilitated the generation of genetically modified rat strains, advancing studies in experimental animals. Among these innovations, Cre-driver rat models have emerged as powerful tools for spatiotemporal control of gene expression. However, their development and characterization remain less advanced compared to mouse models.

In this study, we developed liver-targeting Cre knock-in rats and reporter knock-in rats to evaluate Cre recombinase expression profiles in different genetic contexts. Our results revealed that insertion orientation and promoter origin significantly influence Cre expression patterns. Notably, forward insertion of the *Albumin* (*Alb*) promoter-driven Cre sequence at the *ROSA26* locus resulted in ubiquitous Cre expression, while reverse insertion confined Cre expression predominantly to the liver. Interestingly, Cre expression under an endogenous *Alb* promoter unexpectedly induced expression in non-liver tissues, which may suggest a potential link to the *in vivo* dynamics of albumin.

These findings underscore the importance of rigorous characterization in Cre-based transgenic systems. By elucidating the roles of promoter origin, insertion site, and orientation, our study provides valuable insights for optimizing Cre-driver rat models. These findings pave the way for refining genetic strategies to enhance tissue specificity and reliability in functional genomics and disease modeling.

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Laboratory Animal Research Center

Animal Center
動物センター

Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　士
Associate Professor	Kazuto Yoshimi, Ph.D.	准教授	博士(医科学)	吉　見　一　人
Assistant Professor	Saeko Ishida, D.V.M., Ph.D.	助　教	博士(医学)	石　田　紗恵子

The Laboratory Animal Research Center (LARC) was founded in 1965 as the first modern animal facility in Japan. Currently about 25,616 mice are housed for research of IMSUT, and strictly maintained in the SPF condition. The Animal Center building of LARC was improved in 1998 to perform genome engineering in animals, to make infectious experiments (P2A, P3A), and to house bigger animals, such as rats and rabbits. Techniques of mouse embryo manipulation and generating genetically modified mice, including genome editing technologies, have been introduced into the LARC.

Animal Husbandry and Housing

The Animal Center building is a centralized facility designed, constructed and maintained to meet regulatory standards required for the operation of research animal facilities. We provide barrier housing rooms to support the production and use of geneticall-engineered mice, biohazardous experiments area and equipment room which has X-ray Irradiator, MRI, CT and IVIS imaging system. In 2024, 449 researchers from 39 laboratories are engaged in this facility with about 25,616 mice, 718 rats and 67 hamsters.

Techniques of mouse embryo manipulation (Microbiological cleaning and cryopreservation)

Our Barrie housing rooms are strictly maintained in the SPF condition; therefore, we provide IVF mouse derivation service for all mice shipped to LARC from other institutions or non-approved vendors to keep mice in SPF grade. We make frozen sperm and embryo for reducing number of using animals and laboratory space. In addition, it is useful for making back up of the strains. In 2024, 81 strains of embryos and 51 strains of sperm were stored, and 95 tubes of frozen embryo were used for rederivation.

Amami Laboratory of Medical Science
奄美医科学研究施設

Professor	Tomoji Mashimo, Ph.D.		教　授	博士(人間・環境学)	真　下　知　士
Visiting Associate Professor	Takeshi Annoura, Ph.D.		客員准教授	医学博士	案　浦　　健
Assistant Professor	Shin-Ichi Yokota, D.V.M., Ph.D.		助　教	博士(人間科学)	横　田　伸　一

The Amami Laboratory of Medical Science has a long history originating from the branch office of the Institute for Infectious Disease which was established in 1902. We have made great achievements in filariasis eradication from this island and prevention of Habu bites. Currently, we are maintaining the colonies of New World monkeys, and aiming to overcome endemic infectious diseases in the tropical and subtropical regions through infection experiments using primates.

Reproduction of squirrel monkeys and owl monkeys

Squirrel monkeys (*Saimiri boliviensis*) and Owl monkey (*Aotus lemurinus griseimenbra*) are widely distributed in the tropical rainforest in Central and South America. The advantage of using both species for medical researches resides in its small size and gentle behavior. Squirrel monkeys and owl monkeys are phylogenetically close to each other, and both are well known as the best candidates for malaria model in primates. In our laboratory, squirrel monkeys have a breeding season between winter and early spring. They are polygamy. Their puberty is 3-4 years old in females and 4-5 years old in males. Their gestation period is about 150 days. In contrast, owl monkeys are annual breeding animals. They are monogamy. Their puberty is 3 years old for both sexes. Their gestation period is about 130 days. Five newborns were given in reproductive groups of squirrel monkeys in 2024. On the other hand, owl monkeys have become male-only colonies, and breeding has stopped at present.

Research using non-human primates

Notable aspect of our laboratory is the unique International Joint Usage and Research Center capability of conducting infection experiment using squirrel monkeys, owl monkeys, and cynomolgus monkeys. The 3rd building equipped with animal experimental rooms, which allows for experiments on mosquito-borne infectious diseases in primates was completed last year, and its use is started from this year. We are working with collaborators from several institutions to develop an experimental squirrel monkey infection model to assess the anti-malarial activity of new compounds and vaccines.

Research on the control of snakebite envenoming

Snakebite envenoming is still a serious health problem in many tropical and subtropical countries. It was recognized by the World Health Organization (WHO) as a neglected tropical disease in 2009, and was elevated into Category A of the Neglected Tropical Diseases list in 2017. Amami laboratory used to be an important facility for research and development of antivenom serum for Habu (*Protobothrops flavoviridis*), which is a species endemic to Japan. We are conducting research with collaborators aimed to elucidate the detail components of Habu venom through genome analysis, etc. and that will contribute to control of snakebite envenoming in the world.

Medical Proteomics Laboratory
疾患プロテオミクスラボラトリー

Professor	Mutsuhiro Takekawa, M.D., Ph.D.	教　授	博士(医学)	武　川　睦　寛
Project Professor	Kouhei Tsumoto, Ph.D.	特任教授	博士(工学)	津　本　浩　平
Associate Professor	Masaaki Oyama, Ph.D.	准教授	博士(医学)	尾　山　大　明
Associate Professor	Satoru Nagatoishi, Ph.D.	准教授	博士(生命科学)(大学院工学系研究科)	長門石　　曉
Senior Assistant Professor	Makoto Nakakido, Ph.D.	講　師	博士(生命科学)(大学院工学系研究科)	中木戸　　誠
Assistant Professor	Ryo Matsunaga, Ph.D.	助　教	(生命科学)(大学院工学系研究科)	松　長　　遼
Project Assistant Professor	Hiroshi Sagara, Ph.D.	特任助教	博士(医学)	相　良　　洋

The mission of our laboratory is to develop advanced technologies for integrative proteomic analyses from a physicochemical, structural and systems biology point of view. Currently, we mainly focus on functional protein-protein interaction networks related to a variety of diseases including cancer and infection. We are also engaged in collaborative researches regarding mass spectrometry and electron microscopy, which have made a substantial contribution to many scientific achievements.

<Group I>

1. Integrative analysis of cancer cell signaling networks by high-resolution proteomics and systems biology

Post-translational modifications (PTMs), such as phosphorylation, ubiquitination and acetylation, are known to be widely involved in the regulation of various biological processes through extensive diversification of each protein function at the cellular network level. Previous functional analyses of cancer cell signaling under a variety of experimental conditions revealed many of the key molecules and their associated protein modifications in relation to each type of cancer. In order to systematically discover critical modulators from diversified signaling molecules, we have developed a high-resolution mass spectrometry-based proteomics platform for integrative identification and quantification of multiple post-translational modifications from various types of cancer cells.

1-1. High-resolution proteomic analysis of EGF-regulated ubiquitination dynamics in human cancer cells

Hiroko Kozuka-Hata, Tomoko Hiroki, Aya Kitamura, Aiko Aizawa, Naoaki Miyamura, Kouhei Tsumoto, Jun-ichiro Inoue, and Masaaki Oyama.

Protein ubiquitination is one of the most prevalent post-translational modifications (PTMs) and plays critical roles in regulating protein degradation, signal transduction and DNA repair in cooperation with other PTMs such as phosphorylation and acetylation. Recent mass spectrometry-based proteomics coupled with efficient enrichment technologies for each type of the modified peptides has enabled us to identify precise modification sites and measure their quantitative changes on a global scale. Our previous lysine-modification proteomic analyses of thirteen representative human cancer cell lines led us to identify thousands of ubiquitination (Ub) and acetylation (Ac) sites in total and revealed that their system-wide modification status was mutually different at the cel-

lular network level. In this study, we further applied SILAC (Stable Isotope Labeling by Amino acids in Cell culture) for quantitative description of EGF-dependent lysine-modification site dynamics in HeLa cells in a time-resolved manner. Through integration of large-scale SILAC-encoded data on six time points upon EGF stimulation, we successfully quantified approximately 1,000 kinds of Ub-sites as well as 700 kinds of Ac-sites and found that one-third of these Ub-modified molecules, including several EGF signaling effectors, were subjected to downregulation by proteasomal inhibition.

1-2. Proteome-wide analysis of lysine acetylation and ubiquitination reveals critical signaling regulation in cancer cells

Hiroko Kozuka-Hata, Aya Kitamura, Tomoko Hiroki, Aiko Aizawa, Kouhei Tsumoto, Jun-ichiro Inoue, and Masaaki Oyama.

Post-translational modifications (PTMs), such as phosphorylation, ubiquitination and acetylation, are known to be widely involved in the regulation of various biological processes through extensive diversification of each protein function at the cellular network level. Previous functional analyses of cancer cell signaling under a variety of experimental conditions revealed many of the key molecules and their associated protein modifications in relation to each type of cancer. In order to systematically discover critical modulators from diversified signaling molecules, we have developed a high-resolution mass spectrometry-based proteomics platform for integrative identification and quantification of multiple post-translational modifications from various types of cancer cells. Our large-scale proteomic analysis enabled us to identify more than 5,000 kinds of ubiquitinated sites and 1,600 kinds of acetylated sites from representative human cancer cell lines, leading to identification of approximately 900 novel lysine modification sites in total. Very interestingly, 236 lysine residues derived from 141 proteins were found to be modified with both ubiquitination and acetylation. As a consequence of the subsequent motif extraction analyses, glutamic acid (E) was found to be highly enriched at the position (-1) for the lysine acetylation sites, whereas the same amino acid was relatively dispersed along the neighboring residues of the lysine ubiquitination sites.

1-3. System-wide perturbation of the proteome and phosphoproteome dynamics in glioblastoma stem cells through mTOR signaling inhibition

Hiroko Kozuka-Hata, Tomoko Hiroki, Ryo Koyama-Nasu, Kouhei Tsumoto, Jun-ichiro Inoue, Tetsu Akiyama, and Masaaki Oyama.

As glioblastoma is the most common and aggressive brain tumor with poor prognosis, systematic elucidation of signaling networks causally linked to the tumorigenesis is very crucial for developing more effective treatments for this intractable cancer. In our previous study, we applied a high-resolution mass spectrometry-based proteomics technology in combination with SILAC quantitative methods to understand EGF-dependent phosphoproteome dynamics in patient-derived glioblastoma stem cells. We demonstrated that the phosphorylation levels of the representative mTOR signaling molecules such as RPS6 and PRAS40 were dramatically up-regulated upon EGF stimulation. As EGFR signaling has been reported to play a pivotal role in regulating the maintenance of cancer stem cells, we next carried out mTOR inhibitor-dependent signaling perturbations to unravel stemness-related pathways at the network level.

In the present study, we identified a total of 3,726 proteins including 49 up-regulated and 436 down-regulated factors by Torin 1 treatment. Interestingly, we found that one of the well-known cancer stem cell markers was significantly down-regulated through mTOR signaling inhibition. Our in-depth phosphoproteome analysis also led to identification of 6,250 unique phosphopeptides derived from 2,221 proteins and unveiled a variety of dynamic changes regarding phosphorylation levels of cancer and neural stem cell markers in a comprehensive manner. The integrative view of the mTOR inhibitor-dependent proteome and phosphoproteome dynamics in glioblastoma stem cells presents us with further prospects towards understanding previously unrecognized regulations at the system level.

1-4. System-level analysis of CagA-dependent signaling network dynamics by Helicobacter pylori infection

Hiroko Kozuka-Hata, Masato Suzuki, Kotaro Kiga, Shinya Tasaki, Jun-ichiro Inoue, Tadashi Yamamoto, Chihiro Sasakawa, and Masaaki Oyama.

The signal transduction system within a cell regulates complex biological events in response to bacterial infection. The previous analyses of cell signaling in Helicobacter pylori-infected gastric epithelial cells have revealed that CagA, a major virulence factor of Helicobacter pylori, is delivered into cells via the type IV secretion system and perturbs signaling networks through the interaction with the key signaling molecules such as SHP-2, Grb2, Crk/Crk-L, Csk, Met, and ZO-1. Although the biological activity of tyrosine-phosphorylated CagA has intensively been studied, system-wide effects of the virulence factor on cellular signaling have yet to be analyzed. Here we performed time-resolved analyses of phosphoproteome and CagA-interactome in human gastric AGS

cells by CagA-positive/negative Helicobacter pylori infection. Our highly sensitive nanoLC-MS/MS analyses in combination with the Stable Isotope Labeling by Amino acids in Cell culture (SILAC) technology defined CagA-dependent perturbation of signaling dynamics along with a subset of CagA-associated possible modulators on a network-wide scale. Our result indicated that the activation level of the phosphotyrosine-related signaling molecules in AGS cells was suppressed overall in the presence of CagA during Helicobacter pylori infection. As Helicobacter pylori infection plays pivotal roles in the progression of gastric diseases including carcinogenesis, a comprehensive and fine description of the signaling dynamics would serve as a fundamental platform to theoretically explore for the potential drug targets through analyzing the regulatory mechanisms at the system-level.

2. Mass spectrometry-based annotation of the human short ORFeome

Masaaki Oyama, Hiroko Kozuka-Hata, Sumio Sugano, Tadashi Yamamoto, and Jun-ichiro Inoue.

In parallel with the human genome projects, human full-length cDNA data has also been intensively accumulated. Large-scale analysis of their 5'-UTRs revealed that about half of these had a short ORF upstream of the coding region. Experimental verification as to whether such upstream ORFs are translated is essential to reconsider the generality of the classical scanning mechanism for initiation of translation and define the real outline of the human proteome. Our previous proteomics analysis of small proteins expressed in human K562 cells provided the first direct evidence of translation of upstream ORFs in human full-length cDNAs (Oyama et al., Genome Res, 14: 2048-2052, 2004). In order to grasp an expanded landscape of the human short ORFeome, we have performed an in-depth proteomics analysis of human K562 and HEK293 cells using a two-dimensional nanoLC-MS/MS system. The results led to the identification of eight protein-coding regions besides 197 small proteins with a theoretical mass less than 20 kDa that were already annotated coding sequences in the curated mRNA database. In addition to the upstream ORFs in the presumed 5'-untranslated regions of mRNAs, bioinformatics analysis based on accumulated 5'-end cDNA sequence data provided evidence of novel short coding regions that were likely to be translated from the upstream non-AUG start site or from the new short transcript variants generated by utilization of downstream alternative promoters. Protein expression analysis of the *GRINL1A* gene revealed that translation from the most upstream start site occurred on the minor alternative splicing transcript, whereas this initiation site was not utilized on the major mRNA, resulting in translation of the downstream ORF from the second initiation codon. These findings reveal a novel post-transcriptional system that can augment the human proteome via the alternative use of diverse translation start sites coupled with transcriptional regulation through alternative promoters or splicing, leading to increased complexity of short protein-coding regions defined by the human transcriptome (Oyama et al., Mol Cell Proteomics, 6: 1000-1006, 2007).

3. In-depth proteomic analysis of drug-responsive signaling pathway elements in human cancer cells

Wei QI, Aya Kitamura, Naoaki Miyamura, Tomoko Hiroki, Aiko Aizawa, Kazuki Mori, Hiroko Kozuka-Hata, and Masaaki Oyama.

Abnormal expression of histone deacetylases (HDACs) in human cancer cells was reported to be associated with angiogenesis, migration, chemotherapy resistance as well as cell differentiation and apoptosis in a wide range of previous studies. Therefore, clinical use of HDAC inhibitors has been discussed as a new therapeutic approach against cancer for a long period. In 2006, suberoylanilide hydroxamic acid (SAHA), a pan-inhibitor targeting HDACs and also known as Vorinostat, was approved by the US Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma. In addition to the anticancer activity against hematologic cancers, SAHA also shows a significant antitumor effect on solid tumors through inducing apoptosis, arresting cell cycle or elevating radiation sensitization. In order to unveil the underlying complex mechanism, we used human HeLa cells as the model platform for analyzing SAHA-responsive elements on a proteomic scale. According to the experimental pre-evaluation through western blotting for acetylated histone H3 and microscopic observation of cell growth under a variety of drug-perturbed conditions, we determined to treat cultured cells with SAHA for 24 h to perform an in-depth quantitative proteomic analysis of SAHA-responsive elements in human HeLa cells. After SAHA treatment, the cells were lysed, trypsin-digested and analyzed by high-resolution nanoflow liquid chromatography- tandem mass spectrometry. As a result of ultra-deep proteomic analysis by Orbitrap Eclipse Tribrid system coupled with Ultimate3000 RSLCnano liquid chromatography technology, a total of 5,135 proteins was identified using Proteome Discoverer software. Approximately 8 % of the identified proteins were found to be differentially regulated with more than two-fold changes in response to SAHA treatment by Label Free Quantification (LFQ). The subsequent pathway analysis based on Kyoto Encyclopedia of Genes and Genomes (KEGG) indicated that cell cycle and anti-apoptotic pathway elements including p27 and HO-1 were prominently correlated with SAHA-dependent regulation in human HeLa

cells.

4. Real-Time Search-Assisted Multiplexed Quantitative Proteomics Reveals System-Wide Translational Regulation of Non-Canonical Short Open Reading Frames

Hiroko Kozuka-Hata, Tomoko Hiroki, Naoaki Miyamura, Aya Kitamura, Kouhei Tsumoto, Jun-ichiro Inoue, and Masaaki Oyama.

Abnormal expression of histone deacetylases (HDACs) is reported to be associated with angiogenesis, metastasis and chemotherapy resistance regarding cancer in a wide range of previous studies. Suberoylanilide hydroxamic acid (SAHA) is well known to function as a pan-inhibitor for HDACs and recognized as one of the therapeutic drug candidates to epigenetically coordinate cancer cell fate regulation on a genomic scale. Here, we established a Real-Time Search-assisted mass spectrometric platform for system-wide quantification of translated products encoded by non-canonical short open reading frames (ORFs) as well as already annotated protein coding sequences (CDSs) on the human transcriptome and applied this methodology to quantitative proteomic analyses of SAHA-treated human HeLa cells to evaluate proteome-wide regulation in response to drug perturbation. Very intriguingly, our RTS-based in-depth proteomic analysis enabled us to identify approximately 5000 novel peptides from the ribosome profiling-based short ORFs encoded in the diversified regions on presumed 'non-coding' nucleotide sequences of mRNAs as well as lncRNAs and nonsense mediated decay (NMD) transcripts. Furthermore, TMT-based multiplex large-scale quantification of the whole proteome changes upon differential SAHA treatment unveiled dose-dependent selective translational regulation of a limited fraction of the non-canonical short ORFs in addition to key cell cycle/proliferation-related molecules such as UBE2C, CENPF and PRC1. Our study provided the first system-wide landscape of drug-perturbed translational modulation on both canonical and non-canonical proteome dynamics in human cancer cells.

5. Ultra-deep single-cell proteomic analysis of patient-derived glioblastoma cells

Hiroko Kozuka-Hata, Tomoko Hiroki, Aya Kitamura, Naoaki Miyamura, Tetsu Akiyama, Jun-ichiro Inoue, Kouhei Tsumoto, and Masaaki Oyama.

Single-cell analysis is an essential technique for understanding cellular diversity by analyzing mutually unique data from individual cells. The heterogeneity of cancer cells is known to be deeply involved in drug resistance and poor prognosis, thus there is a strong demand for the development of effective cancer therapies based on single-cell analysis. While single-cell transcriptome analysis has rapidly become widespread with the evolution of next-generation sequencers, single-cell proteome analysis is still an emerging approach due to technical limitations in detection sensitivity. However, since proteins more directly reflect cellular functions compared to RNA molecules, it is highly anticipated that in-depth single-cell proteome analysis, based on recent advancements in mass spectrometry technology, will provide more detailed molecular network information on individual cell regulation. Previously, we conducted a high-precision quantitative phosphoproteome analysis of cancer stem cells (GB2) derived from glioblastoma patients to elucidate the signaling mechanisms leading to very high malignant properties with a five-year survival rate of less than 10%. Our analysis revealed that stimulation by epidermal growth factor (EGF), which controls stemness maintenance of these cells, activated mTORC1 and highly phosphorylated the downstream ribosomal protein S6 (Kozuka-Hata et al., PLoS One, 2012). Since mTOR inhibitors exert antitumor effects by inhibiting the functions of various factors necessary for cancer cell proliferation and angiogenesis, we applied Torin 1, a representative mTOR inhibitor, for EGF signaling perturbation of GB2 cells and newly established an experimental system to evaluate EGF-dependent protein expression changes at the single-cell level. Our integrative single-cell proteomic measurement using advanced Orbitrap MS instruments unveiled Torin 1-dependent global dynamics of more than 6,000 proteins including novel peptides encoded by non-canonical translation at single-cell resolution.

<Group II>

Biomolecular recognition is based on collective and specific non-covalent interactions between discrete biological molecules. Our laboratory studies various protein systems, for instance, antibody-antigen and protein-ligand complexes, to understand quantitatively how these coordinated non-covalent interactions contribute to their specific recognition in biological and artificial systems. We seek to elucidate the molecular mechanisms by which biological molecules obtain high-specificity and affinity from multiple angles using advanced instrumentation. To produce functional molecules with higher performance and better properties, we aim to build a solid foundation from which to develop drugs that modulate specific interactions between biomolecules and ultimately to understand the principles of molecular interactions in our lives.

1. Experimental modification in thermal stability of oligomers by alanine substitution and site saturation mutagenesis of interfacial residues

Hoya M, Matsunaga R, Nagatoishi S, and Tsumoto K.

For certain industrial applications, the stability of protein oligomers is important. In this study, we demonstrated an efficient method to improve the thermal stability of oligomers using the trimeric protein chloramphenicol acetyltransferase (CAT) as the model. We substituted all interfacial residues of CAT with alanine to detect residues critical for oligomer stability. Mutation of six of the forty-nine interfacial residues enhanced oligomer thermal stability. Site saturation mutagenesis was performed on these six residues to optimize the side chains. About 15% of mutations enhanced thermal stability by more than 0.5 °C and most did not disrupt activity of CAT. Certain combinations of mutations further improved thermal stability and resistance against heat treatment. The quadruple mutant, H17V/N34S/F134A/D157C, retained the same activity as the wild-type after heat treatment at 9 °C higher temperature than the wild-type CAT. Furthermore, combinations with only alanine substitutions also improved thermal stability, suggesting the method we developed can be used for rapid modification of industrially important proteins.

2. IL-6 Reduces Spheroid Sizes of Osteophytic Cells Derived from Osteoarthritis Knee Joint via Induction of Apoptosis

Negishi Y, Adili Arepati, Susana de Vega, Momoeda M, Kaneko H, Mehmet Zeynel Cilek, Yoshinaga C, Takafuji K, Otsuka Y, Shimoda M, Negishi-Koga T, Ishijima M, and Okada Y

Osteophytes in osteoarthritis (OA) joints contribute to restriction of joint movement, joint pain, and OA progression, but little is known about osteophyte regulators. Examination of gene expression related to cartilage extracellular matrix, endochondral ossification, and growth factor signaling in articular cartilage and osteophytes obtained from OA knee joints showed that several genes such as COL1A1, VCAN, BGLAP, BMP8B, RUNX2, and SOST were overexpressed in osteophytes compared with articular cartilage. Ratios of mesenchymal stem/progenitor cells, which were characterized by co-expression of CD105 and CD166, were significantly higher in osteophytic cells than articular cells. A three-dimensional culture method for cartilage and osteophyte cells was developed by modification of cultures of self-assembled spheroid cell organoids (spheroids). These spheroids cultured in the media for mesenchymal stem cells containing transforming growth factor-β3 showed characteristic morphologies and gene expression profiles of articular cartilage and osteophytes, respectively. The effects of IL-1β, tumor necrosis factor-α, and IL-6 on the spheroids of articular and osteophytic cells were studied. To the best of our knowledge, they provide the first evidence that IL-6 suppresses the spheroid size of osteophytic cells by inducing apoptosis and reducing extracellular matrix molecules. These data show that IL-6 is the suppressor of osteophyte growth and suggest that IL-6 expression and/or activity are implicated in the regulation of osteophyte formation in pathologic joints.

3. Generation of antibodies to an extracellular region of the transporters Glut1/Glut4 by immunization with a designed antigen

Sumikawa T, Nakakido M, Matsunaga R, Kuroda D, Nagatoishi S, and Tsumoto K.

Monoclonal antibodies are one of the fastest growing class of drugs. Nevertheless, relatively few biologics target multispanning membrane proteins because of technical challenges. To target relatively small extracellular regions of multiple membrane-spanning proteins, synthetic peptides, which are composed of amino acids corresponding to an extracellular region of a membrane protein, are often utilized in antibody discovery. However, antibodies to these peptides often do not recognize parental membrane proteins. In this study, we designed fusion proteins in which an extracellular helix of the membrane protein glucose transporter 1 (Glut1) was grafted onto the scaffold protein Adhiron. In the initial design, the grafted fragment did not form a helical conformation. Molecular dynamics simulations of full-length Glut1 suggested the importance of intramolecular interactions formed by surrounding residues in the formation of the helical conformation. A fusion protein designed to maintain such intramolecular interactions did form the desired helical conformation in the grafted region. We then immunized an alpaca with the designed fusion protein and obtained VHH (variable region of heavy-chain antibodies) using the phage display method. The binding of these VHH antibodies to the recombinant Glut1 protein was evaluated by surface plasmon resonance, and their binding to Glut1 on the cell membrane was further validated by flow cytometry. Furthermore, we also succeeded in the generation of a VHH against another integral membrane protein, glucose transporter 4 (Glut4) with the same strategy. These illustrates that our combined biochemical and computational approach can be applied to designing other novel fusion proteins for generating site-specific antibodies.

4. Unveiling the affinity-stability relationship in anti-measles virus antibodies: a computational approach for hotspots prediction

Paul R, Kasahara K, Sasaki J, Pérez JF, Matsunaga R, Hashiguchi T, Kuroda D, and Tsumoto K.

Recent years have seen an uptick in the use of computational applications in antibody engineering. These tools have enhanced our ability to predict interactions with antigens and immunogenicity, facilitate humanization, and serve other critical functions. However, several studies highlight the concern of potential trade-offs between antibody affinity and stability in antibody engineering. In this study, we analyzed anti-measles virus antibodies as a case study, to examine the relationship between binding affinity and stability, upon identifying the binding hotspots. We leverage in silico tools like Rosetta and FoldX, along with molecular dynamics (MD) simulations, offering a cost-effective alternative to traditional in vitro mutagenesis. We introduced a pattern in identifying key residues in pairs, shedding light on hotspots identification. Experimental physicochemical analysis validated the predicted key residues by confirming significant decrease in binding affinity for the high-affinity antibodies to measles virus hemagglutinin. Through the nature of the identified pairs, which represented the relative hydropathy of amino acid side chain, a connection was proposed between affinity and stability. The findings of the study enhance our understanding of the interactions between antibody and measles virus hemagglutinin. Moreover, the implications of the observed correlation between binding affinity and stability extend beyond the field of anti-measles virus antibodies, thereby opening doors for advancements in antibody research.

5. THOUSAND-GRAIN WEIGHT 6, which is an IAA-glucose hydrolase, preferentially recognizes the structure of the indole ring

Akabane T, Suzuki N, Ikeda K, Yonezawa T, Nagatoishi S, Matsumura H, Yoshizawa T, Tsuchiya W, Kamino S, Tsumoto K, Ishimaru K, Katoh E, and Hirotsu N.

An indole-3-acetic acid (IAA)-glucose hydrolase, THOUSAND-GRAIN WEIGHT 6 (TGW6), negatively regulates the grain weight in rice. TGW6 has been used as a target for breeding increased rice yield. Moreover, the activity of TGW6 has been thought to involve auxin homeostasis, yet the details of this putative TGW6 activity remain unclear. Here, we show the three-dimensional structure and substrate preference of TGW6 using X-ray crystallography, thermal shift assays and fluorine nuclear magnetic resonance (19F NMR). The crystal structure of TGW6 was determined at 2.6 Å resolution and exhibited a six-bladed β-propeller structure. Thermal shift assays revealed that TGW6 preferably interacted with indole compounds among the tested substrates, enzyme products and their analogs. Further analysis using 19F NMR with 1,134 fluorinated fragments emphasized the importance of indole fragments in recognition by TGW6. Finally, docking simulation analyses of the substrate and related fragments in the presence of TGW6 supported the interaction specificity for indole compounds. Herein, we describe the structure and substrate preference of TGW6 for interacting with indole fragments during substrate recognition. Uncovering the molecular details of TGW6 activity will stimulate the use of this enzyme for increasing crop yields and contributes to functional studies of IAA glycoconjugate hydrolases in auxin homeostasis.

6. Structural basis for the recognition of human hemoglobin by the heme-acquisition protein Shr from Streptococcus pyogenes

Senoo A, Hoshino M, Shiomi T, Nakakido M, Nagatoishi S, Kuroda D, Nakagawa I, Tame JRH, Caaveiro JMM, and Tsumoto K.

In Gram-positive bacteria, sophisticated machineries to acquire the heme group of hemoglobin (Hb) have evolved to extract the precious iron atom contained in it. In the human pathogen Streptococcus pyogenes, the Shr protein is a key component of this machinery. Herein we present the crystal structure of hemoglobin-interacting domain 2 (HID2) of Shr bound to Hb. HID2 interacts with both, the protein and heme portions of Hb, explaining the specificity of HID2 for the heme-bound form of Hb, but not its heme-depleted form. Further mutational analysis shows little tolerance of HID2 to interfacial mutations, suggesting that its interaction surface with Hb could be a suitable candidate to develop efficient inhibitors abrogating the binding of Shr to Hb.

7. A high-resolution structural characterization and physicochemical study of how a peptoid binds to an oncoprotein MDM2

Yokomine M, Morimoto J, Fukuda Y, Ueda T, Takeuchi K, Umezawa K, Ago H, Matsuura H, Ueno G, Senoo A, Nagatoishi S, Tsumoto K, and Sando S.

Peptoids are a promising drug modality targeting disease-related proteins, but how a peptoid engages in protein binding is poorly understood. This is primarily due to a lack of high-resolution peptoid-protein complex structures and systematic physicochemical studies. Here, we present the first crystal structure of a peptoid bound to a protein, providing high-resolution structural information about how a peptoid binds to a protein. We previously reported a rigid peptoid, oligo(N-substituted alanine) (oligo-NSA), and developed an oligo-NSA-type peptoid that binds to MDM2. X-ray crystallographic analysis of the peptoid bound to MDM2 showed that the peptoid recognizes the MDM2 surface predominantly through the interaction of the N-substituents, while the main chain acts as a scaffold. Additionally, conformational, thermodynamic, and kinetic analysis of the peptoid

and its derivatives with a less rigid main chain revealed that rigidification of the peptoid main chain contributes to improving the protein binding affinity. This improvement is thermodynamically attributed to an increased magnitude of the binding enthalpy change, and kinetically to an increased association rate and decreased dissociation rate. This study provides invaluable insights into the design of protein-targeting peptoids.

8. Thermodynamic and molecular dynamic insights into how fusion influences peptide-tag recognition of an antibody

Miyanabe K, Yamashita T, and Tsumoto K.

To understand the effect of protein fusion on the recognition of a peptide-tag by an antibody, we fused a CCR5-derived peptide-tag (pep1) to GFP and investigated its recognition by an anti-pep1 antibody, 4B08. First, to characterize the thermodynamic properties associated with the pep1-4B08 binding, isothermal titration calorimetry experiments were conducted. It was found that pep1 fused to the C-terminus of GFP (GFP-CT) enhanced the enthalpic gain by 2.1 kcal mol-1 and the entropic loss only by 0.9 kcal mol-1, resulting in an 8-fold increase in the binding affinity compared to the unfused pep1. On the other hand, pep1 fused to the N-terminus of GFP (GFP-NT) enhanced the enthalpic gain by 3.0 kcal mol-1 and the entropic loss by 3.2 kcal mol-1, leading to no significant enhancement of the binding affinity. To gain deeper insights, molecular dynamics simulations of GFP-NT, GFP-CT, and pep1 were performed. The results showed that the location of the fusion point sensitively affects the interaction energy, the solvent accessible surface area, and the fluctuation of pep1 in the unbound state, which explains the difference in the experimental thermodynamic properties.

9. Functional insights of Tyr37 in framework region 2 directly contributing to the binding affinities and dissociation kinetics in single-domain VHH antibodies

Yamamoto K, Nagatoishi S, Nakakido M, Kuroda D, and Tsumoto K.

Single-domain VHH antibody is regarded as one of the promising antibody classes for therapeutic and diagnostic applications. VHH antibodies have amino acids in framework region 2 that are distinct from those in conventional antibodies, such as the Val-37Phe/Tyr (V37F/Y) substitution. Correlations between the residue type at position 37 and the conformation of the CDR3 in VHH antigen recognition have been previously reported. However, few studies focused on the meaning of harboring two residue types in position 37 of VHH antibodies, and the concrete roles of Y37 have been little to be elucidated. Here, we investigated the functional states of position 37 in co-crystal structures and performed analyses of three model antibodies with either F or Y at position 37. Our analysis indicates that Y at position 37 enhances the dissociation rate, which is highly correlated with drug efficacy. Our findings help to explain the molecular mechanisms that distinguish VHH antibodies from conventional antibodies.

10. Cryo-EM structures elucidate the multiligand receptor nature of megalin

Goto S, Tsutsumi A, Lee Y, Hosojima M, Kabasawa H, Komochi K, Nagatoishi S, Takemoto K, Tsumoto K, Nishizawa T, Kikkawa M, and Saito A.

Megalin (low-density lipoprotein receptor-related protein 2) is a giant glycoprotein of about 600 kDa, mediating the endocytosis of more than 60 ligands, including those of proteins, peptides, and drug compounds [S. Goto, M. Hosojima, H. Kabasawa, A. Saito, Int. J. Biochem. Cell Biol. 157, 106393 (2023)]. It is expressed predominantly in renal proximal tubule epithelial cells, as well as in the brain, lungs, eyes, inner ear, thyroid gland, and placenta. Megalin is also known to mediate the endocytosis of toxic compounds, particularly those that cause renal and hearing disorders [Y. Hori et al., J. Am. Soc. Nephrol. 28, 1783-1791 (2017)]. Genetic megalin deficiency causes Donnai-Barrow syndrome/facio-oculo-acoustico-renal syndrome in humans. However, it is not known how megalin interacts with such a wide variety of ligands and plays pathological roles in various organs. In this study, we elucidated the dimeric architecture of megalin, purified from rat kidneys, using cryoelectron microscopy. The maps revealed the densities of endogenous ligands bound to various regions throughout the dimer, elucidating the multiligand receptor nature of megalin. We also determined the structure of megalin in complex with receptor-associated protein, a molecular chaperone for megalin. The results will facilitate further studies on the pathophysiology of megalin-dependent multiligand endocytic pathways in multiple organs and will also be useful for the development of megalin-targeted drugs for renal and hearing disorders, Alzheimer's disease [B. V. Zlokovic et al., Proc. Natl. Acad. Sci. U.S.A. 93, 4229-4234 (1996)], and other illnesses.

11. Next-Generation Anti-TNFα Agents: The Example of Ozoralizumab

Tsumoto K and Takeuchi T.

Biologic therapy involving anti-tumor necrosis factor-α (anti-TNFα) agents has fundamentally changed the management of patients with immune-mediated inflammatory diseases, including

rheumatoid arthritis, thus benefiting many patients. Nevertheless, the inability of some patients to achieve low disease activity or clinical remission remains a major concern. To address such concerns, next-generation anti-TNFα agents that differ from the immunoglobulin G-format anti-TNFα agents that have been used to date are being developed using antibody-engineering technology. Their unique design employing novel molecular characteristics affords several advantages, such as early improvement of clinical symptoms, optimization of drug bioavailability, enhancement of tissue penetration, and a reduction in side effects. This holds promise for a new paradigm shift in biologic therapy via the use of next-generation anti-TNFα agents. Ozoralizumab, a next-generation anti-TNFα agent that was recently approved in Japan, comprises a variable region heavy-chain format. It has a completely different structure from conventional therapeutic antibodies, such as a small molecular size, an albumin-binding module, and a unique format that produces an avidity effect. Ozoralizumab exhibited rapid biodistribution into joints, provided attenuation of Fcγ receptor-mediated inflammatory responses, and had a high binding affinity to TNFα in non-clinical studies. In clinical trials, ozoralizumab yielded an early improvement in clinical symptoms, a sustained efficacy for up to 52 weeks, and an acceptable tolerability in patients with rheumatoid arthritis. This review focuses on the results of pre-clinical and clinical trials for ozoralizumab and outlines the progress in next-generation antibody development.

12. Characterization of a novel format scFv×VHH single-chain biparatopic antibody against metal binding protein MtsA

Asano R, Takeuchi M, Nakakido M, Ito S, Aikawa C, Yokoyama T, Senoo A, Ueno G, Nagatoishi S, Tanaka Y, Nakagawa I, and Tsumoto K.

Biparatopic antibodies (bpAbs) are engineered antibodies that bind to multiple different epitopes within the same antigens. bpAbs comprise diverse formats, including fragment-based formats, and choosing the appropriate molecular format for a desired function against a target molecule is a challenging task. Moreover, optimizing the design of constructs requires selecting appropriate antibody modalities and adjusting linker length for individual bpAbs. Therefore, it is crucial to understand the characteristics of bpAbs at the molecular level. In this study, we first obtained single-chain variable fragments and camelid heavy-chain variable domains targeting distinct epitopes of the metal binding protein MtsA and then developed a novel format single-chain bpAb connecting these fragment antibodies with various linkers. The physicochemical properties, binding activities, complex formation states with antigen, and functions of the bpAb were analyzed using multiple approaches. Notably, we found that the assembly state of the complexes was controlled by a linker and that longer linkers tended to form more compact complexes. These observations provide detailed molecular information that should be considered in the design of bpAbs.

13. Crystal structures of human CD40 in complex with monoclonal antibodies dacetuzumab and bleselumab

Asano R, Nakakido M, Pérez JF, Ise T, Caaveiro JMM, Nagata S, and Tsumoto K.

CD40 is a member of the tumor necrosis factor receptor superfamily, and it is widely expressed on immune and non-immune cell types. The interaction between CD40 and the CD40 ligand (CD40L) plays an essential function in signaling, and the CD40/CD40L complex works as an immune checkpoint molecule. CD40 has become a therapeutic target, and a variety of agonistic/antagonistic anti-CD40 monoclonal antibodies (mAbs) have been developed. To better understand the mode of action of anti-CD40 mAbs, we determined the X-ray crystal structures of dacetuzumab (agonist) and bleselumab (antagonist) in complex with the extracellular domain of human CD40, respectively. The structure reveals that dacetuzumab binds to CD40 on the top of cysteine-rich domain 1 (CRD1), which is the domain most distant from the cell surface, and it does not compete with CD40L binding. The binding interface of bleselumab spread between CRD2 and CRD1, overlapping with the binding surface of the ligand. Our results offer important insights for future structural and functional studies of CD40 and provide clues to understanding the mechanism of biological response. These data can be applied to developing new strategies for designing antibodies with more therapeutic efficacy.

14. High-throughput system for the thermostability analysis of proteins

Ito S, Matsunaga R, Nakakido M, Komura D, Katoh H, Ishikawa S, and Tsumoto K.

Thermal stability of proteins is a primary metric for evaluating their physical properties. Although researchers attempted to predict it using machine learning frameworks, their performance has been dependent on the quality and quantity of published data. This is due to the technical limitation that thermodynamic characterization of protein denaturation by fluorescence or calorimetry in a high-throughput manner has been challenging. Obtaining a melting curve that derives solely from the target protein requires laborious purification, making it far from practical to prepare a hundred or more samples in a single workflow. Here, we aimed to overcome this throughput

limitation by leveraging the high protein secretion efficacy of Brevibacillus and consecutive treatment with plate-scale purification methodologies. By handling the entire process of expression, purification, and analysis on a per-plate basis, we enabled the direct observation of protein denaturation in 384 samples within 4 days. To demonstrate a practical application of the system, we conducted a comprehensive analysis of 186 single mutants of a single-chain variable fragment of nivolumab, harvesting the melting temperature (Tm) ranging from -9.3 up to +10.8°C compared to the wild-type sequence. Our findings will allow for data-driven stabilization in protein design and streamlining the rational approaches.

15. Impact of single-residue mutations on protein thermal stability: The case of threonine 83 of BC2L-CN lectin

Hoya M, Matsunaga R, Nagatoishi S, Ide T, Kuroda D, and Tsumoto K.

The thermal stability of trimeric lectin BC2L-CN was investigated and found to be considerably altered when mutating residue 83, originally a threonine, located at the fucose-binding loop. Mutants were analyzed using differential scanning calorimetry and isothermal microcalorimetry. Although most mutations decreased the affinity of the protein for oligosaccharide H type 1, six mutations increased the melting temperature (Tm) by >5 °C; one mutation, T83P, increased the Tm value by 18.2 °C(T83P, Tm = 96.3 °C). In molecular dynamic simulations, the investigated thermostable mutants, T83P, T83A, and T83S, had decreased fluctuations in the loop containing residue 83. In the T83S mutation, the side-chain hydroxyl group of serine formed a hydrogen bond with a nearby residue, suggesting that the restricted movement of the side-chain resulted in fewer fluctuations and enhanced thermal stability. Residue 83 is located at the interface and near the upstream end of the equivalent loop in a different protomer; therefore, fluctuations by this residue likely propagate throughout the loop. Our study of the dramatic change in thermal stability by a single amino acid mutation provides useful insights into the rational design of protein structures, especially the structures of oligomeric proteins.

16. PDZD8-FKBP8 tethering complex at ER-mitochondria contact sites regulates mitochondrial complexity

Nakamura K, Aoyama-Ishiwatari S, Nagao T, Paaran M, Obara CJ, Sakurai-Saito Y, Johnston J, Du Y, Suga S, Tsuboi M, Nakakido M, Tsumoto K, Kishi Y, Gotoh Y, Kwak C, Rhee HW, Seo JK, Kosako H, Potter C, Carragher B, Lippincott-Schwartz J, Polleux F, and Hirabayashi Y.

Mitochondria-ER membrane contact sites (MERCS) represent a fundamental ultrastructural feature underlying unique biochemistry and physiology in eukaryotic cells. The ER protein PDZD8 is required for the formation of MERCS in many cell types, however, its tethering partner on the outer mitochondrial membrane (OMM) is currently unknown. Here we identified the OMM protein FKBP8 as the tethering partner of PDZD8 using a combination of unbiased proximity proteomics, CRISPR-Cas9 endogenous protein tagging, Cryo-Electron Microscopy (Cryo-EM) tomography, and correlative light-EM (CLEM). Single molecule tracking revealed highly dynamic diffusion properties of PDZD8 along the ER membrane with significant pauses and capture at MERCS. Overexpression of FKBP8 was sufficient to narrow the ER-OMM distance, whereas independent versus combined deletions of these two proteins demonstrated their interdependence for MERCS formation. Furthermore, PDZD8 enhances mitochondrial complexity in a FKBP8-dependent manner. Our results identify a novel ER-mitochondria tethering complex that regulates mitochondrial morphology in mammalian cells.

17. Development of novel humanized VHH synthetic libraries based on physicochemical analyses

Nakakido M, Kinoshita S, and Tsumoto K.

Due to the high affinity and specificity of antibodies toward antigens, various antibody-based applications have been developed. Recently, variable antigen-binding domains of heavy-chain antibodies (VHH) have become an attractive alternative to conventional fragment antibodies due to their unique molecular characteristics. As an antibody-generating strategy, synthetic VHH libraries (including humanized VHH libraries) have been developed using distinct strategies to constrain the diversity of amino acid sequences. In this study, we designed and constructed several novel synthetic humanized VHH libraries based on biophysical analyses conducted using the complementarity determining region-grafting method and comprehensive sequence analyses of VHHs deposited in the protein data bank. We obtained VHHs from the libraries, and hit clones exhibited considerable thermal stability. We also found that VHHs from distinct libraries tended to have different epitopes. Based on our results, we propose a strategy for generating humanized VHHs with distinct epitopes toward various antigens by utilizing our library combinations.

18. Systematic Preparation of a 66-IgG Library with Symmetric and Asymmetric Homogeneous Glycans and Their Functional Evaluation

Manabe S, Iwamoto S, Nagatoishi S, Hoshinoo A, Mitani A, Sumiyoshi W, Kinoshita T, Yamaguchi Y, and Tsumoto K.

Immunoglobulin G (IgG) antibodies possess a conserved N-glycosylation site in the Fc domain. In FcγRIIIa affinity column chromatography, unglycosylated, hemiglycosylated, and fully glycosylated IgG retention times differ considerably. Using retention-time differences, 66 different trastuzumab antibodies with symmetric and asymmetric homogeneous glycans were prepared systematically, substantially expanding the scope of IgGs with homogeneous glycans. Using the prepared trastuzumab with homogeneous glycans, thermal stability and antibody-dependent cellular cytotoxicity were investigated. In some glycan series, a directly proportional relationship was observed between the thermal unfolding temperature (Tm) and the calorimetric unfolding heat (ΔHcal). Antibody function could be deduced from the combination of a pair of glycans in an intact form. Controlling glycan structure through the combination of a pair of glycans permits the precise tuning of stability and effector functions of IgG. Overall, our technology can be used to investigate the effects of glycans on antibody functions.

19. Triphenylphosphonium-modified catiomers enhance in vivo mRNA delivery through stabilized polyion complexation

Norimatsu J, Mizuno HL, Watanabe T, Obara T, Nakakido M, Tsumoto K, Cabral H, Kuroda D, and Anraku Y.

Nanocarriers based on cationic materials play a central role in the success of mRNA-based therapies. Traditionally, amine-bearing lipids and polymers have been successfully employed for creating mRNA-loaded nanocarriers, though they still present challenges, such as physical and biological instability, limiting both delivery efficiency and therapeutic potential. Non-amine cations could be a promising avenue in addressing these limitations. However, such alternatives remain notably underexplored. Herein, we introduced triphenylphosphonium (TPP) as an alternative cationic moiety for mRNA delivery, leveraging its advantageous properties for nucleic acid complexation. Through the modification of amine-bearing catiomers, we replaced traditional amine-based counterparts with TPP to create innovative polymeric micelles as mRNA nanocarriers. A comprehensive analysis, encompassing physicochemical, thermodynamic, and computational approaches, revealed that the TPP substitution significantly influenced polymer self-assembly, mRNA binding, and the overall stability of mRNA-loaded polymeric micelles. Upon intravenous injection, TPP-bearing micelles demonstrated a remarkable increase in mRNA bioavailability, facilitating efficient protein production in solid tumors. These findings provide a compelling rationale for substituting amines with TPP, emphasizing their potential for advancing mRNA therapeutics.

20. Unveiling the structural mechanisms behind high affinity and selectivity in phosphorylated epitope-specific rabbit antibodies

Kasahara K, Kawade R, Nakakido M, Matsunaga R, Akiba H, Entzminger KC, Maruyama T, Okumura SCJ, Caaveiro JMM, Kuroda D, and Tsumoto K.

Protein phosphorylation is a crucial process in various cellular functions, and its irregularities have been implicated in several diseases, including cancer. Antibodies are commonly employed to detect protein phosphorylation in research. However, unlike the extensive studies on recognition mechanisms of the phosphate group by proteins such as kinases and phosphatases, only a few studies have explored antibody mechanisms. In this study, we produced and characterized two rabbit monoclonal antibodies that recognize a monophosphorylated Akt peptide. Through crystallography, thermodynamic mutational analyses, and molecular dynamics simulations, we investigated the unique recognition mechanism that enables higher binding affinity and selectivity of the antibodies compared to other generic proteins with lower binding affinity to phosphorylated epitopes. Our results demonstrate that molecular dynamics simulations provide novel insights into the dynamic aspects of molecular recognition of posttranslational modifications by proteins beyond static crystal structures, highlighting how specific atomic level interactions drive the exceptional affinity and selectivity of antibodies.

21. Structural basis for the ligand promiscuity of the hydroxamate siderophore binding protein FtsB from Streptococcus pyogenes

Fernandez-Perez J, Senoo A, Caaveiro JMM, Nakakido M, de Vega S, Nakagawa I, and Tsumoto K.

Pathogenic bacteria must secure the uptake of nutritional metals such as iron for their growth, making their import systems attractive targets for the development of new antimicrobial modalities. In the pathogenic bacterium Streptococcus pyogenes, the iron uptake system FtsABCD transports iron encapsulated by siderophores of the hydroxamate class. However, the inability of S. pyogenes to produce these metabolites makes the biological and clinical relevance of this

route unresolved. Herein, we demonstrated that the periplasmic binding protein FtsB recognizes not only the hydroxamate siderophore ferrichrome, as previously documented, but also ferrioxamine E (FOE), ferrioxamine B (FOB), and bisucaberin (BIS), each of them with high affinity (nM level). Up to seven aromatic residues in the binding pocket accommodate the variable backbones of the different siderophores through CH-π interactions, explaining ligand promiscuity. Collectively, our observations revealed how S. pyogenes exploits the diverse xenosiderophores produced by other microorganisms as iron sources to secure this precious nutrient.

22. Malaria parasites require a divergent heme oxygenase for apicoplast gene expression and biogenesis

Blackwell AM, Jami-Alahmadi Y, Nasamu AS, Kudo S, Senoo A, Slam C, Tsumoto K, Wohlschlegel JA, Manuel Martinez Caaveiro J, Goldberg DE, and Sigala PA.

Malaria parasites have evolved unusual metabolic adaptations that specialize them for growth within heme-rich human erythrocytes. During blood-stage infection, Plasmodium falciparum parasites internalize and digest abundant host hemoglobin within the digestive vacuole. This massive catabolic process generates copious free heme, most of which is biomineralized into inert hemozoin. Parasites also express a divergent heme oxygenase (HO)-like protein (PfHO) that lacks key active-site residues and has lost canonical HO activity. The cellular role of this unusual protein that underpins its retention by parasites has been unknown. To unravel PfHO function, we first determined a 2.8 Å-resolution X-ray structure that revealed a highly α-helical fold indicative of distant HO homology. Localization studies unveiled PfHO targeting to the apicoplast organelle, where it is imported and undergoes N-terminal processing but retains most of the electropositive transit peptide. We observed that conditional knockdown of PfHO was lethal to parasites, which died from defective apicoplast biogenesis and impaired isoprenoid-precursor synthesis. Complementation and molecular-interaction studies revealed an essential role for the electropositive N-terminus of PfHO, which selectively associates with the apicoplast genome and enzymes involved in nucleic acid metabolism and gene expression. PfHO knockdown resulted in a specific deficiency in levels of apicoplast-encoded RNA but not DNA. These studies reveal an essential function for PfHO in apicoplast maintenance and suggest that Plasmodium repurposed the conserved HO scaffold from its canonical heme-degrading function in the ancestral chloroplast to fulfill a critical adaptive role in organelle gene expression.

23. Specific recognition mechanism of an antibody to sulfated tyrosine and its potential use in biological research

Ujiie K, Nakakido M, Kinoshita S, Jose Caaveiro M M, Entzminger, C J Okumura, Maruyama, Miyauchi k, Matano T, and Tsumoto K

Post-translational modification of proteins is a crucial biological reaction that regulates protein functions by altering molecular properties. The specific detection of such modifications in proteins has made significant contributions to molecular biology research and holds potential for future drug development applications. In HIV research, for example, tyrosine sulfation at the N-terminus of C-C chemokine receptor type 5 (CCR5) is considered to significantly enhance HIV infection efficiency. However, antibodies specific to sulfated CCR5 still need to be developed. In this study, we successfully generated an antibody that specifically recognized the sulfated N-terminal peptide of CCR5 through rabbit immunization and panning via phage display using a CCR5 N-terminal peptide containing sulfate modification. We used various physicochemical methods in combination with molecular dynamics simulation to screen for residues that could be involved in recognition of the sulfated peptide by this antibody. We also confirmed that this antibody recognized the sulfated full-length CCR5 on the cell surface, which suggested it should be useful as a research tool that could lead to the development of novel therapeutics. Although the antibody binding did not inhibit HIV infection, it could be also described as sulfation site-specific binding, beyond sulfation-specific binding.

24. The pericellular function of Fibulin-7 in the adhesion of oligodendrocyte lineage cells to neuronal axons during CNS myelination

Yamada M, Sasaki B,Yamada N, Hayashi C, Tsumoto K, de Vega S, and Suzuki N

Myelin is an electrical insulator that enables saltatory nerve conduction and is essential for proper functioning of the central nervous system (CNS). It is formed by oligodendrocytes (OLs) in the CNS, and during OL development various molecules, including extracellular matrix (ECM) proteins, regulate OL differentiation and myelination; however, the role of ECM proteins in these processes is not well understood. Our present work is centered on the analyses of the expression and function of fibulin-7 (Fbln7), an ECM protein of the fibulin family, in OL differentiation. In the expression analysis of Fbln7 in the CNS, we found that it was expressed at early postnatal stage and localized in the processes of OL precursor cells (OPCs), in the inner region of myelin, and in axons. The functional analysis using recombinant Fbln7

protein (rFbln7) revealed that rFbln7 promoted OPC attachment activity via β1 integrin and heparan sulfate receptors. Further, rFbln7 induced the adhesion to neurites and the differentiation of OLs. Altogether, our results show that Fbln7 promotes the adhesion between OLs and axons and OL differentiation.

<Group III>

1. Development of new methods for analyzing neural circuits in the retina

Neural circuits in the central nervous system are the basis of various higher-order brain functions. It is also true in case of retina. In the retina, six main classes of neural cells connect systematically to make up complex neural circuits. Characteristics of the retinal neural cells have been examined mainly by the electrophysiological methods and models of cell connectivity have been proposed. Morphological studies of the actual neural connection, which constitute the physiological properties of retinal neurons, have been desired. Until recently the only method to reveal the three-dimensional (3D) connectivity of actual neural cells morphologically was to collect ultrathin serial sections and observe them in transmission electron microscope (TEM). But the technical difficulties discouraged us from such a troublesome procedure. Recent progress in scanning electron microscope (SEM) equipment allowed us to develop a new method to observe ultrathin TEM sections in SEM (thin section scanning electron microscopy: TSSEM). To collect huge number of serial sections stably and efficiently, we have been developing new equipment and techniques. By using these techniques, it became possible to collect more than 1000 serial sections of less than 30 nm thickness much easier. We have analyzed about 500 serial thin sections of zebrafish retinal outer plexiform layer by this method and succeeded in tracing thin processes of bipolar cells into the photoreceptor terminals. In fish retina, four kinds of cone photoreceptor are arranged in a very regular pattern, forming cone mosaics. Therefore, the first step of color recognition circuit is expected to be morphologically analyzed by using TSSEM method.

TSSEM method developed here was expanded to be used in analyzing mitochondrial 3D structure as a collaborative work. Aside from getting 3D information, TSSEM method can provide us precise information of much wider areas of thin sections more effectively and more easily than transmission electron microscopy. Such studies are also in progress as a collaborative work.

2. Collaborative and supportive works as electron microscope core-laboratory

This group is also engaged in collaborative researches using electron microscope. We offer supports for the research projects those need electron microscopic analysis. The services available in this group are the conventional thin section transmission electron microscopy, immuno-electron microscopy, negative staining techniques and scanning electron microscopy. By using individual technique or combination of some of these, we can offer direct visual evidence that cannot be acquired by other methods. This year, 15 projects in 11 laboratories were performed as core-laboratory works.

a. Thin section transmission electron microscopy

Thin section transmission electron microscopy is the most widely used technique to observe the inner structure of cells and tissues. In this method, samples are fixed and embedded in epoxy resin, thin sections of about 70 nm thickness are cut and observed in the electron microscope. In case of immuno-electron microscopy, thin sections are obtained by similar procedure and the antigen epitopes exposed on the surface of the sections are marked by sequential reaction with appropriate primary antibodies and colloidal gold labeled secondary antibodies. This year, thin section electron microscopy and those combined with immuno-electron microscopy were used in many collaborative works.

a-1. Ultrastructural analysis of entry and assembly of Herpes Simplex Virus

We have been performing several studies with research groups in Dr. Kawaguchi[1]'s laboratory: [1]Division of Molecular Virology, Department of Microbiology and Immunology, regarding the infection/replication processes of herpes simplex virus (HSV). Thin section electron microscopy has been used to analyze the function of viral proteins in trans-nuclear membrane processes of the newly formed viruses. By analyzing the virus forming processes in some mutant host cells, we could analyze viral proteins as well as candidate host molecules those may be involved in the trans-nuclear process of the HSV. TSSEM method was also used to observe a specific virus infected single cell.

a-2. Analysis of calcium-binding protein 7 functions on mouse neuromuscular junctions

We have been performing several studies also with research groups in Dr. Yamanashi[2]'s laboratory: [2] Division of Genetics. This year, we analyzed the functions of calcium-binding protein 7 in mouse muscular cell on the morphology of neuromuscular junctions. In this project, fixed pieces of diaphragm were stained with fluorescently labeled alpha bungarotoxin to reveal the acetylcholine receptor rich postsynaptic membrane, observed on fluorescent microscope and then prepared for electron microscopic samples. Flu-

orescently labeled areas of the diaphragm samples were cut into sections and observed in a transmission microscope. Morphological data combined with biochemical experiments showed that the MuSK-mediated signaling induces muscle expression of Cabp7, which suppresses age-related NMJ degeneration likely by attenuating p25 expression, providing insights into prophylactic/therapeutic intervention against age- related motor dysfunction. (ref. Eguchi *et al*)

Some other collaborative research works using thin section electron microscopy and/or immuno-electron microscopy were performed with Dr. Coban[3], in [3]Division of Malaria Immunology, about the function of Paneth cells on Malaria infection, Dr.Takekawa[4] in [4]Division of Cell Signaling and Molecular Medicine, about the changes in nuclear pore size, Dr. Nakahara[5] in [5]Department of Life Science Dentistry, The Nippon Dental University, Dr. Katayama[6], in [6]Laboratory of Viral Infection, Ohmura Satoshi Memorial Institute, Kitasato University and so on.

b. Negative staining techniques

Negative staining techniques are simple and quick method to observe the morphology of the macro-molecules. This year, negative staining techniques were used to analyze exosomes in collaboration with Dr. Hayashi[7] in [7]Division of Vaccine Science, Laboratory of Adjuvant Innovation.

c. Scanning electron microscopy

Conventional scanning electron microscopy is a technique used to examine the surface structure of the cells, tissues or other non-biological materials. Scanning electron microscopy combined with thin section transmission microscopy were used in collaborative work with Dr. Ishikawa[8], [8]Laboratory of Reproductive Systems Biology, about the structure of young and aged mouse oocyte zona pellucida and about the morphology of the sperm and spermatocyte. (ref. Ishikawa-Yamauchi *et al*) Scanning electron microscopy was also used in the work with Dr. Yamagami[9], [9]Division of Ophthalmology, Department of Visual Sciences, Nihon University School of Medicine.

Thin section scanning electron microscope (TS-SEM) methods are also used as a collaborative work with Dr. Kobayashi[10], [10]Division of Protein Metabolism, to analyze the 3D organization of mitochondria in neural stem cells.

Publications

<Group I>

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<Group III>

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Research Center for Asian Infectious Diseases
アジア感染症研究拠点

Director/Professor	Yasushi Kawaguchi, D.V.M., Ph.D.	拠点長／教授	博士(獣医学)	川 口	寧
Project Professor	Xuan Xuenan, D.V.M., Ph.D.	特任教授	農学博士	玄	学南
Project Professor	Mitsue Hayashi, Ph.D.	特任教授	法学博士	林	光江
Visiting Professor	Masaki Imai, D.V.M., Ph.D.	客員教授	博士(獣医学)	今井	正樹
Visiting Professor	Seiya Yamayoshi, D.V.M., Ph.D.	客員教授	博士(医学)	山吉	誠也
Associate Professor	Akihisa Kato, Ph.D.	准教授	博士(医学)	加藤	哲久
Project Associate Professor	Jin Gohda, Ph.D.	特任准教授	博士(薬学)	合田	仁
Project Senior Assistant Professor	Mizuki Yamamoto, Ph.D.	特任講師	博士(医学)	山本	瑞生
Assistant Professor	Naoto Koyanagi, Ph.D.	助 教	博士(生命科学)	小柳	直人
Assistant Professor	Yuhei Maruzuru, Ph.D.	助 教	博士(生命科学)	丸鶴	雄平

Research Center for Asian Infectious Diseases operates two project laboratories (one in Tokyo; one joint lab in Beijing) and a collaborative program (Harbin), supported by AMED, CAS, and CAAS. The center is conducting research on emerging and reemerging infections, aiming to translate its basic studies into practical use. And the project intends to train and educate young Japanese and Chinese scientists for the future generation.

BACKGROUND

China is an important neighbor of Japan, with geopolitical and economic interdependence. And it contains hot spots for emerging and reemerging infections, as exemplified by the occurrence of SARS coronavirus that shocked the world in 2003 and endemic avian influenza virus occasionally jumping from bird to human. The carrier rate of hepatitis viruses is very high and HIV infection is rapidly increasing. In the early 2000's the Institute of Medical Science, the University of Tokyo, (IMSUT) was looking for appropriate counterparts in China to strengthen the studies of emerging and reemerging infections.

IMSUT initially established three collaboration sites in fiscal 2005 in China, two in Beijing and one in Harbin, and had been conducting China-Japan research collaboration, for two 5-year terms (fiscal 2005-2010; 2010-2015), supported by the Ministry of Education, Culture, Sports, Science and Technology under the directorship of Aikichi Iwamoto, former project director. IMSUT thus set up a new sustainable system that allowed IMSUT scientists to work in China, along with Chinese scientists, focusing on the studies of emerging and reemerging infections. In 2015 Yasushi Kawaguchi succeeded A. Iwamoto as project director and launched the project *China-Japan Research Collaboration on Defense against Emerging and Reemerging Infections*, a 5-year J-GRID program of Japan Agency for Medical Research and Development (AMED). In 2020 based on the results of the previous five years, he launched another project *Studies to Control Emerging, Re-emerging and Imported Infectious Diseases to Be Conducted in International Collaboration Sites in China* under a 5-year AMED program *Japan Program for Infectious Diseases Research and Infrastructure*.

In 2005 IMSUT had founded two joint laboratories in collaboration with Institute of Biophysics (IBP) and Institute of Microbiology (IM), which belong to the Chinese Academy of Sciences (CAS), a large national institution consisting of more than 100 research institutes all over China. IMSUT has dispatched Jin Gohda to IM as a principal investigator (PI). Along with his Chinese staffs, PI is conducting basic and translational studies of HIV, MERS coronavirus, dengue virus and SARS-CoV-2. In 2015 IMSUT has set up another

project laboratory in Tokyo, whose studies complement those in Beijing. IMSUT is also conducting a joint research program on avian influenza virus between Yoshihiro Kawaoka at IMSUT and Hualan Chen at Harbin Veterinary Research Institute (HVRI) of Chinese Academy of Agricultural Sciences. The activities in Beijing and Harbin are supported by Mitsue Hayashi of the Beijing Project Office.

This project, making the most of the opportunity of collaboration with the highly advanced Chinese institution, aims to translate our basic studies into practical use in future. During the course of the collaboration the project intends to train and educate young Chinese and Japanese scientists for the future generation and hopes to contribute to the friendship between the two peoples.

PROJECT LABORATORIES AND PROGRAM

Y. Kawaguchi (Director of Research Center for Asian Infectious Diseases; Project Director) manages the Center and the AMED-supported Project, which includes the domestic and overseas laboratories and program. He coordinates our activities and decides the direction of research. He and his group conduct studies of molecular virology and immunology of herpes virus in the Research Center for Asian Infectious Diseases.

a. Project Laboratory at IMSUT and Joint Laboratory at IMCAS

Enveloped viruses, including Flaviviruses, Herpes Simplex Viruses, and Coronaviruses, exhibit pathogenicity and are clinically significant. The J. Gohda and Y. Kawaguchi research groups are conducting studies to develop antiviral molecules targeting enveloped viruses, including SARS-CoV-2 and Flaviviruses.

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is the causative virus for Coronavirus Disease 2019 (COVID-19) and has spread globally since the first reported case in December 2019 in China. To end the ongoing COVID-19 pandemic, the development of antiviral drugs and vaccines targeting SARS-CoV-2 infection is imperative. We have established a dual split protein-based cell fusion assay utilizing the SARS-CoV-2 spike protein to evaluate the antiviral activity of several molecules, advancing the screening of antibodies and small molecules.

This year, we conducted functional analyses of novel antibodies that exhibit neutralizing activity without binding to the conventional pharmaceutical target, the Receptor Binding Domain (RBD). These antibodies target conserved regions across SARS-CoV-2 variants and related coronaviruses, demonstrating long-term potential and effectiveness, including against recent Omicron variants, by targeting regions essential for viral function. Furthermore, these antibodies are suggested to affect the trimeric structure critical for SARS-CoV-2 membrane fusion. From the analysis of small molecules, multiple inhibitors against TMPRSS2, a crucial host protease for virus infection, were identified from synthetic compounds. Notably, while conventional TMPRSS2 inhibitors exhibited low selectivity and inhibited other serine proteases, the newly identified inhibitors appear to selectively target structural features unique to TMPRSS2. These findings suggest potential applications for treating not only SARS-CoV-2 infection but also other viral infections that utilize TMPRSS2 for entry. Additionally, a compound inhibiting virus entry and proliferation within cells was identified from subtropical plant extracts. Analysis of this compound suggests that it inhibits viral replication by activating intracellular inflammatory signaling pathways.

These inhibitory molecules hold promise for future therapeutic development and contribute significantly to understanding the infection mechanisms of SARS-CoV-2. Identifying the target molecules of these agents is expected to advance the discovery of new infection mechanisms and therapeutic targets.

b. Joint Laboratory at IBPCAS

The Joint Laboratory at IBPCAS was closed in March 2020. However, the research collaboration and academic exchange between IMSUT and IBPCAS is still ongoing.

c. Collaborative research program with HVRI

At the end of 2019, a novel coronavirus (severe acute respiratory syndrome coronavirus 2; SARS-CoV-2) was detected in Wuhan, China, that spread rapidly around the world, with severe consequences for human health and the global economy. In China, highly pathogenic avian influenza (HPAI) H5N1 virus transmitted to humans in 1997; since 2013, low pathogenic avian influenza A H7N9 viruses have caused sporadic infections in humans; and in 2016, HPAI H7N9 viruses emerged raising concerns of a pandemic. For these reasons, HVRI (Director, Zhigao Bu) has been conducting collaborative research on influenza virus, SARS-CoV-2, and other emerging viruses from all over Asia.

HVRI focuses on avian influenza viruses that are circulating in Chinese wild waterfowl, domestic poultry, and swine. Specifically, Y. Kawaoka and his group study type A influenza viruses and SARS-CoV-2 viruses, with an emphasis on viral pathogenicity in various hosts, viral evolution, and viral surveillance.

We made two major findings this year. First, an outbreak of highly pathogenic avian influenza viruses of the H5N1 subtype (HPAI H5N1) occurred in dairy cattle in the USA. We characterized A/Texas/37/2024 (TX37), a virus isolated from the eyes of an infected farm worker. Despite causing mild disease in

the infected worker, TX37 proved lethal in mice and ferrets and spread systemically, with high titers in both respiratory and non-respiratory organs. Importantly, TX37 transmitted by respiratory droplets in 17%–33% of transmission pairs, and five of six exposed ferrets that become infected died. Thus, HPAI H5N1 virus derived from dairy cattle transmits by respiratory droplets in mammals without adaptation and causes lethal disease in animal models. Second, from 2018 to 2021, we identified 287 samples containing H6 and other influenza A viruses in Vietnamese live bird markets, revealing high rates of co-infection among birds. Genetic analyses of 132 unique H6 virus sequences showed that they were mostly similar but exhibited reassortment with other avian influenza viruses. The H6 viruses encoded a single basic amino acid at the HA cleavage site, indicating low pathogenicity in poultry. However, their HA gene contained a motif allowing binding to both avian and human receptors, enabling mammalian infection. These findings indicate the need for close monitoring due to their potential for interspecies transmission.

IMSUT PROJECT OFFICE

The office (M. Hayashi) supports the activities of the joint laboratory in Beijing and the joint research program in Harbin. It serves as Secretariat for Steering Committee Meeting and files MOU and Minutes. It helps scientists visiting the joint laboratory/program for collaborative research. It has been gathering the information about emerging infections in China from the Chinese mass media and official announcements, and the gathered information (in Japanese) has been presented and updated on the website of the Project (http://www.rcaid.jp/).

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Laboratory of Molecular Genetics (Frontier Research Unit)
遺伝子解析施設（フロンティア研究領域）

Professor	Makoto Nakanishi, M.D., Ph.D.	教　授　医学博士	中　西　　真
Associate Professor	Kazuo Tatebayashi, Ph.D.	准教授　博士（薬学）	舘　林　和　夫

The Laboratory of Molecular Genetics was established for developing various molecular genetic techniques, spreading them to IMSUT investigators and supporting security management related to experiments carried out using recombinant DNA technologies. Since 2017, this laboratory has integrated the Frontier Research Unit for supporting selected investigators to challenge new fields of bio-medical sciences.

Frontier Research Unit

Protein phosphorylation and dephosphorylation are among the most important intracellular signaling mechanisms, and are mediated, respectively, by protein kinases and protein phosphatases. We study various aspects of cellular signal transduction with a particular emphasis on the role and regulation of protein phosphorylation and dephosphorylation in cellular stress responses, using yeast cells.

1. Molecular mechanisms of the osmotic-enhancement of Pbs2 MAP2K phosphorylation by Ste11 MAP3K in the yeast osmo-regulatory HOG pathway

Kazuo Tatebayashi

The yeast MAPK Hog1 is activated by hyperosmotic stress through the High Osmolarity Glycerol (HOG) pathway, and orchestrates an array of osmo-adaptive changes in transcription, translation, cell cycle, and metabolism. The current widely held model of the HOG pathway is as follows. The upstream portion of the HOG pathway is comprised of the functionally redundant SHO1 and SLN1 branches. In the SHO1 branch, osmosensing complexes composed of Sho1, Opy2, Hkr1, and Msb2 activate the MAP3K Ste11. In the SLN1 branch, the Sln1-Ypd1-Ssk1 phospho-relay mechanism is involved in activation of the functionally redundant MAP3Ks Ssk2 and Ssk22 (Ssk2/22). Ste11 and Ssk2/22 phosphorylate the MAP2K Pbs2 at Ser-514 and/or Thr-518. Phosphorylated Pbs2 then activates Hog1. We found that osmostress not only activates membrane-associated osmosensors but also enhances Hog1 phosphorylation by mono-phosphorylated Pbs2. The lack of the osmotic enhancement of the Pbs2-Hog1 reaction suppresses Hog1 activation by basal MAP3K activities and prevents pheromone-to-Hog1 crosstalk in the absence of osmostress, which ensures the appropriate Hog1 activation only under high osmolarity. Recently, we found that osmostress enhances Pbs2 MAP2K phosphorylation by Ste11 MAP3K as well as Hog1 phosphorylation. Ste11 is phosphorylated and activated by the Ste20/Cla4 kinases upon high osmolarity. The constitutively-active Ste11-DDD mutant carries the substitution mutations of all activating phosphorylation sites for Ste20/Cla4 to phosphomimic Asp (S281D, S285D, and T286D), which circumvent the need for Ste20/Cla4 for Ste11 activation. The endogenous-level expression of Ste11-DDD did not induce the phosphorylation of Pbs2 under unstimulated condition, but induced it upon high osmolarity even in the *ste20Δ cla4-ts* mutant. These results strongly suggest that Pbs2 phosphorylation by activated Ste11 is osmotically-enhanced as Hog1 is.

This year, we examined the underlying mechanisms of the osmotic-enhancement of Pbs2 phosphorylation by Ste11-DDD. Possible key factors are the adaptor protein Ste50, which binds to Ste11, and the transmembrane osmosensor Sho1, which binds to

Pbs2. We found that the deletion of either *STE50* or *SHO1* abrogated the osmostress-induced phosphorylation of Pbs2 by Ste11-DDD. We previously indicated that osmostress induces the interaction of Ste50 and Sho1. Induced Ste50-Sho1 association may enhance Ste11-Pbs2 interaction indirectly, leading to Pbs2 phosphorylation by activated Ste11 under high osmolarity. To examine this possibility, we investigated the effects of the binding deficient mutants of Sho1, Ste50 and Pbs2. Osmotically-enhanced Pbs2 phosphorylation by Ste11-DDD was abrogated by not only reduced interactions of Sho1-Pbs2 and Ste50-Ste11, but inhibition of induced Sho1-Ste50 association. Taken these results together, we propose that osmostress induces the structural change of Sho1 to bind to Ste50-Ste11 complex, tethering Pbs2 and Ste11 together to allow activated Ste11 to phosphorylate Pbs2.

2. Acetic acid-induced stress granules function as a scaffolding complex for MEK Pbs2 to activate SAPK Hog1

Jongmin Lee[1], Kazuo Tatebayashi, and David E. Levin[1]
[1]Department of Molecular and Cell Biology, Boston University Goldman School of Dental Medicine

Stress-activated protein kinases (SAPKs) respond to a wide variety of stressors. In most cases, the pathways through which specific stress signals are transmitted to the SAPK are not known. We show that the yeast SAPK Hog1 is activated by acetic acid through an intracellular mechanism that does not involve stimulation of the High Osmolarity Glycerol (HOG) signaling pathway beyond its basal level. Rather, acetic acid treatment drives the formation of stress granules, which function as a scaffold to bring Hog1 together with Pbs2, its immediately upstream activating kinase, in a stable assembly that leverages the basal activity of Pbs2 to phosphorylate Hog1. Deletion analysis of stress granule components revealed that the assembly is critical for both the acetic acid-induced activation of Hog1 and its association with Pbs2. Activated Hog1 remains associated with stress granules, which may have implications for its targeting.

Publication

Jongmin, J., Tatebayashi, K. and Levin, DE. Acetic acid-induced stress granules function as a scaffolding complex for MEK Pbs2 to activate SAPK Hog1. J. Cell Biol in press

IMSUT Hospital

Department of Hematology/Oncology
血液腫瘍内科

Professor	Yasuhito Nannya, M.D., Ph.D.	教　授	博士(医学)　南谷泰仁
Project Professor	Satoshi Takahashi, M.D., Ph.D.	特任教授	博士(医学)　高橋　聡
Associate Professor	Tokiko Nagamura-Inoue M.D., Ph.D.	准教授	博士(医学)　長村登紀子
Associate Professor	Takaaki Konuma, M.D., Ph.D.	准教授	博士(医学)　小沼貴晶
Associate Professor	Kazuaki Yokoyama, M.D., Ph.D.	准教授	博士(医学)　横山和明
Assistant Professor	Seiko Kato, M.D., Ph.D.	助　教	博士(医学)　加藤せい子
Assistant Professor	Aki Sato, M.D., Ph.D.	助　教	博士(医学)　佐藤亜紀
Assistant Professor	Koji Jimbo, M.D., Ph.D.	助　教	博士(医学)　神保光児

The Division conducts clinical, pathological, and therapeutic research on hematological diseases, including hematopoietic tumors. In the field of genomic medicine, which has been developing in recent years, research is also being conducted for clinical application. In collaboration with HGC, our laboratory conducts research on clinical sequencing, curation through artificial intelligence, automation and efficiency of clinical implementation, and clinical significance of clinical sequencing. In ATL, we have also developed novel clinical markers to elucidate the prognostic significance of HAS-Flow and predict the development of ATL by measuring PVL in HTLV-1 carriers. In the field of adult histiocytosis, we treat a large number of cases as one of the leading clinical facilities in Japan and are responsible for the development of Japanese guidelines. We are also involved in the elucidation of clonal progression in histiocytosis. We are also working to improve the clinical practice of transplantation through the analysis of HSCT data.

1 Allogeneic hematopoietic cell transplantation for patients with acute myeloid leukemia not in remission.

Flow cytometric profiles with CD7 and CADM1 in CD4+ T cells are promising indicator for prognosis of aggressive ATL

Koji Jimbo1, Toyotaka Kawamata2, Yoshihiro Inamoto3, Ayumu Ito3, Kazuaki Yokoyama1, Aki Sato1, Takahiro Fukuda3, Kaoru Uchimaru1,4, Yasuhito Nannya1

1. Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.
2. Department of Hematology, Tokyo Metropolitan Bokutoh Hospital, Tokyo, Japan.
3. Department of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan.
4. Laboratory of Tumor Cell Biology, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan.

Adult T-cell leukemia/lymphoma (ATL) is a poor prognosis hematological malignancy originating from human T-cell leukemia virus (HTLV)-1-infected CD4＋ T cells. Flow cytometric plots of CADM1 and CD7 in CD4＋ T cells are useful for separating HTLV-1-uninfected T cells and ATL cells. They are indicators of clonal evolution of HTLV-1 infected cells and disease progression of asymptomatic carriers or indolent ATL. However, the impacts of the plots on the clinical course or prognosis of ATL, especially in

aggressive ATL, remain unclear. We focused on the N fraction (CD4 + CADM1 + CD7-) reflecting ATL cells and analyzed the flow cytometric profiles and clinical course of 497 samples from 92 HTLV-1-infected patients that were mainly aggressive ATL. The parameters based on N fractions showed significant correlations with known indicators of ATL disease status (sIL-2R, LDH, abnormal lymphocytes, etc.) and sensitively reflected the treatment response of aggressive ATL. The parameters based on N fractions significantly stratified the prognosis of aggressive ATL at four different time points: before treatment, after one course of chemotherapy, at the best response after chemotherapy, and before allo-HCT. Even after mogamulizumab administration, which shows potent effects for peripheral blood lesions, the N fraction was still a useful indicator for prognostic estimation. In summary, this report shows that CADM1 versus CD7 plots in CD4 + T cells are useful indicators of the clinical course and prognosis of aggressive ATL. Therefore, this CADM1 and CD7 profile is suggested to be a useful prognostic indicator consistently from HTLV-1 carriers to aggressive ATL.

2 Prognosis of aggressive adult T-cell leukemia/lymphoma with central nervous system infiltration and utility of CD7 versus CADM1 flow-cytometric plots of cerebrospinal fluid

Koji Jimbo1,2, Tomohiro Ishigaki2,3, Masataka Sakashita2, Shohei Andoh2, Hirona Ichimura2, Ayumu Ito4, Kazuaki Yokoyama2, Aki Sato2, Takahiro Fukuda4, Kaoru Uchimaru2,5, Yasuhito Nannya1,2

1. **Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.**
2. **Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.**
3. **Department of Laboratory Medicine, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.**
4. **Department of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan.**
5. **Laboratory of Tumor Cell Biology, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan.**

The prognosis of adult T-cell leukemia/lymphoma (ATL) with primary central nervous system (CNS) involvement has been unclear since the advent of new therapies. Recently, we have shown that flow cytometric CD7/CADM1 analysis of CD4 + cells (HAS-Flow) is useful to detect ATL cells that are not morphologically diagnosed as ATL cells. We investigated the role of CNS involvement in ATL using cytology and HAS-Flow by analyzing cerebrospinal fluid (CSF) from 73 aggressive ATL cases. Based on the findings in CSF, the study subjects were classified into CNS + (cytologically malignant, n = 18), CNS- (cytologically non-malignant and ATL cell population negative in HAS-Flow, n = 44), and CNS-Micro (cytologically non-malignant and ATL cell population positive in HAS-Flow, n = 11) groups. As expected, the CNS + group had a shorter overall survival than the CNS- groups (P<0.001). However, the CNS-Micro group showed no adverse impact on overall survival compared to the CNS- group (P = 0.506), even without additional CNS-targeted treatments. HAS-Flow also demonstrated clinical utility in the diagnosis of CSF lesions in ATL patients with cerebral white matter lesions and in the detection of ATL cells on post-treatment CSF examination in patients with CNS involvement. Our study demonstrates that ATL with CNS involvement have a poor prognosis and that CSF HAS-Flow is useful to assist in the diagnosis of suspected CNS involvement and to detect ATL cells with high sensitivity after treatment.

3 Mathematical Model for Long-Term Kinetics of Proviral Load in HTLV-1 Carriers: Prospective Risk Estimation for the Development of Adult T-cell Leukemia/lymphoma

Koji Jimbo1,2, Masanori Nojima3, Keiko Toriuchi4, Makoto Yamagishi5, Makoto Nakashima6, Yoshihisa Yamano6,7, Kaoru Uchimaru2,4, Yasuhito Nannya1,2

1. **Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.**
2. **Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.**
3. **Center for Translational Research, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.**
4. **Laboratory of Tumor Cell Biology, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan.**
5. **Laboratory of Viral Oncology and Genomics, Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, The University of Tokyo, Tokyo, Japan.**
6. **Department of Rare Diseases Research, Institute of Medical Science, St. Marianna University School of Medicine, Kanagawa, Japan**
7. **Department of Neurology, St. Marianna University School of Medicine, Kanagawa, Japan.**

In HTLV-1 asymptomatic carriers (ACs), high proviral load (PVL) is a risk for developing ATL. PVL values has been assumed to be unchanged for each ACs over at least 10 years of observation, and the same

threshold for risk of developing ATL has been applied regardless of age. To determine the dynamics of PVL for each ACs, we applied mathematical analysis for the population data including 1371 samples from 252 ACs (median of 4 samples per case, range:2-15) at wide range of ages (16-79). This study was conducted with cooperation of JSPFAD. Analysis of PVL from the whole samples showed clear trend to increase with age, and trajectory analysis using linear function model for PVL logarithm data revealed six groups based on the intercept and slope of the linear model. Of these, one group (12%) with a high initial value and a high rate of increase was designated as the high risk group, followed by two groups as the high-intermediate (21%) and low-intermediate risk groups (22%) respectively, and three groups with low initial values were designated as the low risk group, of which two groups showed no significant increase along with age. To examine clinical significance of this model, we applied this model to 15 patients who developed from ACs to ATL (not included the model generation), and showed that 12 cases were classified in the high risk, 2 in the high-intermediate risk, and 1 in the low-intermediate risk group, while none were included in the low risk group. This model is expected to be used to prospectively estimate the risk of developing ATL in ACs based on age and PVL value.

4 Multi-hit somatic mosaicism of TP53 pathogenic variants in a patient mimicking Li-Fraumeni syndrome

Kazuaki Yokoyama1, Nozomi Yokoyama2, Aya Shinozaki-Ushiku3, Mika Ito1, Hiroyuki Takamori4, Satoshi Takahashi5, Eigo Shimizu6, Seiya Imoto6, Tomoki Todo7, Yasuhito Nannya1,4

1. **Department of Hematology/Oncology, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan**
2. **Department of Laboratory Medicine, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.**
3. **Department of Pathology, The University of Tokyo Hospital, Tokyo, Japan**
4. **Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.**
5. **Division of Clinical Precision Research Platform, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan**
6. **Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo, Japan**

We present a case of a 38-year-old female initially suspected of having Li-Fraumeni syndrome based on her history of early-onset anaplastic oligodendroglioma and subsequent therapy-related myelodysplastic syndrome. Genetic analysis revealed two pathogenic TP53 variants: c.818G>A p.(Arg273His) and c.659A>C p.(Tyr220Ser). Further investigation using multiple tissue samples demonstrated varying variant allele frequencies, confirming somatic mosaicism rather than germline mutations. This case represents the first report of biallelic TP53 mosaicism, highlighting the challenges in distinguishing between germline and somatic mosaic variants and the implications for cancer risk assessment, surveillance, and genetic counseling (manuscript in preparation).

5 Collaborative Research with Fujitsu Ltd.: Development of AI-based Pathogenicity Prediction System for Gene Fusion in Cancer Research Project: Pathogenicity Prediction of Gene Fusion in Structural Variations: A Knowledge Graph-Infused Explainable Artificial Intelligence (XAI) Framework

Principal Investigators:
From IMSUT: Yasuhito Nannya, Seiya Imoto
From Fujitsu Research: Masaru Fuji
Project Members:
From IMSUT:
Kazuaki Yokoyama, Miho Ogawa, Hidehito Fukushima, Hiroyuki Takamori
From Fujitsu Research:
Shin-Ichiro Tago, Katsuhiko Murakami, Sho Takishita, Hiroaki Morikawa, Rikuhiro Kojima

Project Summary:

This collaborative research between The Institute of Medical Science, The University of Tokyo (IMSUT) and Fujitsu Ltd. aims to develop an innovative AI system for predicting pathogenicity in cancer-related structural variants. By combining IMSUT's expertise in cancer genomics with Fujitsu's advanced AI technology, the project successfully developed a highly accurate prediction system that can also explain its decision-making process, marking a significant step forward in genomic medicine (PMID: 38791993).

6 Genetic Debulking Before Allogeneic Stem Cell Transplantation for Myelodysplastic Syndrome Using the Molecular International Prognostic Scoring System

Masataka Sakashita1, Hirona Ichimura1, Shohei Andoh1, Koji Jimbo1, Aki Sato1, Kazuaki Yokoyama1, Seiko Kato1, Takaaki Konuma1, Tomokazu Seki1, Iku Kamitani1, Hidehito Fukushima1, Hiroyuki Takamori1, Satoshi Takahashi1, Seishi Ogawa2 and Yasuhito Nannya1

1. **Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.**
2. **Department of Pathology and Tumor Biology, Kyoto University, Kyoto, Japan**

Allogeneic hematopoietic stem cell transplantation (aHSCT) is the only curative therapy for patients with myelodysplastic syndrome (MDS). The Revised International Prognostic Scoring System (IPSS-R) is most frequently used for risk stratification of MDS. However, it remains unclear whether bridging therapy before aHSCT improves disease outcomes. Recently, the Molecular International Prognostic Scoring System (IPSS-M), which considers the genetic profile of MDS, has been developed. Using clinical and genetic data, we are currently analyzing the impact of lowering the IPSS-M score before aHSCT.

7 A prospective analytical study of minimal residual disease after allogeneic hematopoietic stem cell transplantation using circulating tumor DNA in acute myeloid leukemia: KSGCT1702

Miho Ogawa[1], Kazuaki Yokoyama[1]
1: Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

【Background】

Post-allogeneic relapse of AML/MDS is an important clinical issue, and there is a need to develop a non-invasive method to detect relapse at an early stage

【Purpose】

To evaluate the usefulness of the minimal residual disease assay, which measures driver mutations identified by comprehensive genetic analysis in post-transplant AML/MDS patients by VAF of circulating tumor DNA in post-transplant patients' serum, in transplant patients in the Kanto Hematopoietic Stem Cell Group.

【Subjects】

Patients diagnosed as AML/MDS cases according to WHO Classification 2008, aged 20 to 65 years, who may undergo allogeneic hematopoietic stem cell transplantation with myeloablative pretreatment at KSGCT participating centers, and whose written consent has been obtained from the patient

Enrollment period: June 2018 - June 2021

Number of patients: 70 patients

Primary endpoint: comparison of one-year cumulative relapse rate by the presence or absence of ctDNA persistence after transplantation

【Method】

Target cases will be enrolled and next-generation sequencing will be performed on tumor and control (oral mucosa) specimens. Driver gene mutations will be identified and a Droplet Digital PCR (ddPCR) assay will be designed. Cell free DNA will be extracted from serum samples before and after bone marrow transplantation, and driver gene mutations will be quantitatively measured using ddPCR.

【Progress】

A total of 70 cases from 12 centers were included in this study. Of these, 12 cases were lost to follow-up before allogeneic haematopoietic stem cell transplantation and 58 cases underwent allogeneic haematopoietic stem cell transplantation. Fifteen cases relapsed within 1 year of transplantation and 10 cases died within 1 year of transplantation, including 1 case of death before transplantation and 4 cases of death due to relapse.

Next-generation sequencing (NGS) was performed in 58 patients to identify driver gene mutations. This identified driver gene mutations in 51 cases that could be targeted for MRD measurement.

The median age of the 51 patients was 53 years (range: 25-65 years) and 60.8% (31 cases) were male. The diagnosis was acute myeloid leukaemia (AML) in 88.2% (45 cases) and myelodysplastic syndrome (MDS) in 11.8% (6 cases). In addition, umbilical cord blood was used as the source of transplantation in 54.9% (28 cases) and the disease status at the time of transplantation was complete remission (CR) in 54.9% (28 cases).

In addition, 74.5% (38 cases) had chromosomal abnormalities at diagnosis and 33.3% (17 cases) had an unfavourable prognosis according to the European LeukemiaNet (ELN) 2022 risk classification.

In the 51 cases in which we identified drivers for MRD measurement, we identified 60 variants for Point mutation and insertion deletion and 10 variants for Fusion as driver gene mutations.

Point mutaion and insertion deletion were more common in NPM1 and NRAS, while fusion was more common in KMT2A related and CBFB-MYH11.

MRD was measured in cell free DNA at 30, 60, and 90 days after transplantation for driver gene mutations in 45 patients. 22, 12, and 13 patients were MRD positive at 30, 60, and 90 days, respectively. MRD-positive cases at 30, 60, and 90 days post-transplant tended to have higher cumulative recurrence rates. Statistical analysis, including survival analysis, and preparation for submission for publication are currently underway.

8 A prospective analytical study of minimal residual disease after allogeneic hematopoietic stem cell transplantation using circulating tumor DNA in acute lymphoid leukemia: KSGCT1901

Miho Ogawa[1], Kazuaki Yokoyama[1]
1: Department of Hematology/Oncology, Research Hospital, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

【Background】

Post-allogeneic relapse of ALL is an important clinical issue, and there is a need to develop a non-invasive method to detect relapse at an early stage

【Purpose】

To evaluate the usefulness of the minimal residual disease assay, which measures driver mutations iden-

tified by comprehensive genetic analysis in post-transplant ALL patients by VAF of circulating tumor DNA in post-transplant patients' serum, in transplant patients in the Kanto Hematopoietic Stem Cell Group.

【Subjects】

Patients 16 years of age or older, acute lymphoblastic leukemia according to WHO classification 2016, any history of chemotherapy at the time of transplantation, specimens with tumor volume of at least 20% available, potential for allogeneic transplantation, and written consent obtained from the patient.

Enrollment: July 2020 - March 2023

Primary endpoint:

Comparison of one-year cumulative recurrence rate by the presence or absence of residual ctDNA after transplantation

Target cases: 55 cases

【Method】

Target cases will be enrolled and next-generation sequencing will be performed on tumor and control (oral mucosa) specimens. Driver gene mutations will be identified and a Droplet Digital PCR (ddPCR) assay will be designed. Cell free DNA will be extracted from serum samples before and after bone marrow transplantation, and driver gene mutations will be quantitatively measured using ddPCR.

【Progress】

There were 54 cases enrolled, 5 cases of pre-transplant dropout, and 43 cases of transplantation performed. NGS of tumor samples was performed in 40 cases and driver gene mutations were identified in 24 cases. A Droplet Digital PCR assay is being designed for each case.

9 Common progenitor origin for Rosai–Dorfman disease and clear cell sarcoma

Aki Sato[1*], Nozomi Yusa[2], Hiroyuki Takamori[3], Eigo Shimizu[4], Kazuaki Yokoyama[1], Satoshi Ichikawa[5,6], Hisayuki Yokoyama[5,7], Yuki Kasahara[8], Kodai Enda[9], Fumiyoshi Fujishima[9,10], Ryo Ichinohasama[11], Yasunori Ota[12], Seiya Imoto[4], Yasuhito Nannya[1,3]

[1]Department of Hematology and Oncology, The Institute of Medical Science Research Hospital, The University of Tokyo, Tokyo, Japan

[2]Department of Laboratory Medicine, The Institute of Medical Science Research Hospital, The University of Tokyo, Japan

[3]Division of Hematopoietic Disease Control, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

[4]Division of Health Medical Intelligence, Human Genome Center, The Institute of Medical Science, The University of Tokyo, Tokyo, Japan

[5]Department of Hematology, Tohoku University Hospital, Sendai, Japan

[6]Department of Hematology and Rheumatology, Tohoku Medical and Pharmaceutical University Hospital, Sendai, Japan

[7]Division of Hematology and Cell Therapy, Yamagata University Hospital, Yamagata, Japan

[8]Department of Medical Oncology, Tohoku University Hospital, Sendai, Japan

[9]Department of Pathology, Tohoku University Hospital, Sendai, Japan

[10]Division of Diagnostic Pathology, Tohoku Medical and Pharmaceutical University, Sendai, Japan [11]Division of Hematopathology, Tohoku University Hospital, Sendai, Japan

[12]Department of Diagnostic Pathology, The Institute of Medical Science Research Hospital, The University of Tokyo, Tokyo, Japan

Histiocytic neoplasms (HNs) in adults have been reported to be associated with a high prevalence of coexisting hematological and solid malignancies. While part of coexisting HNs and hematological malignancies share identical genetic alterations, the genetic association between HNs and solid malignancies has not been scarcely reported. We report a case of Rosai–Dorfman disease (RDD) complicated by coexisting clear cell sarcoma (CCS). RDD is a rare HN. CCS is an ultrarare soft tissue sarcoma with a poor prognosis. Mutation analysis with whole-exome sequencing revealed six shared somatic alterations including *NRAS* p.G12S and *TP53* c.559 + 1G>A in both the RDD and CCS tissue. This is the first evidence of a clonal relationship between RDD hematological and solid malignancies using mutational analysis. We hypothesize that neural crest cells, which originate in CCS, are likely the common cells of origin for RDD and CCS. This case helps to unravel the underlying clinicopathological mechanisms of increased association of solid malignancies in histiocytic neoplasms.

10 Clinical and prognostic features of Langerhans cell histiocytosis in adults

Aki Sato[1*] | Masayuki Kobayashi[2] | Nozomi Yusa[3] | Miho Ogawa[4]| Eigo Shimizu[5] |Toyotaka Kawamata[1] | Kazuaki Yokoyama[1] | Yasunori Ota [6] | Tatsuo Ichinohe[7] | Hitoshi Ohno[8] | Yasuo Mori[9]| Emiko Sakaida[10] | Tadakazu Kondo[11] | Seiya Imoto[5] |Yasuhito Nannya[1,4]| Kinuko Mitani[12] and Arinobu Tojo[13]

[1]Department of Hematology and Oncology, Institute of Medical Science Research Hospital, University of Tokyo, Tokyo, Japan

[2]Department of Hematology, Tokyo Metropolitan Bokutoh Hospital, Tokyo, Japan

[3]Department of Applied Genomics, Institute of Medical Science Research Hospital, University of Tokyo, Tokyo, Japan

[4]Division of Hematopoietic Disease Control, Institute of Medical Science, University of Tokyo, Tokyo, Japan

[5]Division of Health Medical Intelligence, Human

Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan
[6]Department of Diagnostic Pathology, Institute of Medical Science Research Hospital, University of Tokyo, Tokyo, Japan
[7]Department of Hematology and Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan
[8]Department of Hematology, Tenri Hospital, Nara, Japan
[9]Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan
[10]Department of Hematology, Chiba University Hospital, Chiba, Japan
[11]Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan
[12]Department of Hematology and Oncology, Dokkyo Medical University, Tochigi, Japan
[13]Institute of Innovation Advancement, Tokyo Medical and Dental University, Tokyo, Japan

Langerhans cell histiocytosis (LCH) is a rare disease characterized by clonal expansion of CD1a+ CD207+ myeloid dendritic cells. The features of LCH are mainly described in children and remain poorly defined in adults; therefore, we conducted a nationwide survey to collect clinical data from 148 adult patients with LCH. The median age at diagnosis was 46.5 (range: 20–87) years with male predominance (60.8%). Among the 86 patients with detailed treatment information, 40 (46.5%) had single system LCH, whereas 46 (53.5%) had multisystem LCH. Moreover, 19 patients (22.1%) had an additional malignancy. *BRAF* V600E in plasma cell-free DNA was associated with a low overall survival (OS) rate and the risk of the pituitary gland and central nervous system involvement. At a median follow-up of 55 months from diagnosis, six patients (7.0%) had died, and the four patients with LCH-related death did not respond to initial chemotherapy. The OS probability at 5 years post-diagnosis was 90.6% (95% confidence interval: 79.8–95.8). Multivariate analysis showed that patients aged ≥60 years at diagnosis had a relatively poor prognosis. The probability of event-free survival at 5 years was 52.1% (95% confidence interval: 36.6–65.5), with 57 patients requiring chemotherapy. In this study, we first revealed the high rate of relapse after chemotherapy and mortality of poor responders in adults as well as children. Therefore, prospective therapeutic studies of adults with LCH using targeted therapies are needed to improve outcomes in adults with LCH.

11 Yanada M1,2, Yamasaki S3, Kondo T4, Kawata T5, Harada K6, Uchida N7, Doki N8, Yoshihara S9, Katayama Y10, Eto T11, Tanaka M12, Takada S13, Kawakita T14, Nishida T15, Ota S16, Serizawa K17, Onizuka M6, Kanda Y18, Fukuda T19, Atsuta Y20,21, Konuma T22.

1 Nagoya City University East Medical Center, Nagoya, Japan.
2 Aichi Cancer Center, Nagoya, Japan.
3 Kyusyu University Beppu Hospital, Beppu, Japan.
4 Kobe City Medical Center General Hospital, Kobe, Japan.
5 Hyogo Prefectural Amagasaki General Medical Center, Amagasaki, Japan.
6 Tokai University School of Medicine, Isehara, Japan.
7 Toranomon Hospital, Tokyo, Japan.
8 Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital, Tokyo, Japan.
9 Hyogo Medical University Hospital, Nishinomiya, Japan.
10 Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima, Japan.
11 Hamanomachi Hospital, Fukuoka, Japan.
12 Kanagawa Cancer Center, Yokohama, Japan.
13 Saiseikai Maebashi Hospital, Maebashi, Japan.
14 National Hospital Organization Kumamoto Medical Center, Kumamoto, Japan.
15 Japanese Red Cross Aichi Medical Center Nagoya Daiichi Hospital, Nagoya, Japan.
16 Sapporo Hokuyu Hospital, Sapporo, Japan.
17 Kindai University Hospital, Osaka-, Sayama, Japan.
18 Jichi Medical University, Shimotsuke, Japan.
19 National Cancer Center Hospital, Tokyo, Japan.
20 Japanese Data Center for Hematopoietic Cell Transplantation, Nagoya, Japan.
21 Aichi Medical University, Nagakute, Japan.
22 The Institute of Medical Science, The University of Tokyo, Tokyo, Japan.

Allogeneic hematopoietic cell transplantation (HCT) is the last option for long-term survival for patients with chemotherapy-refractory acute myeloid leukemia (AML). By using the Japanese nationwide registry data, we analyzed 6927 adults with AML having undergone first allogeneic HCT while not in complete remission (CR) between 2001 and 2020. The 5-year overall survival (OS), relapse, and non-relapse mortality (NRM) rates were 23%, 53%, and 27%, respectively. Multivariate analysis identified several factors predictive of OS mainly through their effects on relapse (cytogenetics, percentage of blasts in the peripheral blood, and transplantation year) and NRM (age, sex, and performance status). As regards disease status, relapsed disease was associated with a higher

risk of overall mortality than primary induction failure (PIF). The shorter duration of the first CR increased the risks of relapse and overall mortality for the relapsed group, and the longer time from diagnosis to transplantation did so for the PIF group. Our experience compiled over the past two decades demonstrated that >20% of patients still enjoy long-term survival with allogeneic HCT performed during non-CR and identified those less likely to benefit from allogeneic HCT. Future efforts are needed to reduce the risk of posttransplant relapse in these patients.

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3. 南谷泰仁. Current Therapy 骨髄増殖性腫瘍の分子病態 p26-30, 2024
4. 小川弥穂、南谷泰仁　EBM血液疾患の治療2025　がんゲノム情報に基づくAML診療の現状と展望 p110-114
5. 南谷泰仁　Medical Practice　白血病・リンパ腫におけるゲノム医療
6. 南谷泰仁　医学のあゆみ　ゲノム解析時代の血液腫瘍学　造血器腫瘍におけるゲノム異常に基づく治療薬アクセス
7. 南谷泰仁　日本内科学会雑誌　骨髄異形成症候群__病態解明と治療の進歩
8. 南谷泰仁　臨床と研究　特集・白血病治療の最前線　がんゲノム時代の白血病研究　骨髄異形成症候群
9. 南谷泰仁　最新のがん薬物療法　2025-2026　造血器腫瘍に対するがん遺伝子パネル検査
10. 南谷泰仁　腫瘍内科　骨髄異形成症候群におけるゲノム情報
11. 南谷泰仁　新白血病学　骨髄異形成症候群の染色体異常・遺伝子異常：概論
12. 南谷泰仁　医学のあゆみ　骨髄系腫瘍診療におけるパネル検査の臨床的有用性
13. 佐藤亜紀　日本臨床　血液症候群（第3版）IV　その他の組織球症 p480-485
14. 佐藤亜紀　医学のあゆみ　ゲノム解析時代の血液腫瘍学　組織球腫瘍におけるゲノム異常と臨床的有用性 p73-78
15. 塩田曜子、佐藤亜紀　臨床血液　ランゲルハンス細胞組織球症における晩期合併症の特徴 p352-362
16. 小沼貴晶 移植後シクロホスファミドによる移植片対宿主病予防－BMT CTN 1703試験を踏まえて－　血液内科　科学評論社　2024年88巻第5号 521-527
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IMSUT Hospital

Department of Infectious Diseases and Applied Immunology

感染免疫内科

Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授　博士(医学)	四　柳　　宏
Senior Assistant Professor	Eisuke Adachi, M.D., D.M.Sc.	講　師　博士(医学)	安　達　英　輔
Senior Assistant Professor	Michiko Koga, M.D., D.M.Sc.	講　師　博士(医学)	古　賀　道　子
Assistant Professor	Yoshiaki Kannno, M.D., D.M.Sc.	助　教　博士(医学)	菅　野　芳　明
Assistant Professor	Makoto Saito, M.D., D.Phil.	助　教　博士(医学)	齋　藤　　真

Founded in 1981, IMSUT Hospital started its HIV clinic in 1986, and as of 2024, 1343 HIV-infected patients have visited us. Currently, a total of 568 patients are actively under our clinical management. In addition to HIV infection, we also provide treatment for other infections such as hepatitis and malaria and addressed cases of mpox, an emerging and re-emerging infection prevalent among HIV-MSM. The results of a molecular epidemiological analysis of syphilis, which has become endemic in recent years, are presented.

1. Treatment of HIV/AIDS and sexually-transmitted diseases in IMSUT hospital

Statistical characteristics of HIV/AIDS at IMSUT Hospital indicate that fourteen new patients with HIV infection visited our hospital this year (from January 1 to December 31, 2024), and a total of 568 patients are currently under medical management in our outpatient clinic. Of these, 560 people living with HIV (PLWH) are receiving antiretroviral therapy, with the majority achieving well-controlled plasma HIV viral loads. This success is attributed to the high level of medication adherence among PLWH attending our clinic. Figure 1 illustrates the number of PLWH attending IMSUT Hospital since 1995.

This year, we participated in the VOGUE Study (a Phase 3b, multi-center, randomized, parallel-group, open-label, non-inferiority study evaluating the efficacy, safety, and tolerability of oral dolutegravir/lamivudine once daily as a first-line regimen compared to oral bictegravir/emtricitabine/tenofovir alafenamide once daily for virologic suppression and maintenance in antiretroviral therapy-naive adults living with HIV). Additionally, we participated in the M19-965 Study (NCT06032546), which investigates budigalimab and another experimental monoclonal antibody (ABBV-382) targeting the alpha-4 beta-7 integrin receptor, either as monotherapy or in combination.

2. Epidemic of multiple Treponema pallidum strains in men who have sex with men in Japan: efficient multi-locus sequence typing scheme and indicator biomarkers

Wakana Sato, Ayako Sedohara, Michiko Koga, Hiroshi Yotsuyanagi, Yasutoshi Kido[1] and Eisuke Adachi

[1]Graduate School of Medicine, Osaka Metropolitan University

The challenges in culturing Treponema pallidum have hindered molecular-biological analysis. This study aims to establish a molecular epidemiological analysis of syphilis among Japanese men who have

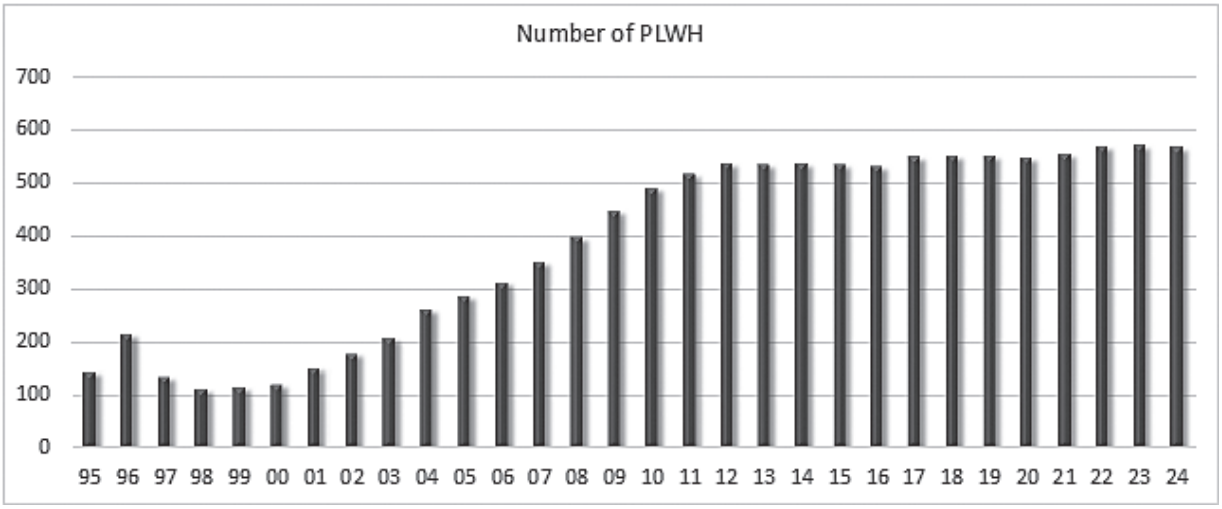

Figure 1. Number of PLWH attending IMSUT Hospital

sex with men (MSM) and to investigate the relationship between bacteremia and associated pathophysiology. We used whole blood specimens from syphilis-diagnosed individuals in Tokyo, collected between February 2019 and June 2022. All individuals were MSM, and most were people with HIV (97.2%). We used a multi-locus sequence typing (MLST) scheme for epidemiological analysis. Sequences for MLST (TP0136, TP0548, and TP0705) were obtained. Out of 71 whole blood samples, 26 samples (36.6%) were positive for TP0136, and we sequenced three loci for MLST in 22 samples (31.0%). The most frequently detected sequence type (ST) was ST3 (n = 9), followed by ST6 (n = 6). Phylogenetic analysis revealed that 12 samples belonged to the SS14-like group (60%), and 8 samples belonged to the Nichols-like group (40%). Treponema pallidum subsp. endemicum (TEN), the cause of bejel was detected in three samples (12%). There was a significant association between TP0136 detection rate and C- reactive protein (CRP) (77.0% at a cut-off:0.5 mg/dL).

Both SS14-like and Nichols-like strains were circulating concurrently, and TEN could have been sexually transmitted among MSM with HIV. Elevated CRP may indicate the presence of the pathogen in the blood.

3. Favorable Virological Outcome, Characteristics of Injection Site Reactions, Decrease in Renal Function Biomarkers in Asian People with HIV Receiving Long-Acting Cabotegravir Plus Rilpivirine

Eisuke Adachi, Amato Otani, Makoto Saito, Michiko Koga, Hiroshi Yotsuyanagi

Long-acting cabotegravir plus rilpivirine has revolutionized the concept of antiretroviral therapy, but as the causes of virological failure and satisfaction can depend on patient background, real-world data are needed. In this single-center study, we reviewed clinical records of people with HIV (PWH) who received injectable cabotegravir plus rilpivirine between June 2022 and January 2023. We assessed virological and safety outcomes, including injection site reactions (ISRs) and changes in serum creatinine and cystatin C. Seventy-four patients were included. There were no virological failures. Approximately 80% of individuals achieved HIV-RNA undetectable in all visits up to 14 months (median 13 months) after switching. Pain upon injection was significantly more common at the rilpivirine injection site, while delayed pain was significantly more common at the cabotegravir injection site. The serum creatinine (mean difference-0.12 mg/dL, $p < .0001$) and the cystatin C (mean difference -0.077 mg/dL, $p < .0001$) decreased significantly after switching, and in multivariable regression analysis, baseline characteristics did not affect the decrease in these renal function markers. Long-acting cabotegravir plus rilpivirine showed excellent antiviral efficacy and safety in PWH in Japan. ISRs were characterized differently at the cabotegravir and rilpivirine injection sites. Although cystatin C showed decrease after the regimen switch, further confirmation is needed whether cabotegravir plus rilpivirine can improve renal function.

4. RBD-mRNA vaccine against COVID-19 (DS-5670) Comparative study to investigate immunological response to additional vaccination

This single-dose clinical trial compared the test drug, Dythyrona intramuscular injection, with the control drug, Comirnaty intramuscular injection, as part of planned therapeutic use. The study also evaluated the RBD-type mRNA vaccine against the full-length mRNA vaccine as booster vaccinations, focusing on identifying surrogate markers and biomarkers, such as differences in B cell responses primarily associated with antibody production. These findings aim

to enhance the understanding of immunological responses.

5. Multiple-clone infections of Mpox: Insights from a single primary lesion

Objectives

Natsuko Kaku[1], Mayo Yasugi[1], Yu Nakagama[1], Eisuke Adachi, Yasutoshi Kido[1]

The 2022 Mpox epidemic transitioned to human-to-human transmission through primary lesions, showing a higher rate of genomic mutations than typical for a DNA virus. While Orthopoxviruses are traditionally considered to cause monoclonal infections in a single patient, we explored whether Mpox virus (MPXV) exhibits genomic plasticity in primary lesions. Five clones, A3, A4, B3, C2, and C5, were isolated from a primary skin lesion of an adult male patient with Mpox and human immunodeficiency virus on antiretroviral therapy, who was not immunocompromised. Whole-genome sequencing was performed to analyze single nucleotide variations in each clone. The tissue culture infectious dose 50 (TCID50) and cytopathic effects (CPE) were measured at one, three, and six days post-infection as in vitro virus phenotypes. Clones A4, C2, and C5 had dominantly identical sequences, while minor clones A3 and B3 had probably three and certainly one additional unique mutation, respectively. Four mutations in A3 and B3 were located in the viral open reading frames, with A3 causing amino acid substitutions at two sites in B21R and B3 resulting in one amino acid change in I6L. Although there were no significant differences in TCID50 levels among the clones, B3 showed a greater CPE than the others at three and six days post-infection. The coexistence of multiple clones with distinct genotypes and viral characteristics within a primary lesion suggests genomic plasticity in MPXV, indicating that genomic diversity may arise early in infection.

6. Bioterrorism-related information website management and crisis management

“バイオテロ対応ホームページ” (a website providing information on bio-terrorism in Japanese), which was developed in 2008 to provide information on clinical diagnosis and testing procedures for bioterrorism-related diseases for medical institutions, and was opened to the public in 2016 in anticipation of international situations and mass gathering events in Japan.

7. Pre- and post-travel treatment of imported infectious diseases and tropical diseases at IMSUT Hospital

The pandemic of COVID-19 had unprecedented impact of our life; global transport and travelling was one of the most affected areas. In mid 2022, the number of returnees and travelers consulted gradually increased, and two cases of malaria patients visited the hospital. For the tropical and parasitic diseases, dozens of important medicines essential for treatment of them are not licensed in Japan. Research Group on Chemotherapy of Tropical Diseases, Research on Publicly Essential Drugs and Medical Devices, Grant from Japan Agency for Medical Research and Development had been established to cope with this situation. We are the medical institution of the research group using these orphan drugs if needed, and collecting clinical data.

8. Treatment of hepatitis in IMSUT hospital:

About 300 HIV-non-infected patients with liver diseases such as viral hepatitis and NAFLD are under medical management in our outpatient clinic. Several patients were introduced from outside for the treatment of chronic hepatitis C with direct acting anti-virals (DAA) and successfully achieved the sustained viral response (SVR). In addition, we treated HIV-infected patients who developed acute hepatitis C with DAAs, who achieved SVR.

9. Breakthrough hepatitis B virus reactivation after switching to cabotegravir plus rilpivirine in a person with attenuated hepatitis B surface antibody after infection

Eisuke Adachi, Ayako Sedohara, Kotaro Arizono, Kazuaki Takahashi, Amato Otani, Yoshiaki Kanno, Makoto Saito, Michiko Koga and Hiroshi Yotsuyanagi

A person with HIV who initiated antiretroviral therapy due to acute hepatitis B fifteen years ago, with attenuated hepatitis B surface antibody, experienced breakthrough HBV reactivation four months after switching from bictegravir/emtricitabine/tenofovir alafenamide to cabotegravir plus rilpivirine. An immune escape mutation, E164V, was identified in the isolated HBV-DNA. It is expected that the patient will undergo seroconversion of HBsAg again with further ART containing F/TAF. Nonetheless, ART devoid of tenofovir or lamivudine is not a viable option for this patient in the future. For many years, tenofovir or lamivudine-containing ART have been widely used worldwide, however, future ART will increasingly use regimens that do not include anti-HBV drugs. Healthcare providers must contemplate the potential scenario, as in this case, wherein the detection of anti-HBs might be a consequence of HBV suppression through ART containing anti-HBV drugs rather than indicative of a functional cure. Switching to ART regimens devoid of anti-HBV effects should be approached with caution.

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IMSUT Hospital

Department of Rheumatology and Allergy
アレルギー免疫科

Associate Professor Motohisa Yamamoto, M.D., D.M.Sc.
Assistant Professor Masaaki Uehara, M.D., D.M.Sc.

准教授 博士(医学) 山 本 元 久
助 教 博士(医学) 上 原 昌 晃

Our department is founded in 2001 to tackle systemic autoimmune inflammatory diseases including rheumatoid arthritis, systemic lupus erythematosus, and IgG4-related disease. We provide patients personalized and evidence-based medical service. We challenge cutting edge science of autoimmune, rheumatic and allergic diseases and novel treatments for patients with these disorders. As part of an elite teaching hospital, we also contribute to preparing the next generation of leading academic physicians, scientists and clinician-educators.

1. Clinical activities in IMSUT Hospital

Yuta Ichii, Tomonao Tanaka, Satsuki Aochi, Masaaki Uehara, Motohisa Yamamoto

Rheumatologists at our division provide state-of-the-art diagnosis and treatment for systemic autoimmune diseases (the total number of patients was approximately 3,000 per year). Our physicians have active basic and clinical research projects and also are involved in the training of rheumatology specialists.

Rheumatologic services offered at IMSUT Hospital include:

- Outpatient consultations
- Outpatient specialty care for patients with rheumatic diseases
- Hospital consultations
- Education on rheumatologic diseases and treatments
- Training of residents and young doctors for rheumatologists
- Clinical trials
- Community medicine

2. Establish of new registry for patients with IgG4-related disease and develop novel diagnostic and therapeutic approaches for IgG4-related disease

Yuta Ichii, Tomonao Tanaka, Satsuki Aochi, Masaaki Uehara, Motohisa Yamamoto

IgG4-related disease is a new disease concept, established this century. As a chronic fibro-inflammatory disorder, IgG4-related disease is characterized by elevated serum levels of IgG4 and abundant infiltration of IgG4-bearing plasma cells into and fibrosis of the involved organs. Whether the disorder is an autoimmune disease remains unclear; nevertheless, consultation with rheumatologists regarding patients with IgG4-related disease is increasing owing to the various organ dysfunction involved and the abnormal immune responses observed. We tackle elucidating the pathogenesis of IgG4-related disease and developing novel treatments. At first, we established a new registry system for the patients with IgG4-related disease (TOMORROW registry), and started to enroll IgG4-related disease patients. We cooperate with national policies and also provide the data to the Rare Disease Data Registry of Japan (RADDAR-J), which was established by AMED. We will organize the clinical figures of IgG4-related disease and develop a

more accurate diagnostic and therapeutic approach by a TOMORROW registry.

Furthermore, using the obtained blood and tissue samples, we will carry out a multi-omics analysis. We will link the results to the individual clinical data, and promote personalized medicine that predicts therapeutic response and prognosis using artificial intelligence. To achieve this, we are currently conducting RNA-Seq of both salivary gland specimens and peripheral blood mononuclear cells, microbiome analysis of saliva, and analysis of the relationship between therapeutic response and HLA. The search for therapeutic target molecules for drug discovery is underway.

3. Development of AI-based diagnostic, therapeutic methods, and prognostic algorithms for rheumatic diseases

Tomonao Tanaka, Masaaki Uehara, Motohisa Yamamoto

Currently, rheumatic diseases are diagnosed using patterned diagnostic criteria based on a combination of physical, hematological, and imaging findings. In addition, treatment strategies for rheumatic diseases are determined after carefully considering the distribution and extent of disability. We have successfully developed a diagnostic algorithm for IgG4-related disease using AI based on clinical data collected in a multicenter study and reported it. In collaboration with Showa University, we are now developing an algorithm to predict treatment response, complications, and prognosis for rheumatoid arthritis.

4. Development of preventive methods for glucocorticoid-induced myopathy and osteonecrosis

Masaaki Uehara, Motohisa Yamamoto

The administration of glucocorticoids to patients with rheumatic diseases often results in glucocorticoid-induced myopathy. We previously found that administration of branched-chain amino acids (BCAA) to such patients improves the loss of skeletal muscle, especially slow-twitch muscle. We also found that the serum concentration of the specific amino acids reflects the slow-twitch muscle improvements. Based on this, we propose the need for separate muscle recovery methods for slow- and fast-twitch muscles and investigate the best method for each.

On the other hand, when a large amount dose of glucocorticoid is used for remission induction, the risk of osteonecrosis of the femoral head occurs. Currently, osteonecrosis of the femoral head is one of the complications that there is no way to prevent. In collaboration with the Department of Orthopaedic Surgery, Sapporo Medical University School of Medicine, we are working to develop a method to prevent osteonecrosis of the femoral head. Currently, several candidate drugs have been identified and clinical trials have been completed.

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IMSUT Hospital

Department of Oncology and General Medicine

腫瘍・総合内科

Head, Professor	Narikazu Boku, M.D., D.M.Sc.	教　授	博士(医学)	朴　　成和
Professor	Hiroshi Yotsuyanagi, M.D., D.M.Sc.	教　授	博士(医学)	四　柳　　宏
Project Senior Assistant Professor	Koichi Kimura, M.D., D.M.Sc.	特任講師	博士(医学)	木　村　公　一
Assistant Professor	Keisuke Baba, M.D., D.M.Sc.	助　教	博士(医学)	馬　場　啓　介

The department of oncology and general medicine started in July 2021 taking over the department of general medicine. The members specialize in medical oncology, hepatology, cardiology, endocrinology/metabolism. Our aim is to practice total human medical care including cancer patients in collaboration with other departments in the IMSUT hospital and conduct clinical research.

1. Treatment of patients with advanced cancer.

Boku N, Baba K

Patients with various, mainly gastrointestinal, cancers were treated by standard chemotherapy including cytotoxic chemotherapy, molecular target agents, immune checkpoint inhibitors, in combination with surgery and radiation therapy. The number of chemotherapy cases has been increasing: 150 chemotherapy regimens for 109 patients in 2024, including 55 new patients. With help of the special patient support team including nurses, pharmacists and nutritionists, the quality and system of patient care during chemotherapy has been improved. We enrolled 8 patients to 8 prospective multi-institutional clinical trials (5 patients in 4 clinical trials for new drug development) (Table). As a result of our in-house clinical trial, topical steroid could reduce the incidence of hand-hoot syndrome in colorectal cancer patients receiving adjuvant chemotherapy with capecitabine plus oxaliplatin. As a principal investigator, we are now preparing a multi-institutional randomized phase III trial comparing topical diclofenac with placebo for preventing capecitabine induced hand-foot syndrome, for which we have achieved a grant from the Japan Agency for Medical Research and Development (AMED). This trial is planned to be launched in April 2025. As a basic research, we investigate the synergism of molecular target agents based on the phosphoproteomic analysis for developing new personalized treatment, for which we have obtained the Grants-in-Aid for Scientific Research from Japan Society for the Promotion of Science.

2. Multicenter clinical and experimental studies of muscular dystrophy.

Kimura K

Multicenter clinical studies for patients with muscular dystrophy have been conducted in collaboration with NHO (National Hospital Organization) hospitals; Sendai-nishitaga National Hospital (Miyagi), Niigata National Hospital (Niigata), Matsumoto Medical Center (Nagano), Shimoshizu National Hos-

Table: List of on-going clinical trials

Phase I/II study of S-1 at the dose recommended by the BBT formula in combination with oxaliplatin and nivolumab for advanced gastric cancer
Randomized phase II trial of trifluridine/tipiracil plus ramucirumab versus trifluridine/tipiracil in previously treated advanced gastric adenocarcinoma
A phase II study of mFOLFOX6 + Nivolumab as first-line treatment in gastric cancer with severe peritoneal metastasis
A prospective study of triplet therapy with encorafenib, Binimetinib and cetuximab for early relapse BRAF V600E-mutated Stage II/III colorectal cancer during or after adjuvant chemotherapy
A randomized phase III study to compare ONO-4538 in combination with ipilimumab, fluoropyrimidine- and platinum-based chemotherapy versus chemotherapy in advanced gastric cancer
A randomized phase II study to compare ONO-4578 with placebo in combination with nivolumab, fluoropyrimidine- and platinum- based chemotherapy with those of the treatment with placebo in combination with nivolumab and chemotherapy in advanced gastric cancer
A phase 1b/2 study evaluating bemarituzumab in combination with other anti-cancer therapies in advanced gastric cancer
A phase I study of a recombinant measles virus for Nectin-4 (+) tumor

pital (Chiba), Hakone National Hospital (Kanagawa), Osaka-toneyama Medical Center (Osaka), Iou National Hospital (Ishikawa), Hiroshima-nishi Medical Center (Hiroshima), Akita National Hospital (Akita), and Aomori National Hospital (Aomori). Furthermore, we performed several animal experiments using CRISPR/CAS9 genome-designed rats in collaboration with department of veterinary physiology (The University of Tokyo), Kobe University (Hyogo), and National Institute of Advanced Industrial Science and Technology (AIST, Ibaraki). Other animal experiments using dogs, pigs and knockout mice were performed in collaboration with National Center of Neurology and Psychiatry (NCNP, Tokyo). We contribute to the publication of Japanese clinical guidelines of Duchenne muscular dystrophy and Fukuyama muscular dystrophy.

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IMSUT Hospital

Department of Applied Genomics
ゲノム診療科
Department of Clinical Genomics
ゲノム診療部

Professor Yoichi Furukawa, M.D., Ph.D.

教 授 博士(医学) 古 川 洋 一

Our department has been working on the application of human genome information in clinics. As clinical services in IMSUT Hospital, we provide genetic counseling, genetic tests for human diseases, and a surveillance program for patients with hereditary colorectal cancer. In addition, we have been carrying out two research projects; 1) determination of genetic alterations in human tumors, and elucidation of the mechanisms underlying their development, and 2) clinical sequence for the implementation of genomic medicine.

1. Genetic test of human neoplasms

Yoichi Furukawa

As a part of clinical service, we have performed genetic analysis of human neoplasms including colorectal cancer. A total of 21 cases were analyzed by WGS in 2024. The results were utilized for the precise classification of neoplasms, evaluation of disease status, selection of therapeutic drugs, and evaluation of the response to treatment.

2. Genetic counseling and related activities

Yoichi Furukawa, Takashi Okada, Yataro Daigo, Koichiro Yuji, Reiko Sada, Mitsuko Nakazawa, Miho Iigaya[1], Yoshinari Miyamoto[2], Masae Ono[3], Mayumi Tamari[4], Shiro Ikegawa[5], Hidewaki Nakagawa[6], Natsuko Watanabe[7], Ai Yoshihara[7], Toru Akiyama[8]: [1]Kitasato University, [2]National Center for Global Health and Medicine, [3]Yotsuba Mirai Clinic, [4]Jikei Medical University, [5]National Rehabilitation Center for Children with Disabilities, [6]Center for Integrative Medical Sciences, RIKEN, [7]Ito Hospital, [8]Jichi Medical University.

In IMSUT hospital, we provided genetic counseling and genetic tests to clients who visited our counseling clinic. In 2024, we had a total of 40 counseling cases with various hereditary diseases such as muscular dystrophy, Huntington's disease, Ehlers-Danlos syndrome, hereditary breast and ovarian cancer, and Lynch syndrome. In the counseling, we provided appropriate information about the diseases to the clients and took their psychological care in collaboration with a clinical psychologist. Genetic testing was performed in cases with informed consent after thoughtful discussion about its merit and demerit.

Systematic surveillance programs are provided for the patients susceptible for hereditary tumors.

3. Identification of pathogenic germline variants in patients with biliary tract cancer: insights from 799 Japanese cases

Kiyoko Takane[1], Kiyoshi Yamaguchi[1], Tsuneo Ikenoue, Yoichi Furukawa

[1]Division of Clinical Genome Research, Advanced

Clinical Research Center

Biliary tract cancer (BTC) is a group of malignancies that include intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC), gallbladder carcinoma (GBC), and ampulla of vater carcinoma (AVC). Although BTC is relatively rare, it is a highly aggressive disease with a poor prognosis, largely due to its frequent diagnosis at late-stages and limited treatment options. The incidence of BTC is particularly high in East Asia and South America, likely due to a combination of environmental, lifestyle, and genetic factors. Despite advances in diagnostic technologies and treatment modalities, the overall survival rates for BTC patients remain dismal compared to other gastrointestinal cancers.

Recent studies suggest that germline variants in homologous recombination repair (HRR) genes may contribute to BTC development. Therefore, in this study, we analyzed 799 Japanese BTC cases from Biobank Japan using amplicon sequencing, focusing on key HRR genes including *BRCA1*, *BRCA2*, *PALB2*, *BARD1*, *BRIP1*, *RAD51C*, *RAD51D*, *RAD50*, *FANCM*, *ATM*, *CHEK2*, and *NBN*. Germline variants were assessed using ClinVar, gnomAD, and TogoVar databases, and consequently pathogenic variants were identified in 30 cases. The variants included those in *BRCA1* (8 cases), *BRCA2* (5 cases), *PALB2* (6 cases), *BRIP1* (2 cases), *RAD51D* (2 cases), *RAD50* (1 case), *FANCM* (1 case), *ATM* (4 cases), and *CHEK2* (1 case). As we expected, a significant correlation between pathogenic HRR variants and family history of cancer was observed. This finding underscores the potential importance of hereditary cancer predisposition in BTC and highlights the need for genetic screening in patients with family history of malignancies. Such efforts may allow earlier detection of BTC and more personalized therapeutic interventions.

4. Clinical sequencing for the implementation of genomic medicine

Kiyoko Takane, Kiyoshi Yamaguchi, Yoichi Furukawa, Eigo Shimizu[1], Rika Kasajima[1], Kotoe Katayama[1], Seiya Imoto[1], Tetsuo Shibuya[2], Kazuaki Yokoyama[3], Yasuhito Nanya[3], Koichiro Yuji[4], Rui Yamaguchi[5], Satoru Miyano[6]: [1]Division of Health Medical Intelligence, [2]Division of Medical Data Informatics, Human Genome Center, [3]Department of Hematology/Oncology, [4]Project Division of International Advanced Medical Research, Advanced Clinical Research Center, IMSUT, [5]Division of Cancer Systems Biology, Aichi Cancer Center Research Institute, [6]M&D Data Science Center, Institute of Integrated Reseach, Institute of Science ToKyo

The application of Next-Generation Sequencing (NGS) technology in clinical medicine has revolutionized molecular diagnostics by enabling multiple gene testing, or analysis of the entire exon or whole genome with a limited amount of DNA. In collaboration with Human Genome Center and Advanced Clinical Research Center, we have been working on the genetic diagnosis of patients with suspected hereditary cancer predisposition, and the implementation of precision medicine for patients with rare or intractable cancer.

We have applied NGS technology for molecular diagnostics of hereditary colon cancer syndromes such as familial adenomatous polyposis (FAP), Lynch syndrome (LS), and polymerase proofreading-associated polyposis (PPAP). In addition to short read-sequencing, we took advantage of MinION, a long-read sequencer of Oxford nanopore platform, for the detection of pathogenic structural variants (SVs) because not only single nucleotide variants (SNVs) and short insertions and deletions (indels) but also structural variations (SVs) are responsible for the predisposition of hereditary cancer. Utilizing MinION, we have successfully identified the breakpoint of a pathogenic SV that could not be determined by short-read sequencing technology.

We have been also working on the implementation of genomic data in clinics. Patients with various types of cancer who gave written informed consent for genetic analysis were enrolled in this study. Genetic alterations in their tumors were identified using NGS, and the data were subsequently analyzed by QIAGEN Clinical Insights (QCI). Actionable variants were reviewed and discussed in a tumor board to determine recommended therapeutic options. This multidisciplinary board comprised physicians, medical oncologists, genetic counselors, geneticists, bioinformaticians, and ethics experts, and convened online every two weeks.

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IMSUT Hospital

Department of Radiology
放射線科

Associate Professor	Hiroyuki Akai, M.D., D.M.Sc.	准教授	博士(医学)	赤 井 宏 行
Senior Assistant Professor	Toshihiro Furuta, M.D., D.M.Sc.	講 師	博士(医学)	古 田 寿 宏
Assistant Professor	Shimpei Kato, M.D., D.M.Sc.	助 教	博士(医学)	加 藤 伸 平
Project Assistant Professor	Naomasa Okimoto, M.D., D.M.Sc.	特任助教	博士(医学)	沖 元 斉 正

Department of Radiological Technology
放射線部

Associate Professor	Hiroyuki Akai, M.D., D.M.Sc.	准教授	博士(医学)	赤 井 宏 行
Head Radiologic Technologist	Kenji Ino, RT	放射線技師長		井 野 賢 二

The Department of Radiology undertakes radiology service at IMSUT hospital. Our expertise includes general diagnostic radiology, neuroradiology, clinical nuclear medicine, and radiation therapy. Board-certified radiologists at the Department of Radiology conduct all examinations of CT, MRI, and nuclear medicine. Radiological reports are made by the radiologists. In addition, several clinical studies are being conducted in collaboration with other departments or institutions. We also investigate the technical aspects of molecular imaging with intact small animals for its application to preclinical studies using an optical imaging system and MRI.
The Department of Radiological Technology constitutes the hospital radiology service together with the Department of Radiology. Plain radiography, dual-energy X-ray absorptiometry, and barium studies are also available at the Department of Radiological Technology, other than CT, MRI, and radioisotope examinations. More than 10,000 patients visit our department every year. Radiologic technologists at the department make an effort to provide high-quality medical images in daily practice as well as to reasonably reduce radiation exposure of a patient during the examination.

Faster acquisition of magnetic resonance imaging sequences of the knee via deep learning reconstruction: a volunteer study.

Akai H, Yasaka K[1], Sugawara H[2], Furuta T, Tajima T[3], Kato S, Yamaguchi H, Ohtomo K[3], Abe O[1], Kiryu S[3].

[1] Department of Radiology, Graduate School of Medicine, University of Tokyo, [2] Department of Diagnostic Radiology, McGill University, [3] Department of Radiology, International University of Health and Welfare Narita Hospital

In the present study, we aimed to evaluate wheth-

er deep learning reconstruction (DLR) can accelerate the acquisition of magnetic resonance imaging (MRI) sequences of the knee for clinical use. Twenty-seven healthy volunteers (age: 40.6 ± 11.9 years) were enrolled. Using a 1.5-T MRI scanner, sagittal fat-suppressed T2-weighted imaging (fs-T2WI), coronal proton density-weighted imaging (PDWI), and coronal T1-weighted imaging (T1WI) were performed. DLR was applied to images with a number of signal averages (NSA) of 1 to obtain 1DLR images. Then 1NSA, 1DLR, and 4NSA images were compared subjectively, and by noise (standard deviation of intra-articular water or medial meniscus) and contrast-to-noise ratio between two anatomical structures or between an anatomical structure and intra-articular water. As a result, according to objective evaluations, PDWI 1DLR images showed the smallest noise and significantly higher contrast than 1NSA and 4NSA images. For fs-T2WI, smaller noise and higher contrast were observed in the order of 4NSA, 1DLR, and 1NSA images. According to the subjective analysis, structure visibility, image noise, and overall image quality were significantly better for PDWI 1DLR than 1NSA images; moreover, the visibility of the meniscus and bone, image noise, and overall image quality were significantly better for 1DLR than 4NSA images. Fs-T2WI and T1WI 1DLR images showed no difference between 1DLR and 4NSA images. Three 1DLR image sequences were obtained within 200 s (approximately 12 minutes for 4NSA image). To sum up, compared to PDWI 4NSA images, PDWI 1DLR images were of higher quality, while the quality of fs-T2WI and T1WI 1DLR images was similar to that of 4NSA images.

Dataset augmentation with multiple contrasts images in super-resolution processing of T1-weighted brain magnetic resonance images.

Kageyama H, Yoshida N[4], Kondo K[5], Akai H.
[4] Department of Radiological Technology, Faculty of Medical Technology, Niigata University of Health and Welfare, [5] Graduate Division of Health Sciences, Komazawa University

This study investigated the effectiveness of augmenting datasets for super-resolution processing of brain Magnetic Resonance Images (MRI) T1-weighted images (T1WIs) using deep learning. By incorporating images with different contrasts from the same subject, this study sought to improve network performance and assess its impact on image quality metrics, such as peak signal-to-noise ratio (PSNR) and structural similarity (SSIM). This retrospective study included 240 patients who underwent brain MRI. Two types of datasets were created: the Pure-Dataset group comprising T1WIs and the Mixed-Dataset group comprising T1WIs, T2-weighted images, and fluid-attenuated inversion recovery images. A U-Net-based network and an Enhanced Deep Super-Resolution network (EDSR) were trained on these datasets. Objective image quality analysis was performed using PSNR and SSIM. Statistical analyses, including paired t test and Pearson's correlation coefficient, were conducted to evaluate the results. Augmenting datasets with images of different contrasts significantly improved training accuracy as the dataset size increased. PSNR values ranged 29.84–30.26 dB for U-Net trained on mixed datasets, and SSIM values ranged 0.9858–0.9868. Similarly, PSNR values ranged 32.34–32.64 dB for EDSR trained on mixed datasets, and SSIM values ranged 0.9941–0.9945. Significant differences in PSNR and SSIM were observed between models trained on pure and mixed datasets. Pearson's correlation coefficient indicated a strong positive correlation between dataset size and image quality metrics. Using diverse image data obtained from the same subject can improve the performance of deep-learning models in medical image super-resolution tasks.

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IMSUT Hospital

Department of Palliative Medicine and Advanced Clinical Oncology

先端緩和医療科

Project Senior Assistant Professor　Tetsuya Ito, M.D., Ph.D.

特任講師　博士（医学）　伊　藤　哲　也

Our goal is set to improve patients' quality of life by controlling symptoms related to the disease and treatment. We will perform a multidisciplinary approach to medical care based on cancer treatment and palliative medicine.

Palliative medicine to improve QOL of patients with life-threatening illness and their families.

Tetsuya Ito[1], Keishi Mori[1], Noriko Fujiwara[1], Aya Watanabe[1], Tomoe Honda[1], Yasuki Hijikata[1]
[1] Dept. of Palliative Medicine / Advanced Clinical Oncology, IMSUT Hosp.

Patients with life-threatening illness including cancer and their families are facing challenges, that interfere with their quality of life.

Regardless of the stage of the disease, we aim to address problems of patients and families, whether physical, psychological, social or spiritual, and eventually improve their quality of life under multidisciplinary collaboration.

At the same time, we will conduct research activities to build evidence on palliative medicine and disseminate new findings.

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IMSUT Hospital

Department of Diagnostic Pathology
病理診断科
Department of Pathology
病理部

Associate Professor	Yasunori Ota, M.D., Ph.D.	准教授　博士(医学)	大　田　泰　徳
Project Assistant Professor	Tamami Denda, Ph.D.	特任助教　博士(保健学)	傳　田　珠　美

Our mission

1. *We provide an accurate and high-quality pathological diagnosis to the patient in this research hospital, The Institute of Medical Science, The University Of Tokyo.*
2. *Make diagnosis by morphological approach using microscope to the laboratory materials.*

Overview

We study about the hematological malignancy and transplantation pathology. We emphasize many clinical cases and write case reports about human diseases.
We also perform pathological and cytological diagnosis of many specimens submitted by various departments.

1. HHV8 negative effusion-based lymphoma has been adopted for the new WHO classification.

Effusion-based lymphoma is found in pleura or ascites and usually lack of evidence for nodular lesion. Conventional findings about EBL are bad clinical course and many patients are infected by HIV. However, some of Japanese patients were not infected HIV and good clinical course. We reported some case reports about EBL in Japan and are going to promote multi-institutional joint research in Japan. We therefore conducted a retrospective study of 95 patients with EBL, regardless of HHV8 status, in Japan. Of 69 patients with EBL tested for HHV8, a total of 64 were negative. The median age of patients with primary HHV8-negative EBL at diagnosis was 77 years (range, 57-98 years); all 58 tested patients were negative for HIV. Primary HHV8-negative EBL was most commonly diagnosed in pleural effusion (77%). Expression of at least 1 pan B-cell antigen (CD19, CD20, or CD79a) was observed in all cases. According to the Hans algorithm, 30 of the 38 evaluated patients had nongerminal center B-cell (non-GCB) tumors. Epstein-Barr virus–encoded small RNA was positive in 6 of 45 patients. In 56 of 64 HHV8-negative patients, systemic therapy was initiated within 3 months after diagnosis. Cyclophosphamide, doxorubicin, vincristine, and prednisolone (CHOP) or CHOP-like regimens with or without rituximab (n = 48) were the most common primary treatments. The overall response and complete response rates were 95% and 73%, respectively. Three patients did not progress without systemic treatment for a median of 24 months. With a median 25-month follow-up, the 2-year overall survival and progression-free survival rates were 84.7% and 73.8%. Sixteen patients died; 12 were lym-

phoma-related deaths. Thus, most EBL cases in Japan are HHV8-negative and affect elderly patients. The non-GCB subtype is predominant. Overall, primary HHV8-negative EBL exhibits a favorable prognosis after anthracycline-based chemotherapy.

This disease concept has been adopted for the new WHO classification of hematolymphoid tumor.

2. Clear cell sarcoma can arise from Rosai Dorfman disease.

Histiocytic neoplasms (HNs) in adults have been reported to be associated with a high prevalence of coexisting haematological and solid malignancies. While a proportion of coexisting HNs and haematological malignancies share identical genetic alterations, the genetic association between HNs and solid malignancies has scarcely been reported. We report a case of Rosai–Dorfman disease (RDD) complicated by coexisting clear cell sarcoma (CCS). RDD is a rare HN. CCS is an ultra rare soft tissue sarcoma with a poor prognosis. Mutation analysis with whole-exome sequencing revealed six shared somatic alterations including NRAS p.G12S and TP53 c.559 + 1G>A in both the RDD and CCS tissue. This is the first evidence of a clonal relationship between RDD and solid malignancies using mutational analysis. We hypothesize that neural crest cells, which originate in CCS, are likely the common cells of origin for RDD and CCS. This case helps to unravel the underlying clinicopathological mechanisms of increased association of solid malignancies in HNs.

3. Medical Activities

We have performed microscopic diagnosis of many pathological and cytological samples. We also provided immunohistochemical analysis and in situ hybridization in order to improve the diagnostic accuracy and decide the treatment.

Pathological diagnosis	n = 1678
Biopsy	n = 1045
Surgical resection	n = 317
Bone marrow aspiration	n = 205
Intraoperative diagnosis	n = 26
Consultation	n = 44
Other	n = 41
Immunohistochemistory	n = 392
Cytological diagnosis	n = 370
Autopsy	n = 2

4. Pathology Core Laboratory II

Pathology Core Laboratory II handles a large number of specimens, including mouse, cultured cells and human tissue samples collected at the IMSUT hospital. We have performed preparation of pathological specimen and pathological analysis (n = 150).

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IMSUT Hospital

Department of Gastroenterology
消化器内科

Professor	Hiroaki Ikematsu, M.D., Ph.D.	教　授	博士(医学)	池　松　弘　朗
Associate Professor	Yoshihiro Hirata, M.D., D.M.Sc.	准教授	博士(医学)	平　田　喜　裕
Assistant Professor	Tatsunori Minamide, M.D.	助　教		南　出　竜　典

The department of Gastroenterology, which focuses on endoscopy and treatment, started in October 2023. We are committed to quality endoscopy, and our mission is to provide careful observation, accurate diagnosis using magnifying endoscopes, and advanced treatments such as endoscopic submucosal dissection (ESD). In addition, collaborating with various departments for comprehensive, minimally burdensome patient care.

1. Introduction

We primarily focus on endoscopic examinations for the early detection and treatment of esophageal, gastric, and colorectal cancers. Our mission is to provide painless endoscopic procedures, thorough and minimally overlook observations, precise diagnoses using magnification endoscopy, and advanced endoscopic treatments, particularly Endoscopic Submucosal Dissection (ESD). We aim to provide prompt endoscopic examinations and treatments for patients referred from other hospitals and clinics. In addition, we aim to tailor our approach to each specific lesion, choosing the most suitable treatment options and collaborate with Oncology, Surgery, Radiology, and other departments to provide treatment that that impose minimal burden on the patient's overall well-being. The outpatient clinic treats all gastrointestinal diseases and also offers Helicobacter pylori outpatient clinic.

2. Endoscopic examination

Endoscopic examinations are conducted for screening, investigating symptomatic cases, and precisely diagnosing and treating lesions. Additionally, procedures such as gastric fistula construction, ileus tube insertion, and stent placement are also performed.

When a lesion is detected, a magnifying endoscope is used to closely examine the surface microvasculature and pit patterns. Endoscopic treatment options encompass cold polypectomy, hot snare polypectomy using bipolar snare, EMR (Endoscopic Mucosal Resection), and ESD (Endoscopic Submucosal Dissection), selected based on the size, morphology, and depth diagnosis of the detected lesion.

The number of endoscopies and ESDs during the year were as follows; upper endoscopy: 892 cases, colonoscopy: 875 cases, gastric ESD: 22 cases, colorectal ESD: 20 cases. The number of upper endoscopies and colonoscopies increased by more than 100 cases and the number of ESDs also tripled compared to last year.

We plan to significantly increase both the number of endoscopic examinations and treatments in the next year and beyond.

3. Treatment of drug-resistant Helicobacter pylori infection

Some patients fail to respond to first- and second-line Helicobacter pylori (H. pylori) eradication therapy, but third-line eradication is not always done.

Meanwhile, penicillin allergy patients do not take routine eradication medicines because insurance coverage regimens in Japan include amoxicillin. In H. pylori out-patient clinic, we make correct diagnosis of infection by multiple modalities, give eradication therapy for these refractory patients, and achieve high rates of successful eradication.

In 2024, 55 patients (18 with drug allergies, 18 with drug-resistant H. pylori) visited our hospital and received eradication treatment. Of these patients, 93% were successfully eradicated.

4. Clinical research

We are engaged in clinical research to address clinical questions in the field of gastrointestinal endoscopy. In January 2025, we initiated patient enrollment for a randomized controlled trial evaluating the utility of Narrow-band Imaging (NBI) or iodine staining in surveillance after endoscopic resection of esophageal squamous cell carcinoma (EVEREST trial). Assistant Professor Minamide serves as the research coordinator for this multicenter collaborative trial. We will continue to conduct clinical research to develop novel diagnostic and therapeutic approaches.

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IMSUT Hospital

Department of Surgery
外科

Professor	Dai Shida, M.D., Ph.D.	教　授	博士(医学)	志　田　　大
Associate Professor	Susumu Aikou, M.D., Ph.D.	准教授	博士(医学)	愛　甲　　丞
Assistant Professor	Naoki Sakuyama, M.D., Ph.D.	助　教	博士(医学)	柵　山　尚　紀
Assistant Professor	Satoko Monma, M.D.	助　教		門　間　聡　子
Assistant Professor	Junko Mukohyama, M.D., Ph.D.	助　教	博士(医学)	向　山　順　子
Assistant Professor	Ai Sadatomo, M.D., Ph.D.	助　教	博士(医学)	佐田友　　藍
Assistant Professor	Yuka Ahiko, M.D.	助　教		阿　彦　友　佳
Assistant Professor	Haruna Onoyama, M.D., Ph.D.	助　教	博士(医学)	小野山　温　那

The mission of our department is to provide surgical treatment for various gastrointestinal diseases, including colorectal and gastric cancers. Since the participation of Prof. Shida and Dr. Ahiko in September 2020, we mainly perform laparoscopic surgery instead of open surgery for these diseases. Additionally, we began performing robotic surgery for rectal cancer in April 2021, followed by robotic surgery for colon cancer in September 2022.

1. Introduction

We specialize in the treatment of gastrointestinal cancers, with a particular focus on the surgical treatment of colorectal and gastric cancers. As certified surgeons under the Japan Society for Endoscopic Surgery's Endoscopic Surgical Skill Qualification System (Dr. Shida and Dr. Aikou), as well as qualified console surgeons for robotic surgery (da Vinci system) (Dr. Shida, Dr. Aikou, Dr. Ahiko, Dr. Sakuyama, Dr. Onoyama, Dr. Monma, Dr. Sadatomo, and Dr. Mukohyama), we actively perform minimally invasive surgeries that reduce the physical burden on our patients. Additionally, starting in October 2022, after Dr. Kojima S (Visiting Lecture in our department) joined our team, we began offering laparoscopic surgery for inguinal hernias.

This year, Dr. Ahiko and Dr. Onoyama retired in March 2024. Dr. Sadatomo joined the department for a six-month period from April to September 2024. Dr. Ito Go has joined our team as a senior resident (specialist trainee) starting in April 2024. And, Dr. Mukohyama joined our team in May 2024.

2. Treatment for gastrointestinal malignancy

Colorectal cancers and gastric cancers are what we mainly treat.

For colorectal cancer, if appropriate preoperative testing is conducted and the surgery is tailored to the stage of the disease, it is possible to completely cure more than 70% of patients, even with advanced cancer. For rectal cancer, in order to improve the QOL (quality of life) after surgery as much as possible, we select not only autonomic nerve-sparing surgery but also anus-sparing surgery if the cancer can be sufficiently resected. As qualified surgeons (endoscopic surgical skill qualification system) of the Japan Society for Endoscopic Surgery (Dr. Shida and Dr. Aikou) and certified robotic surgery proctors (Dr. Shida), we actively perform minimally invasive surgeries. In addition to robotic surgery for rectal cancer, we began performing robotic-assisted surgery for colon cancer in September 2022.

For gastric cancer (including gastric cancer and gastric GIST), we select the surgical method with policy of 'leaving the remaining stomach as much as possible', because stomach surgery limits the amount of food that patients eat after surgery which leads to weight loss and weakness. Under the policy of "preserving the stomach whenever possible," we focus on minimally invasive surgeries with small incisions to minimize the burden on the patient. Our goal is to perform patient-friendly surgeries that do not compromise the oncological outcomes.

To reduce the time patients feel anxious after their diagnosis, our department aim to 'perform surgery and discharge patients **within one month** of the initial consultation for colorectal and gastric cancers'. Our entire staff is dedicated to providing the best possible care for our patients.

3. Surgical treatment for inguinal hernia

For inguinal hernias, we also use laparoscopic surgery in October, 2022. Compared to traditional open surgery, the laparoscopic approach results in smaller incisions, and we perform the surgery using the "TEP (Total Extraperitoneal) technique," which does not require entry into the abdominal cavity, reducing the risk of intra-abdominal complications such as adhesions, bowel obstruction, or organ injury.

4. Surgical treatment for other benign diseases

We also treat a variety of benign diseases such as acute appendicitis, cholecystitis, and colonic diverticulitis.

5. Endoscopic examination and treatment with chemotherapy

In collaboration with the Department of Oncology and General Medicine (Prof. Boku N. and Dr. Baba K.) and the Department of Gastroenterology (Prof. Ikematsu H., Dr. Hirata Y. and Dr. Minamide T.), we have performed many cases of upper gastrointestinal endoscopy and colonoscopy as well as chemotherapy.

6. Launch of Robotic Surgery

Robotic surgery involves performing laparoscopic procedures with robotic assistance, where the surgeon controls the robot (the robot does not perform the surgery autonomously). Under high-definition 3D visualization, the use of a robot with complex, articulated joints enables more delicate and precise operations, enhancing the benefits of traditional laparoscopic surgery. Laparoscopic surgery has a limitation in that the instruments used, such as forceps and electric scalpels, are rigid and cannot bend, which restricts fine manipulation deep within the body. This limitation is overcome by robotic assistance. In robotic surgery, with high-resolution 3D visualization and multi-jointed, flexible instruments, there is a higher likelihood of making precise cuts along tissue lines and preserving function by avoiding damage to nerves and other structures.

In April 2021, we began performing robotic surgery for rectal tumors, including rectal cancer, rectal GIST, and rectal neuroendocrine tumors (NET). At that time, our hospital received "facility certification for robotic surgery for rectal tumors." As a result, we started offering robotic surgery as part of standard, insurance-covered care.

With the revision of the Japanese medical reimbursement system in 2022, robotic-assisted surgery became available for colon cancer as part of standard insurance coverage, in addition to rectal cancer. In September 2022, we expanded our services to include robotic surgery for colon cancer. Our hospital also received "facility certification" from the Ministry of Health, Labour and Welfare for colon cancer.

7. Robotic Colon Cancer Surgery Focusing on the Outermost Layer of the SMA Plexus

In robotic surgery for colon cancer, we focus on the outermost layer of the SMA (superior mesenteric artery) plexus to perform a more reliable and safer dissection. This surgical technique has been introduced in both English and Japanese publications in 2024.

Publications

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IMSUT Hospital

Department of Anesthesia
麻酔科

2024.4.1~

Professor	Masahiko Bougaki, M.D., Ph.D.	教　授　博士(医学)	坊　垣　昌　彦
Assistant Professor	Fumiko Seto, M.D., Ph.D.	助　教　博士(医学)	瀬　戸　富美子

~2024.3.31

Associate Professor	Ryo Orii, M.D., Ph.D.	准教授　博士(医学)	折　井　　亮
Assistant Professor	Miho Asahara, M.D., Ph.D.	助　教　博士(医学)	浅　原　美　保

Our department is dedicated to delivering safe and effective anesthetic management to surgical patients at IMSUT Hospital. As the number of surgical procedures performed under general anesthesia continues to grow, our team of anesthesiologists plays a pivotal role in perioperative medicine. This encompasses preoperative patient evaluation, intraoperative anesthetic management, and postoperative pain management, ensuring comprehensive care and optimal outcomes for our patients.

Preoperative Evaluation and Anesthetic Planning

Comprehensive patient evaluation, which includes a thorough assessment of comorbidities alongside an understanding of the planned surgical procedure, is essential for developing an appropriate anesthetic plan. All patients undergoing major surgical procedures are preoperatively interviewed by our team members, ensuring that anesthetic management is tailored to their needs and based on informed decisions.

To enhance the efficiency and quality of this process, a dedicated Perioperative Anesthetic Clinic was established in June 2024. This clinic streamlines preoperative evaluations, facilitating better communication, personalized care, and optimal preparation for surgical patients.

Intraoperative Anesthetic Management

Our department managed over 400 cases of general anesthesia for major surgical procedures. Approximately one-third of these cases involved robotic-assisted surgeries for conditions such as colon cancer, rectal cancer, prostate cancer, and renal tumors, which often required extended operation times.

Epidural anesthesia was utilized in about one-fourth of the general anesthesia cases, providing effective pain control and enhanced postoperative recovery. Additionally, spinal anesthesia was employed for several minor surgical procedures in the perineal region, tailored to the specific needs of these cases.

Postoperative Pain Management

Effective postoperative pain management is critical for facilitating early mobilization and promoting enhanced recovery following major surgery. For these procedures, our department provides intravenous patient-controlled analgesia (iv-PCA) or patient-controlled epidural analgesia (PCEA), tailored to each patient's needs.

Peripheral nerve blocks are employed in selected cases to further optimize pain relief. Non-opioid analgesics are also incorporated into all cases as part of a multimodal analgesia approach. We continuously

strive to improve postoperative pain management with individualized care to enhance patient outcomes.

Publications

Kashiwa K, Kurosawa H, Fujishiro K, Kubo H, Inokuchi R, Bougaki M, Kawamura G, Sato M, Konoeda C, Nakajima J, Doi K. Increased white blood cell count is associated with an increased demand for unfractionated heparin during veno-arterial extracorporeal oxygenation in lung transplantation. J Extra Corpor Technol. 56:108-113, 2024.

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IMSUT Hospital

Department of Joint Surgery
関節外科

Project Professor　Minoru Tanaka, M.D., Ph.D.

特任教授　博士（医学）　田　中　　実

The Department of Joint Surgery was established in 2006 to evaluate and treat hemophilic arthropathy. We have provided specialized care to hemophilia patients across Japan for nearly two decades, addressing their unique orthopedic needs. Our services have included comprehensive joint evaluations, surgical interventions such as joint arthroplasties and arthroscopic synovectomies, and pioneering research in perioperative bleeding control and the pathogenesis of hemophilic arthropathy. In collaboration with the Department of Orthopedic Surgery at the University of Tokyo, we have also contributed to the development of innovative therapies, including mesenchymal stem cell treatments for hemophilic arthropathy. Over the years, more than 270 surgical procedures have been performed, including those addressing complex cases involving coagulation disorders such as factor VII deficiency and Von Willebrand disease.

New Organizational Structure in 2024

The Department of Joint Surgery has transitioned to a new organizational structure. While maintaining our commitment to hemophilia outpatient care, the department has shifted its focus towards tumor rehabilitation in collaboration with the Rehabilitation Department at the University of Tokyo Hospital. This collaboration has strengthened comprehensive rehabilitation services, including cancer rehabilitation. Through this partnership, we have enhanced our ability to deliver high-quality care to a diverse patient population. Despite a limited number of occupational therapists (OT) and physical therapists (PT), we remain dedicated to providing the highest standard of care. By maintaining close collaboration with nursing staff, we ensure that our patients receive the support they need as part of a coordinated team effort.

Future Directions

The Department of Joint Surgery aims to enhance its rehabilitation services further while continuing to uphold its legacy of excellence in hemophilic arthropathy care. Currently, we support a diverse patient population, including those recovering from hematologic and neurosurgical conditions. Moving forward, we will continue to explore new opportunities for research and collaboration, ensuring that our patients receive cutting-edge treatments and therapies. We remain dedicated to doing our best for our patients and improving their quality of life.

IMSUT Hospital

Department of Surgical Neuro-Oncology
脳腫瘍外科

Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士(医学)	藤　堂　具　紀
Project Professor	Minoru Tanaka, M.D., Ph.D.	特任教授	博士(医学)	田　中　　実
Assistant Professor	Hirotaka Ito, M.D., Ph.D.	助　教	博士(医学)	伊　藤　博　崇
Assistant Professor	Seisaku Kanayama, M.D.	助　教		金　山　政　作
Assistant Professor(Thoracic surgeon)	Yoshinori Sakata, M.D., Ph.D.	助　教	博士(医学)(呼吸器外科医)	坂　田　義　詞

All kinds of brain tumors, especially malignant glioma, are treated at our department. Malignant glioma is incurable by standard therapy alone, therefore refined, personalized treatment regimens utilizing non-standard radiation therapy and chemotherapy are considered. In addition, G47Δ, the first oncolytic virus therapy drug for malignant glioma in the world, developed by this department, is commercially available and used for treatment since November 2021. Based on scientific evidence and findings from basic research, we conduct advanced medical practices in addition to standard therapy.

Introduction

Department of Surgical Neuro-Oncology was established in 2011. Our department started treating out-patients in October 2011 and in-patients in April 2012. Our department focuses on malignant tumors of the brain, such as gliomas or metastatic brain tumors. Glioblastoma is one of the most aggressive and malignant cancers of the central nervous system. The standard upfront treatment includes resection to remove as much of the tumor as possible while preserving function, followed by radiation of 60Gy and temozolomide. Established good prognostic factors are limited but include young age, high Karnofsky Performance Status (KPS), high mini-mental status examination score, O^6-methylguanine methyltransferase promoter methylation, and resection of > 98% of the tumor. Nevertheless, glioblastoma is refractory to conventional therapies and has a poor prognosis with a 5-year survival rate of less than 5%. Therefore, we should consider refined and personalized treatment approaches for selected patients: high dose radiation therapy of 80Gy for newly diagnosed glioblastoma or extended field stereotactic radiosurgery for recurrent gliomas. We also conduct translational research based on scientific evidence. We are developing recombinant herpes simplex virus type I (HSV-1), which has genetic modifications in the viral genome so that the viruses replicate selectively in cancer cells while eliciting an immune response against tumor-associated proteins. Clinical trials using a third-generation, triple-mutated oncolytic herpes simplex virus type 1 (HSV-1), G47Δ, were performed in patients with glioblastoma from 2015 to 2020 and malignant pleural mesothelioma from 2018 to 2021. A clinical trial targeting patients with olfactory neuroblastoma has been ongoing since 2013. Additionally, an investigator-initiated trial utilizing T-hIL12 for malignant melanoma, conducted in collaboration with Shinshu University, has been underway since January 2020.

Drug approval of a replication-competent, HSV-1, G47Δ for malignant glioma

Genetically engineered, conditionally replicating HSV-1is promising therapeutic agents for solid carcinomas. We developed G47Δ by introducing an additional genetic mutation to a second generation, dou-

ble-mutated oncolytic HSV-1, G207, used in the phase I clinical trial for glioblastoma in the United States in 1998. We conducted a phase II clinical trial of G47Δ in patients with recurrent or residual glioblastoma since December 2014 to June 2020. The patients received repeated stereotactic injections with G47Δ every 4 weeks, 6 injections being the maximum total. In the final analysis, the 1-year survival rate after initiation of G47Δ treatment (the primary endpoint) was 84%. The most common side effect of G47Δ was fever followed by vomiting, nausea, lymphopenia, and leukopenia. A new drug application (NDA) for G47Δ for malignant glioma has been submitted to the Ministry of Health, Labour and Welfare in December 2020. In June 2021, G47Δ was approved as the world's first oncolytic virus drug for malignant glioma. Since its commercial release as Delytact in November 2021, the Department has begun treating patients with malignant gliomas and evaluating its safety and efficacy in real-world clinical practice.

A clinical study of G47Δ in patients with progressive olfactory neuroblastoma

Olfactory neuroblastoma is an uncommon malignant neuroectodermal tumor, which is thought to originate from the olfactory membrane of the sinonasal tract. Patients should receive aggressive treatment with combined treatment such as surgery, radiation therapy, and chemotherapy because there is no effective treatment once it recurs: An aggressive en bloc resection, with combined radiation therapy was recommended. We have been conducting a phase I clinical trial of G47Δ in patients with progressive olfactory neuroblastoma since August 2013. G47Δ was repeatedly inoculated to the residual tumor in nasal cavity every 4 weeks until tumor progression or excessive toxicity occurred. The primary endpoint was safety, and the secondary endpoints included efficacy analysis. Participant recruitment has been completed, and data analysis is currently underway.

A clinical study of G47Δ in patients with progressive malignant pleural mesothelioma

Malignant pleural mesothelioma is a rare asbestos-induced malignancy with an estimated incidence of approximately 2,000 new cases diagnosed in Japan. Worldwide, nearly 80% of mesothelioma deaths occur in ten countries, with Japan, the United Kingdom, and the United States being in the top three. It is expected to continue to increase over the next several decades. Median survival ranges from 9 to 18 months and correlates with stages. Radiotherapy can be used for different indications in mesothelioma: palliation, as a preventive treatment, and as part of multimodality treatment. Combination doublet chemotherapy of cisplatin, with either pemetrexed or raltitrexed, has shown a more prolonged survival compared with cisplatin alone in randomized phase III trials. Carboplatin is an acceptable alternative to cisplatin and may be better tolerated in the elderly population. We conducted a phase I clinical trial of G47Δ for inoperable, recurrent or progressive malignant pleural mesothelioma from 2018 to 2021. A fixed dose of G47Δ was administered into the pleural cavity every 4 weeks, maximum 6 times. The primary endpoint was safety, and the secondary endpoints included efficacy analysis. We completed the enrollment and confirmed the safety of repeated intrapleural administration with G47Δ.

A phase 1/2 clinical trial of a recombinant herpes simplex type 1 with human IL-12 expression, T-hIL12, in patients with malignant melanoma

Malignant melanoma is a tumor produced by the malignant transformation of melanocytes. Melanocytes are derived from the neural crest; consequently, melanomas, although they usually occur on the skin, can arise in other locations where neural crest cells migrate, such as the gastrointestinal tract and brain. The 5-year relative survival rate for patients with stage 0 melanoma is 97%, compared with about 10% for those with stage IV disease. We started a phase 1/2 clinical trial of T-hIL12 in patients with malignant melanoma since January 2020 jointly with Shinshu University. T-hIL12 is a G47Δ-based recombinant herpes simplex type I with human IL-12 expression. This IL-12-mediated antitumor immunity could be T-cell-mediated. The main inclusion criteria in phase 1 are 1) histologically confirmed malignant melanoma with stage 3 or 4, 2) patients who have at least one metastatic skin lesion with 10 mm or larger (the longest diameter), or at least one metastatic lymph node with 15 mm or larger (the shortest axis), 3) patients who were administered with anti-PD-1 antibody, or targeted molecular drugs, 4) the size and distribution of all the metastatic lesions are recognized with clinical findings including imaging studies (CT, MRI), 5) age >= 20 years, 6) more than 30 days have passed from the previous treatment, 7) Eastern Cooperative Oncology Group (ECOG) performance Status (PS) of 0-2, 8) patients without severe disorders (severe myelosuppression, liver dysfunction, chronic renal dysfunction), whereas in phase 2 they are eight items, which are defined in the same way as in the phase 1 except for 3) of phase1. The 3rd inclusion criterion of phase2 is 3) patients who have not been administered with anti-PD-1 antibody or targeted molecular drugs. T-hIL12 will be administered into the tumor of skin or lymph node metastases in patients with advanced stage of malignant melanoma. The assigned dose will be repeatedly inoculated into the metastases 2 or 4 times, with an interval of 14 (14 -28) days. The primary endpoint in phase 1 is safety, and in phase 2 a response rate (RECIST 1.1). The Phase 1 part of our phase 1/2 clinical trial of T-hIL12 in patients with malignant melanoma has concluded, and the Phase II

part is progressing as planned.

Routine activities

Patients with brain tumors are treated by four faculty neurosurgeons and one resident. A total of 119 operations were carried out in 2024 including 118 gliomas and one meningioma. More than 100 cases of oncolytic virus therapy were performed. Standard craniotomies and image guided stereotactic biopsies of deep seated lesions, as well as high-tech brain tumor resections are performed. The high-tech equipment regularly used in brain tumor resection surgeries includes an operative microscope, a 3-D neuro-navigation system, intraoperative motor evoked potential (MEP and SEP) recording, intraoperative ultrasonography and an ultrasonic surgical aspirator.

Patients with newly diagnosed malignant glioma have been treated with high dose or standard dose radiation therapy and concomitant chemotherapy. Temozolomide was administered to glioma patients during radiation therapy followed by a maintenance therapy every 28 days for as long as possible. The overall survival of patients with glioblastoma was 30.3 months (95% confidence interval, 24.5-36.1 months. The five-year overall survival rate was 26.5%.

Recurrent malignant glioma patients are treated with innovative non-standard therapies whenever possible. Recurrent glioma patients who have small lesions, receive extended field stereotactic radiosurgery. To enhance the efficacy of stereotactic radiosurgery (SRS), the irradiation field is enlarged to include as many tumor cells invasive to the surrounding tissue as possible. We demonstrated 93% local control in patients who received 20 Gy to a 0.5-1.0 cm extended field SRS compared to 47% of patients who were treated with 20 Gy to the gadolinium-enhancing margin only.

Treatment of primary central nervous system lymphoma

Primary central nervous system lymphoma patients will first undergo biopsy for pathological diagnosis. Standard treatment includes high-dose methotrexate-based chemotherapy, often combined with agents such as rituximab, procarbazine, and vincristine (R-MPV regimen), followed by consolidation therapy with high-dose cytarabine (Ara-C). whole-brain radiation therapy may still be considered in certain cases, depending on individual patient factors and disease characteristics.

Development of next-generation oncolytic HSV-1 for malignant glioma

As a next-generation oncolytic HSV-1 that follows G47Δ, we are currently developing G47Δ-based oncolytic HSV-1 that expresses bevacizumab (anti-VEGF monoclonal antibody), T-BV. The protocol for the phase I clinical trial of T-BV for grade 4 malignant glioma has been drafted. We expect to start the clinical trial in the near future.

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IMSUT Hospital

Department of Urology
泌尿器科

Professor	Haruki Kume, M.D., Ph.D.	教　授	博士(医学)	久　米　春　喜
Project Associate Professor	Sayuri Takahashi, M.D., Ph.D.	特任准教授	博士(医学)	髙　橋　さゆり
Project Assistant Professor	Yuji Hakozaki, M.D., Ph.D.	特任助教	博士(医学)	箱　崎　勇　治
Project Assistant Professor	Jun Takahashi, M.D.	特任助教		髙　橋　　潤

Our department of Urology was established in ISMUT hospital on July 1st, 2020 to improve the occupancy rate by introducing advanced medical treatments such as robotic surgery. We successfully performed 1,241 cases of urological surgery including 181 cases of robotic surgery, which resulted in increase of the revenue. Further, we have been engaged in basic research on castration resistant prostate cancer to discover novel drugs by the method of molecular and cell biology.

1. Basic research

The canonical and non-canonical WNT signaling pathways regulate prostate cancer progression, but the precise mechanism by which this regulation occurs has yet to be established. We investigated the expression levels of WNTs in prostate cancer tissues and found that WNT5A is expressed at higher levels in cancer cells and in the stroma of non-cancerous regions than in prostate cancer epithelium of patient samples. WNT5A was also high expressed in prostate stroma-derived WPMY1 cells. WNT5A knockdown in WPMY1 cells resulted in reduced expression of cancer-related genes, and several cytokines according to microarray analysis. WNT5A expression was directly regulated by the ligand-inducible androgen receptor bound to the WNT5A promoter. Receptor tyrosine kinase-like orphan receptor 1 (ROR1), a WNT5A receptor, was highly expressed in prostate cancer cell lines and patients. shRNA-mediated ROR1 knockdown in PC3 cells abrogated WNT5A-induced cell proliferation. These results suggest that WNT5A in stromal cells regulates prostate cancer proliferation and invasion, at least in part, via the ROR1 signaling pathway. Our results suggest that cell-to-cell communication between stromal cells and prostate cancer cells enhances prostate cancer progression, and ROR1 may be a novel therapeutic target for prostate cancer.

2. Clinic

Since we established the department of Urology in IMSUT Hospital in July,2020, the number of patients has been increased by introduced from urological clinics, hospitals, and other departments of our hospital. Totally 2,002 patients visited our department in 2024 for the purpose of thorough examinations for diagnosis or surgical treatments.

We totally performed 399 surgical operations in 2024: 35 cases of Robotic-assisted prostatectomy, six Robotic-assisted partial nephrectomy, nine Laparoscopic nephroureterectomy or nephrectomy, four Radical cystectomy with ileal conduit urinary diversion or with cutaneous ureterostomy, 14 open surgery, 79 Trans-urethral resection of bladder tumor, nine Trans-urethral resection of prostate, 27 Trans-urethral lithotripsy, seven Ureteroscopy, 50 Ureteral stenting, and nine Botox injection for overactive bladder

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IMSUT Hospital

Department of Medical Informatics
医療情報部

Associate Professor	Hiroyuki Akai, M.D., D.M.Sc.	准教授　博士(医学)	赤　井　宏　行
Senior Assistant Professor	Toshihiro Furuta, M.D., D.M.Sc.	講　師　博士(医学)	古　田　寿　宏

Department of Medical Informatics is engaged in the management of hospital information systems, including infrastructure for the system and the electric medical records, at the Institute of Medical Science (IMSUT) Hospital. Hospital information system enables medical staff to securely provide patient care and helps to conduct clinical research. The current hospital information system has been renewed for better patient care since 2017.
We also devote ourselves to the development and improvement of infrastructure for a regional community-based medical cooperation network between IMSUT hospital and other healthcare providers.

1. Management and operation of the hospital information system and network

Hiroyuki Akai, Toshihiro Furuta, Shimpei Kato

We offer services related to the hospital information system of the IMSUT hospital. We work together with the IT service room of IMSUT, and the Information Technology Center of the University of Tokyo. We are obliged to maintain the hospital information service and the network system for better medical care, ensuring that patient medical records are saved in a standard format and are easily transferrable to other healthcare providers.

Our missions are as follows:

- Supervision, development, operation, and management of the hospital information system
- Education on the hospital information system to the medical staff
- Development and management of the network infrastructure for securely dealing with patient personal information and clinical records
- Day-to-day management and operation of the hospital information system and network
- General work concerning the operation of the hospital information system and network

2. IT support to a community-based healthcare provider network

Hiroyuki Akai, Toshihiro Furuta, Shimpei Kato

"Community-based integrated care systems" is a keyword for the Japanese healthcare system in this decade. IMSUT hospital belongs to its community-based healthcare provider network, and we continuously improve infrastructure for cooperation in the network.

Our hospital information system has been renewed since 2017, and in late 2023, we updated the system. The latest electronic healthcare record system will help refer patients from hospital to clinic and clinic to hospital in the network. Also, we constructed a network with the University of Tokyo Hospital (UTH) that can send medical images of our hospital to the Picture Archiving and Communication System of UTH.

IMSUT Hospital

Department of Cell Processing and Transfusion

セルプロセッシング・輸血部

Clinical Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.	病院教授	博士(医学)	長 村 登紀子
Associate Professor	Kazuaki Yokoyama, M.D., Ph.D.	准教授	博士(医学)	横 山 和 明
Project Assistant Professor	Kazuhiro Sudo, Ph.D.	特任助教	博士(医学)	須 藤 和 寛

Our department was established in 1990 to manage transfusion medicine and cell processing for hematopoietic stem-cell transplantation. In addition to transfusion related works, our department has been supporting the cell processing for translational studies preformed in IMSUT-Cell Resource Center (IMSUT-CRC), established in 1997. Our recent projects include the Research Cord Blood Bank (RCBB); the National BioResource Project (NBRP) supported by the Ministry of Education, Culture, Sports, Science and Technology; and umbilical cord derived mesenchymal stromal cells (UC-MSC). We have been studying the immunological effects of UC-MSC administration for treatment-resistant severe acute graft-versus host disease, acute cerebral injury, and radiation injury.

1. Transfusion medicine and related tests

Abe Y, Ogami K, Iwasawa N, Yokoyama K, Nagamura-Inoue T

Our department controls and supports transfusion medicine through blood typing, irregular antibody testing, and cross-matching tests on blood transfusion products including concentrated red blood cells, platelets, and frozen plasma. The blood type of some patients with hematopoietic disorders and post–stem cell transplantation is undetectable.

2. Cell Processing and quality tests for Hematopoietic stem cell transplantation (HSCT) and clinical trials.

Nagamura-Inoue T, Yokoyama K, Takahashi A, Ogami K, Miharu Y

For autologous peripheral blood stem cell transplantation (PBSCT), we perform apheresis for patients with myeloma and malignant lymphoma after mobilization by granulocyte colony-stimulating factor with or without the CXCR-4 inhibitor, Plerixafor. We test CD34-positive cells in the graft of PBSC, bone marrow, and cord blood as the quality tests for HSCT. We process the cells for clinical trials including collection (apheresis), cryo- preservation, and thawing with or without washing upon the requests.

3. Exploring the therapeutic application of UC-MSCs for severe acute graft-versus-host disease (aGVHD) and non-infectious pulmonary complications (NIPC) after hematopoietic stem cell transplantation

Huang X, Nagamura-Inoue T, Takahashi A, Hori A, Mori Y, Nagamura F, Yokoyama K

We investigated the immunosuppressive mechanisms of UC-MSCs on inflammatory cells. A phase I dose-escalation trial, IMSUT-CORD for steroid-resist-ant aGVHD using allogeneic umbilical cord-de-

rived mesenchymal stromal cells (IMSUT-CORD) have been safely completed (Int J Hematol. 2022 Nov; 116(5):754-769.). From 2022 to 2023, phase II clinical trial of NIPC treated with UC-MSCs have been implement- ed. We continued to prepare the next clinical trials of NIPC (phase III). We are investigating the mechanism of the effectiveness of UC-MSCs in severe aGVHD and NIPC in vitro and in vivo.

4. Study of therapeutic application of UC-MSCs to neurological injuries

Cho T, Sei K, Mori Y, Mukai T, Nagamura-Inoue T

Based on the efficacy of proof of concept using UC-MSCs for cerebral palsy by Mukai T et al, a clinical trial (Phase I/II) for cerebral palsy treated with UC-MSCs was implemented from 2021 to 2023, and completed safely. Nowadays, we investigated the efficiency of UC-MSCs for the treatment of acute encephalitis (AE) mimicking the infant viral encephalitis. We found the improvement of the neuron degeneration and part of behavior abnormalities in AE by intravenous injection of UC-MSCs. We also study the cerebral palsy (Periventricular leukoencephalopathy; PVL) rat model treated with UC-MSCs.

5. Research and Development of UC-MSCs (IMSUT-CORD) treatment for new application of UC-MSCs to acute radiation injury, ARDS, cleft palate, hemorrhagic arthropathy, and acceleration of engraftment of HSC

Sudo K, Hu D, Mori Y, Takahashi A, Miharu Y, Hori A, Nagamura-Inoue T

We have been exploring UC-MSCs (IMSUT-CORD) treatment for new application of UC-MSCs to acute radiation injury, cleft palate, hemorrhagic arthropathy, and acceleration of engraftment of using mice models in collaboration with companies.

6. The Research Cord Blood Cell Resource / National BioResource Project (NBRP)

Shibuya Y, Sakai R, Miharu Y, Takahashi A, Nagaya N, Nagamura-Inoue T

The Research Cord Blood bank / resource was established in 2004 and supported by the Ministry of Education, Culture, Sports, Science and Technology for the development of regenerative medicine, immunological cell therapy, infection research, modified gene cell therapy, and drug discovery. Since July 2012, this project has been incorporated into the National BioResource Project (NBRP). The research umbilical cord blood (CB) bank provides processed and cryopreserved CB units (nucleated cells, mononuclear cells, and CD34 + cells) to researchers worldwide via the RIKEN Bioresource Center. The website is at http://www.nbrp.jp/.

7. Institute of Medical Science, University of Tokyo, Cell Resource Center (IMSUT-CRC)

Takahashi A, Miharu Y, Hori A, Mori Y, Nagamura-Inoue T

To promote cell therapy in translational research, IMSUT-CRC was established in 1997 (originally called the Room for Clinical Cellular Technology, or RCCT). To date, the following projects have been implemented:1) CB cell processing for banking in the manner of the Tokyo Cord Blood Bank (1997–2008), 2) research cord blood bank (2004–), 3) dendritic cell therapies (1998–2001), 4) regenerative therapy of alveolar bone derived from bone marrow mesenchymal cells (2005–2011), 5) gene therapy for renal cancer (1998), 6) CB and UC-MSC banking (IMSUT-CORD; 2012–), 7) aAVC-WT1 cell therapy (2017–), and (8) dendritic cell (DC) therapy using DCs pulsed with neoantigen (2020–).

Visit our website: http: //www.ims.u-tokyo.ac.jp/dcpt/english/

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IMSUT Hospital

Surgical Center
手術部

Project Professor　Minoru Tanaka, M.D., Ph.D.

特任教授　博士（医学）　田　中　　実

IMSUT hospital provides seamless support for translational research. Our mission is the management and operation of the surgical center to achieve a safe and organized environment where surgical procedures can be performed in high quality. A da Vinci surgical system (da Vinci Xi), a robotic technology that allows surgeons to perform minimally invasive procedures, was introduced in November 2020. Robot-assisted Radical Prostatectomies (RARP) for prostate cancer and robotic rectal surgery for tumors including rectal cancer and GIST are performed. The Medtronic Stealth Autoguide Platform was introduced in April 2023.

Introduction
IMSUT hospital provides seamless support for translational research.

The aim is to apply knowledge gained from basic science to clinical and community health-care settings. Our mission is the management and operation of the surgical center to achieve a safe and organized environment where surgical procedures can be performed in high quality. Our activities include the management of clean areas, the establishment of protocols for infection control, maintenance of equipment such as astral lamps, surgical microscopes and fiberscopes, and organizing of daily and weekly operations.

A da Vinci surgical system (da Vinci Xi), a robotic technology that allows surgeons to perform minimally invasive procedures, was introduced in November 2020, and Robot-assisted Radical Prostatectomies (RARP) for prostate cancer started. Department of surgery initiated Robotic rectal surgery for tumors including rectal cancer and GIST in 2021. The Medtronic Stealth Autoguide Platform was introduced in April 2023. It is a robotic guidance system intended for instrument holders' spatial positioning and orientation. It is based on a pre-operative plan and feedback from an image-guided navigation system with 3D imaging software. Oncolytic virus therapy using the Stealth Autoguide Robotic system was started. In 2024, the robotic-assisted surgery program further expanded. The number of robot-assisted surgeries reached 142 cases, including 101 in the Department of Surgery and 41 in the Department of Urology. Of these, Robot-assisted Radical Prostatectomies (RARP) accounted for 34 cases, and Robot-assisted Pancreaticoduodenectomies (RAPAN) accounted for 7 cases. To support this expansion, medical engineering (ME) staff participated in the 2024 robotic surgery training program, enhancing their operational skills and knowledge of robotic systems.

Collaboration and Training

Collaboration with ward nurses was strengthened by conducting a joint initiative to observe and understand the practical aspects of robotic-assisted surgeries, including patient positioning for complex procedures. This initiative deepened team understanding and improved perioperative care coordination.

Medical Engineering Division

Medical engineer staffs increased accordingly, and a ME Division was newly established in the Sur-

gical Center. Three of four maintained at a NASA class 1,000 clean level and specifically designed for neurosurgery and joint surgery. For prompt and sustained supply of sterilized materials, we keep the surgical tools for each department in sets of designated purposes.

Equipment in the surgical center

The center is equipped with C-arm x-ray TV systems, surgical microscopes, ultrasonic aspirators, image guided navigation systems, intraoperative ultrasound imaging systems, intraoperative nerve simulation monitoring systems, etc. The endoscopic procedure room is located separately but adjacent to the surgical center.

TV monitoring system

Each operating room is equipped with a TV camera, so that the rooms can be monitored in the control center as well as by pad devices carried by managing anesthesiologists.

Induction of electronic ordering system

We are accommodating an electronic ordering system for the surgical center that allows a real time ordering by clinical departments and a computerized management of operation schedules.

Facts in the fiscal year 2024

Total number of operations	701
Planned operations	688
Emergency operations	13
General anesthesia	376
Spinal	25
Epidural	124
Local	227
Others	92

IMSUT Hospital

Department of Laboratory Medicine
検査部

Clinical Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.		部長 / 病院教授	博士(医学)	長 村 登紀子
Assistant Professor	Tomohiro Ishigaki, M.D., Ph.D.		副部長 / 助教	博士(医学)	石 垣 知 寛
Project Senior Assistant Professor	Koichi Kimura, M.D., Ph.D.		特任講師	博士(医学)	木 村 公 一
Chief Technologist	Hironori Shimosaka		技師長	臨床検査技師	下 坂 浩 則

The Department of Laboratory Medicine has seven divisions: clinical hematology, biochemistry/serology, microscopy, pathology, microbiology, physiology, and a TR verification laboratory.
Clinical laboratory tests are necessary for all clinical practice steps, including disease diagnosis, stage evaluation, treatment determination, and assessment after therapy. Our department conducts most of the clinical laboratory examinations in our hospital under stringent quality control and provides investigational laboratory analysis in collaboration with many other departments.
To facilitate translational research projects in this research hospital, we established a special division named TR Verification Laboratory. This division has contributed to evaluating the safety of experimental therapeutic approaches and biopharmaceutical products for clinical trials.
As a central medical department, we also participate in many clinical trials and support many research studies conducted in our hospital.

* Only achievements related to clinical laboratory medicine are shown here, and other ones overlapping across affiliations have been omitted. Please refer to each respective affiliation.

1. Establishment of a new cytometric method to evaluate the immune function of chimeric antigen receptor T (CAR-T) cells and application for clinical testing

Tomohiro ISHIGAKI.

CAR-T cell therapy, a groundbreaking approach to hematological malignancies, can be hindered by relapses due to CAR-T cells' poor immune response or exhaustion after proliferation. Hence, evaluating the immune functions is a crucial clinical need. In this study, we have harnessed molecular imaging flow cytometry (MI-FCM) to develop a new evaluation method with joint researchers.

Our study involved the creation of eight CAR-T clones through the genetic modification of PBMCs from eight healthy donors using lentivirus to express an anti-CD19 CAR. We then assessed the cytotoxicity of these clones in vitro using a luciferase assay. Subsequently, we stimulated them with CD19 antigen for an hour and meticulously analyzed the intensity, area, and distribution (spot counts) of the CAR expression using an imaging flow cytometer. We further evaluated the cytotoxicity of some clones in vivo using a xenotransplantation model of CAR-T cells and B-cell leukemia cell-line (NALM6) cells in NOG mice.

We found superficial CAR antigens aggregate in immune responses and could detect the concentration as a decrease in CAR expression area. The percentage of CAR T-cells with a single-spot CAR concentration was significantly correlated with killing activity. We also confirmed that the clone with the

lowest concentration percentage couldn't prolong survival in xenotransplantation models. We also found that this method could be applied to remnants of CAR-T cell therapy products.

In conclusion, quantifying the superficial CAR concentration by MI-FCM could be useful for evaluating their immune function. [The 66th annual meeting of the American Society of Hematology (ASH), 2024.] [The 86th annual meeting of JSHEM, 2024.] [The 84th annual meeting of JCA, 2024.]

2. Evaluation of superior migration ability of umbilical cord-derived mesenchymal stromal cells (MSCs) toward activated lymphocytes compared to those of bone marrow and adipose-derived MSCs.

Tokiko NAGAMURA-INOUE.

Upon inflammation and tissue damage, mesenchymal stromal cells (MSCs) are activated and migrate to suppress inflammation and repair tissues. Though migration is the first important step for MSCs to get functional, the migration potency of umbilical cord-derived MSCs remains poorly understood. We compared the migration potencies of umbilical cord-derived(UC-), bone marrow-derived (BM-), and adipose tissue-derived (AD-) MSCs toward allogeneic stimulated mononuclear cells (MNCs) in mixed lymphocyte reaction (MLR). UC-MSCs showed significantly faster and higher proliferation potencies and higher migration potency toward unstimulated MNCs and MLR than BM- and AD-MSCs. The amounts of CCL2, CCL7, and CXCL2 in the supernatants were significantly higher in UC-MSCs co-cultured with MLR than in MLR alone and BM- and AD-MSCs co-cultured with MLR. The amount of CCL8 was higher in BM- and AD-MSCs than in UC-MSCs, and the amount of IP-10 was higher in AD-MSCs co-cultured with MLR than in UC- and BM-MSCs. The migration of UC-MSCs toward the MLR was partially attenuated by platelet-derived growth factor, insulin-like growth factor 1, and matrix metalloproteinase inhibitors in a dose-dependent manner. UC-MSCs showed faster proliferation and higher migration potency toward activated or non-activated lymphocytes than BM- and AD-MSCs. The functional chemotactic factors may vary among MSCs derived from different tissue sources, although the roles of specific chemokines in the different sources of MSCs remain to be resolved. [Akiko HORI, Tokiko NAGAMURA-INOUE, et al. Front Cell Dev Biol. 2024.]

3. Highly sensitive detection and analysis of cells in the spinal fluid of aggressive adult T-cell leukemia/lymphoma with central nervous system infiltration.

Tomohiro ISHIGAKI.

The prognosis of adult T-cell leukemia/lymphoma (ATL) with primary central nervous system (CNS) involvement has been unclear since the advent of new therapies. We have shown that flow cytometric CD7/CADM1 analysis of CD4-positive cells is useful for detecting ATL cells that are not morphologically diagnosed as ATL cells. We investigated the role of CNS involvement in ATL using cytology and flow cytometry by analyzing cerebrospinal fluid (CSF) from aggressive ATL cases. Based on the findings in CSF, the study subjects were classified into CNS + (cytologically malignant), CNS- (cytologically non-malignant and ATL cell population negative in flow cytometry), and CNS-Micro (cytologically non-malignant and ATL cell population positive in flow cytometry) groups. As expected, the CNS + group had a shorter overall survival than the CNS- group. However, the CNS-Micro group showed no adverse impact on overall survival compared to the CNS- group, even without additional CNS-targeted treatments. Flow cytometry also demonstrated clinical utility in diagnosing CSF lesions in ATL patients with cerebral white matter lesions and detecting ATL cells on post-treatment CSF examination in patients with CNS involvement. Our study demonstrates that ATL with CNS involvement has a poor prognosis and that highly-sensitive flow cytometric analysis of CSF is useful to assist in the diagnosis of suspected CNS involvement and to detect ATL cells after treatment. [Koji JIMBO, Tomohiro ISHIGAKI, et al, Ann Hematol. 2024-2025.]

4. Retrospective analysis and search for clinical laboratory parameters associated with cardiac deterioration and shorter survival in Becker Muscular Dystrophy (BMD).

Koichi KIMURA and Tomohiro ISHIGAKI.

Becker muscular dystrophy (BMD) is an X-linked recessive disorder caused by a mutation in the dystrophin gene. It is most common in muscular dystrophies. BMD has a later onset and milder symptoms than Duchenne muscular dystrophy (DMD). Still, cardiac diseases are now one of the leading causes of morbidity and mortality in these patients. We have retrospectively reviewed biochemical examination results and echocardiographic findings. We found a clinical laboratory parameter that could be associated with cardiac deterioration and shorter survival.

5. Laboratory contribution as a central medical department and support for many clinical investigations and trials in this hospital.

Hironori SHIMOSAKA and Clinical laboratory members (clinical hematology, biochemistry/serology, physiology, and microbiology team)

We participate in clinical trials and research led by other hospital departments. Our laboratory members officially contributed to 9 clinical investigations and trials conducted in this hospital, including treatments using new drugs and new cell therapy. We also contributed to many other basic and clinical studies.

IMSUT Hospital

Center for Clinical Safety and Infection Control
医療安全・感染制御センター

Head, Professor　Yasuhito Nannya, M.D., D.M.Sc.

教　授　博士(医学)　南　谷　泰　仁

The Center for Clinical Safety and Infection Control consists of the Department of Medical Safety Management and the Department of Infection Prevention and Control and supports for providing safe medical care.

Department of Medical Safety Management
医療安全管理部

Head, Associate Professor	Susumu Aikou, M.D., D.M.Sc.	准教授	博士(医学)	愛　甲　　丞
Associate Professor	Motohisa Yamamoto, M.D., D.M.Sc.	准教授	博士(医学)	山　本　元　久
Nurse Manager	Nozomi Linzbichler	看護師長		リンツビヒラ希
Director of Pharmacy	Seiichiro Kuroda	薬剤部長		黒　田　誠一郎

Department Medical Safety Management is responsible for carrying out medical safety in order to prevent incidents and accidents beforehand and deliver safe medical care to patients. At our hospital, we mainly have focused on hematological malignancies, infectious diseases, immune diseases, but in recent years, robotic surgery and chemotherapy are also increasing. We try to respond appropriately to such medical activities.

Department of Infection Prevention and Control
感染制御部

Head, Senior Assistant Professor	Eisuke Adachi, M.D., D.M.Sc.	講　師	博士（医学）	安達英輔
Nurse Manager	Fumie Kameda	看護師長		亀田史絵
Head of Nursing Department	Mika Kogayu	看護部長		小粥美香
Pharmacist	Naoki Furukawa	薬剤師		古川直樹
Pharmacist	Mika Yamamura	薬剤師		山村美佳
Clinical laboratory technician	Takashi Momoda	臨床検査技師		百田尭史
Clinical laboratory technician	Hiroko Shibata	臨床検査技師		柴田浩子

Department of Infection Prevention and Control builds ICT (Infection Control Team) and AST (Antimicrobial StewardshipTeam) to promote the practice of hospital infection control and prevent the spread of antimicrobial resistant organisms. The ICT consists of a infection control doctor, an infection control nurse, a pharmacist, a clinical laboratory technician and an administrative staff.

IMSUT Hospital

Center for Translational Research
トランスレーショナルリサーチ・治験センター

Professor	Fumitaka Nagamura, M.D., D.M.Sc.	教　授　博士(医学)	長　村　文　孝
Associate Professor	Masanori Nojima, M.D., Ph.D., M.P.H.	准教授　博士(医学)	野　島　正　寛

Our major mission is to support the conduct of clinical trials, especially for sponsor-investigator clinical trial based on Translational Research (TR). Our roles on TR varies from the advice for acquiring intellectual property, preparation for clinical trials, assistance for conducting clinical trials, and so on. Our center consists of coordinator section, administrative section, data management/biostatistics section, and project management section

1. Promotion of Translational Research at IMSUT Hospital

All members of staff.

We have an unwavering commitment to deliver novel therapies through the conduct of translational research. To advance basic research findings into clinical application, we offer investigators the following services:

1)Planning research and development (R & D) strategies, including selecting target diseases, planning product designs, and clarifying development pathways;
2)Offering opportunities to consult an appointed patient attorney about the acquisition and maintenance of intellectual property rights as well as patent strategies;
3)Providing information necessary in the preclinical phase of R & D, such as information on drug regulatory affairs and preclinical studies;
4)Encouraging investigators to consult regulatory advisors of Pharmaceuticals and Medical Devices Agency (PMDA) in a timely manner;
5)Participating in investigator-regulator meetings to help investigators deal with the issues pointed out in the meetings;
6)Advising on clinical trial design so that feasible and scientifically appropriate trials are conducted;
7)Reviewing clinical study protocols, consent forms, and related documents in prior to Institutional Review Board examination to ensure the quality of clinical trials conducted at IMSUT Research Hospital;
8)Assigning Translational Research Coordinators (TRCs) to each translational research project in the clinical trial phase; TRCs help patients participating in clinical trials to understand study protocols and to cope with negative emotions including fear, confusion, and depression; TRCs assist investigators

2. Statistics and Quality control in Clinical Trials

Masanori Nojima, Motoki Amai, Mitsumi Tokunaga, Fumitaka Nagamura

We have planned and performed data management, monitoring, and statistical works in clinical trials.

[Data management]: Planning, EDC and CRF preparation, registration, allocation, database management, data cleaning, coding

[Monitoring]: Monitoring for drug management.

[Statistics]: Planning and perform for statistical analyses, Sample size calculation.

3. Support for the investigator-initiated clinical trials under an Investigational New Drug Application

All members of staff

Our mission is to develop efficient approaches for conducting investigator-initiated clinical trials under Investigational New Drug application (IND) to promote translational research. In 2024, we supported four sponsor-investigator clinical trials by site management as well as project management including the preparation. These four clinical trials were: oncolytic virus for malignant melanoma, adjuvant vector vaccine for SARS-CoV2 for heavily treated patients with B cell malignancies (other institute), gene modified measles virus vaccine for nectin-4 positive tumors, and dendritic cell therapy for ATLL.

4. Management of "Translational Research Network Program" of Japan Agency for Medical Research and Development.

Miwako Okada, Fumitaka Nagamura

Ministry of Education, Culture, Sports, Science and Technology launched "Translational Research Network Program" to promote translational research based on the results of basic science in academia. This program was transferred to Japan Agency for Medical Research and Development in 2015 and has been expected to support TRs from basic science to seek obtaining intellectual property to the early stage of clinical trial. In 2024, we supported 34 basic researches (24: other than IMSUT), 15 preclinical studies (8: other than IMSUT), and 12 clinical studies (4: other than IMSUT) under this program. The number of studies we assist has been increasing year by year. Efficient operation of the organization is required.

5. Statistical consulting for basic research

Masanori Nojima

Consulting for study design and statistical analysis in any type of clinical research including clinical research, basic medical/biological research. We have collaborated with other members in IMSUT and other institutions through the consulting.

Publications

1. Koga M, Fukuda A, Nojima M, Ishizaka A, Itoh T, Eguchi S, Endo T, Kakinuma A, Kinai E, Goto T, Takahashi S, Takeda H, Tanaka T, Teruya K, Hanai J, Fujii T, Fujitani J, Hosaka T, Mita E, Minami R, Moro H, Yokomaku Y, Watanabe D, Watanabe T, Yotsuyanagi H. Non-acquired immunodeficiency syndrome defining malignancies in people living with haemophilia and human immunodeficiency virus after direct-acting antiviral era. Glob Health Med. 2024 Oct 31;6(5):316-323. doi: 10.35772/ghm.2024.01036. PMID: 39483444; PMCID: PMC11514628.
2. Nakase H, Wagatsuma K, Kobayashi T, Matsumoto T, Esaki M, Watanabe K, Kunisaki R, Takeda T, Arai K, Ibuka T, Ishikawa D, Matsuno Y, Sakuraba H, Ueno N, Yokoyama K, Saruta M, Hokari R, Yokoyama J, Tamano S, Nojima M, Hisamatsu T; MEFV-IBDU Group. Involvement of Mediterranean fever gene mutations in colchicine-responsive enterocolitis: a retrospective cohort study. EBioMedicine. 2024 Dec;110:105454. doi: 10.1016/j.ebiom.2024.105454. Epub 2024 Nov 19. PMID: 39566399; PMCID: PMC11612373.
3. Wakisaka A, Kimura K, Morita H, Nakanishi K, Daimon M, Nojima M, Itoh H, Takeda A, Kitao R, Imai T, Ikeda T, Nakajima T, Watanabe C, Furukawa T, Ohno I, Ishida C, Takeda N, Komai K. Efficacy and Tolerability of Ivabradine for Cardiomyopathy in Patients with Duchenne Muscular Dystrophy. Int Heart J. 2024;65(2):211-217. doi: 10.1536/ihj.23-563. PMID: 38556332.
4. Terunobu Iwai, Ryosuke Ikeguchi, Tomoki Aoyama, Takashi Noguchi, Koichi Yoshimoto, Daichi Sakamoto, Kazuaki Fujita1, Yudai Miyazaki, Shizuka Akieda, Tokiko Nagamura-Inoue, Fumitaka Nagamura, Koichi Nakayama, Shuichi Matsuda. Nerve regeneration using a Bio 3D conduit derived from umbilical cord–Derived mesenchymal stem cells in a rat sciatic nerve defect model. PLoS ONE 19(12):e0310711. https://doi.org/10.1371/journal.pone.0310711
5. Hiroko Yaegashi, Yukikazu Hayashi, Makoto Takeda, Shih-Wei Chiu, Haruhiko Nakayama, Hiroyuki Ito, Atsushi Takano, Masahiro Tsuboi, Koji Teramoto, Hiroyuki Suzuki, Tatsuya Kato, Hiroshi Yasui, Fumitaka Nagamura, Yataro Daigo, Takuhiro Yamaguchi. Efficiency of eSource Direct Data Capture in Investigator-Initiated Clinical Trials in Oncology. Ther Innov Regul Sci. 2024 Jul 2. doi: 10.1007/s43441-024-00671-0. Online ahead of print.
6. Akiko Hori, Atsuko Takahashi, Yuta Miharu, Satoru Yamaguchi, Masatoshi Sugita, Takeo Mukai, Fumitaka Nagamura, Tokiko Nagamura-Inoue. Superior migration ability of umbilical cord-derived mesenchymal stromal cells (MSCs) toward activated lymphocytes in comparison with those of bone marrow and adipose-derived MSCs. Front. Cell Dev. Biol. 12:1329218. doi: 10.3389/fcell.2024.1329218.

IMSUT Hospital

Therapeutic Vector Development Center
治療ベクター開発センター

Professor	Tomoki Todo, M.D., Ph.D.		教　授	博士(医学)	藤　堂　具　紀
Project Professor	Minoru Tanaka, M.D., Ph.D.		特任教授	博士(医学)	田　中　　実

The Therapeutic Vector Development Center (TVDC), formerly known as the Core Facility for Therapeutic Vectors, was established in 2002 as the first facility in Japanese academic institutions for the clinical-grade production of viral and cellular vectors. TVDC is designed to support clinical trials requiring the production of recombinant viral vectors, genetic modification, and/or ex vivo manipulation of patient-derived tissues or cells under Good Gene, Cellular, and Tissue-based Products Manufacturing Practice (GCTP) conditions.

Maintenance of the Standard Operating Procedures (SOPs)

The GCTP (former cGMP) compliance is maintained by the regularly revised SOPs that document all the elements of laboratory work, including both tangible and intangible factors like equipment, facility design, personnel, etc.

ISO certification

The management system of TVDC was re-qualified as ISO 9001-certified in 2023, which has been regularly performed by an independent organization to meet the requirement for ISO 9001 standard.

Validation of TVDC

The TVDC consists of two units; 1) the Vector Unit, the primary suite for viral vector production and ex vivo transduction; 2) the Cell Unit, the suite for cell processing capable of generating therapeutic cells such as dendritic cells for immunotherapy and gene therapy. Each unit has two independent compartments maintained at a Class 10,000 clean level. The facility and equipment are regularly validated in accordance with the SOPs to fulfill the cGMP standard. In 2024, a new Vector Unit (Vector Unit II) was built on the 3rd floor of Building No.1.

Production of clinical grade oncolytic HSV-1

Multiple lots of clinical-grade oncolytic herpes simplex virus type 1 (HSV-1), including G47Δ and various armed third-generation oncolytic HSV-1, have been produced in the Vector Unit (recent Vector Unit I) by the laboratory specialists of the Division of Innovative Cancer Therapy.

Oncolytic MV project

A clinical-grade oncolytic measles virus (MV) was produced by the Laboratory Animal Research Center and stored in the Vector Unit.

IMSUT Hospital

IMSUT CORD
臍帯血・臍帯バンク

Clinical Professor	Tokiko Nagamura-Inoue, M.D., Ph.D.		病院教授	博士(医学)	長 村 登紀子
Professor	Fumitaka Nagamura, M.D., Ph.D.		教 授	博士(医学)	長 村 文 孝
Project Assistant Professor	Kazuhiro Sudo, Ph.D.		特任助教	博士(医学)	須 藤 和 寛

Human umbilical cord blood (CB) and umbilical cord tissue (UC) are attractive sources of somatic stem cells for gene and cell therapies. Especially, the UC has been rapidly utilized as an abundant source of mesenchymal stromal cells (MSCs), which migrate toward inflamed or damaged tissue to reduce inflammation and support tissue repair. Both CB and UC can be provided as "off-the-shelf" cell products for immunotherapies and regenerative medicine. IMSUT CORD is the CB and UC-derived cell bank established in IMSUT hospital in 2016. The aim of IMSUT CORD is to collect, process /culture, cryopreserve, stock, and release CB- and UC-derived cells—including mesenchymal stromal cells (MSCs)—for clinical and research use. We have released CB and UC-derived MSCs to researchers under material transfer agreements to expedite translational studies. We have supplied UC-MSC products for clinical trials for severe acute graft-versus-host disease (GVHD; 2018– 2020), COVID-19-related ARDS (2020–2022), cerebral palsy (PVL;2021–2023), and noninfectious pulmonary complication after allogeneic hematopoietic stem cell transplantation NIPC; 2022–2023). Our main processing facility has been moved from IMSUT cell resource center to new IMSUT-HLC cell processing facility since 2021.

1. Establishing a stable perinatal appendage–derived cell supply system as the source of allogeneic somatic stem cells for research and clinical use

Sudo K, Takahashi A, Hori A, Miharu Y, Sakai T, Shibuya Y, Nagaya N, Ogami K, Mukai T, Nagamura F, Nagamura-Inoue T

Human umbilical cord blood (CB) and umbilical cord tissue (UC) are attractive sources of somatic stem cells for gene and cell therapies. CB and UC can be obtained noninvasively from donors. CB, a known source of hematopoietic stem cells for transplantation, has attracted attention as a new source of immune cells, including universal chimeric antigen receptor T cell therapy (CAR-T) and, more recently, universal CAR-natural killer cells. UC-derived mesenchymal stromal cells (UC-MSCs) have a higher proliferation potency than those derived from adult tissues and can be used anon-HLA restrictively. We have established a CB/UC bank at the IMSUT hospital (IMSUT CORD) to collect CB and UC tissue after informed consent from the mothers in collaboration with the obstetricians. After receiving them, we stock the UC-tissue, and to manufacture master cells and product cells for research and clinical use.

To maintain quality control, we have introduced the ISO 9001:2015 quality management standards in IMSUT CORD since 2018. We have transferred the manufacturing and testing technologies to the client companies, where they apply our techniques and

standards in their clinical trials including therapies for acute GVHD, cerebral palsy, and COVID-19 related acute respiratory distress syndrome (ARDS). The IMSUT CORD mission is to supply domestic UC-MSCs and CB as a source of allogeneic somatic stem cells in research and clinical use. We have supplied clinical-grade UC-MSC products for clinical trials including severe acute graft-versus-host disease (GVHD; 2018-2020), COVID-19-related ARDS (2020-2022), cerebral palsy (PVL) (2021-2023), and non-infectious pulmonary disease after allogeneic hematopoietic stem cell transplantation NIPS; 2022-2023), after approval by the review board of IMSUT CORD and PMDA. We are currently preparing for a clinical trial for treating peripheral nerve injury using allograft bio3D conduit made with UC-MSC products with Kyoto University (AMED project 2022-2024). Since 2021, our main manufacturing location has been moved from the IMSUT-Cell Resource Center (IMSUT-CRC) to a new facility, the IMSUT-HLC Cell Processing Facility (IMSUT-HLC CPF), where the manufacturing license was obtained in 2023.

Visit our website: https://plaza.umin.ac.jp/imsut-cord/

Publications

1) Iwai T, Ikeguchi R, Aoyama T, Noguchi T, Yoshimoto K, Sakamoto D, Fujita K, Miyazaki Y, Akieda S, Nagamura-Inoue T, Nagamura F, Nakayama K, Matsuda S. Nerve regeneration using a Bio 3D conduit derived from umbilical cord-Derived mesenchymal stem cells in a rat sciatic nerve defect model. PLoS One. 19(12): e0310711, 2024
2) Iwatake M, Nagamura-Inoue T, Doi R, Tanoue Y, Ishii M, Yukawa H, Matsumoto K, Tomoshige K, Nagayasu T and Tsuchiya T. Designer umbilical cord-stemcells induce alveolar wall regeneration in pulmonary disease models, Frontiers in Immunology, 15,1384718, 2024
3) Hori A, Takahashi A, Miharu Y, Yamaguchi S, Sugita M, Mukai T, Nagamura F, and Nagamura-Inoue T. Superior migration ability of umbilical cord-derived mesenchymal stromal cells (MSCs) toward activated lymphocytes in comparison with those of bone marrow and adipose-derived MSCs, Front Cell Dev Biol. 12:1329218, 2024

IMSUT Hospital

Department of Nursing
看護部

Director	Mika Kogayu, RN., MSN
Deputy Director	Minayo Hisahara, RN
Deputy Director	Masako Ozawa, RN
Nurse Manager	Hatsuko Narita, RN
Nurse Manage	Tomoko Sato, RN. MSN
Nurse Manager	Nozomi Linzbichler, RN
Nurse Manager	Yukari Tsuru, RN
Nurse Manager	Fumie Kameda, RN
Nurse Manager	Junko Sunada, RN., MSN
Nurse Manager	Emiko Sugiyama, RN
Nurse Manager	Chiharu Shimazu

看護部長	修士(看護学)	小　粥　美　香
副看護部長		久　原　みな代
副看護部長		小　澤　昌　子
看護師長		成　田　初　子
看護師長	修士(看護学)	佐　藤　朋　子
看護師長		リンツビヒラ希
看護師長		都　留　由香里
看護師長		亀　田　史　絵
看護師長	修士(心理学)	砂　田　純　子
看護師長		杉　山　栄美子
看護師長		嶋　津　千　陽

Department of Nursing seeks to provide high-quality nursing care and contribute to the team approach to patient centered care to meet diversified needs, along with changes in social circumstances and with the progress of medical science.

One of our missions is to "make a difference in patient outcomes through nursing care." As nurses, we provide optimal care so that patients can receive high-quality treatment. Patients should be able to live a valuable and meaningful life. As healthcare providers, we strive to prevent infections, bedsores, and other complications. We also do our best to ensure the safety and quality of life of our patients.

We have introduced a career ladder system, e-running, to support nurses' active learning and growth. This allows nurses to continue learning and maintain their motivation to complete their career development as nurses. Excellent knowledge and evidence-based nursing skills are also very important in patient care. We are accelerating the use of a competency model for the development of head nurses.

We have introduced a pair system as a nursing delivery system, and are working to improve the quality of nursing, the effectiveness of OJT (on-the-job training), and the efficiency of nursing services.

Conference Presentation

都留由香里、杉原望、岩崎宏美、小沼貴晶「造血細胞移植後の紫外線対策に関する調査」日本造血・免疫細胞療法学会、東京国際フォーラム、2024.3.21～23

小林路世、慢性疾患看護専門看護師が捉える慢性病者のセクシュアルヘルスと支援の実際、日本慢性看護学会、神戸国際会議場、2024.8.10～11

上山美香、小林路世、古賀道子、織田ひとみ、中澤光子、渡辺直子、四柳宏、菊池正、砂田純子、独居で視力障害があり永久人工肛門を造設した

PLWHに対する外来での関わり、京王プラザホテル、日本エイズ学会・学術総会、2024.11.28〜30

Maiko Noguchi-Watanabe, PhD, Reiko Yamahana, MS, RN,Junko Sunada, RN, CN ,Effect of homecare nurses' remote consultation with advanced nurses on patient symptoms: A feasibility study, The Gerontological Society of America(GSA) Annual Scientific Meeting,Seattle,Unaited States,November13-16

IMSUT Hospital

Department of Pharmacy
薬剤部

Director	Seiichiro Kuroda
Chief	Yohei Iimura
Chief	Sonoe Minegishi-Higashino
Pharmacist	Masaaki Ishibashi
Pharmacist	Mika Yamamura-Noguchi
Pharmacist	Mai Yokota

薬剤部長	黒田誠一郎
薬剤主任	飯村洋平
薬剤主任	峰岸園恵
薬剤師	石橋正祥
薬剤師	山村実佳
薬剤師	横田舞

The Department of Pharmacy seeks to provide high-quality pharmaceutical care services. We contribute to the team approach to patient-oriented medical care and provides a drug distribution service. We are also trying to contribute to propel the right use of medicines for patients.

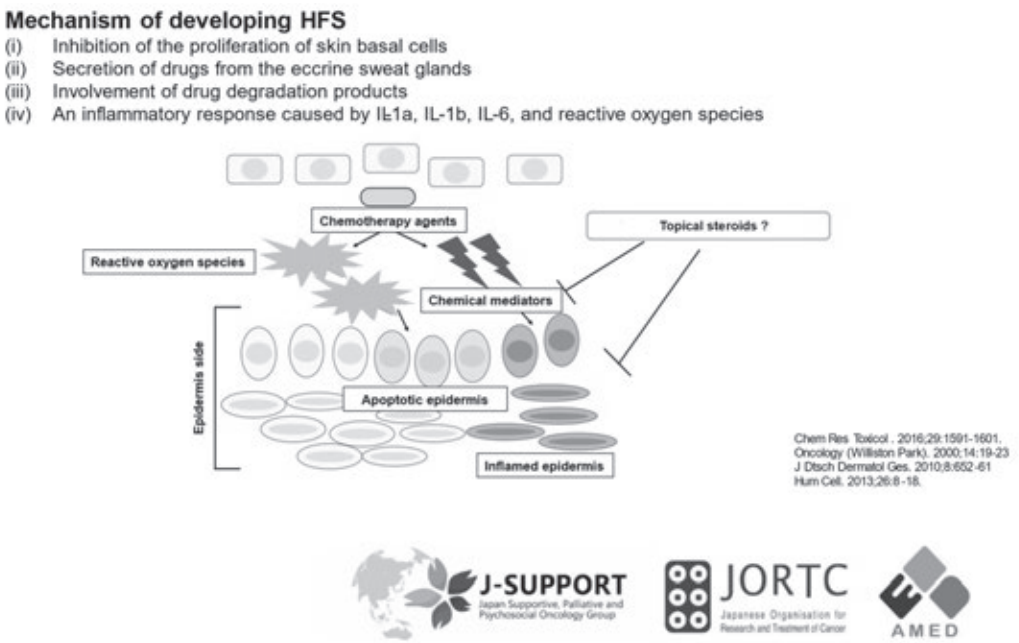
<Publication>

1. Kuroda Seiichiro ,Kiyomi A, Imai S, Sugiura.M. :Japanese Journal of Infection Prevention and Control 2023; 38(3): 114-122.
2. Kuroda Seiichiro, Okuno.M, Kiyomi A, Imai S, Sugiura M. :Japanese Journal of Infection Prevention and Control 2024; 39 (4): 126-132.

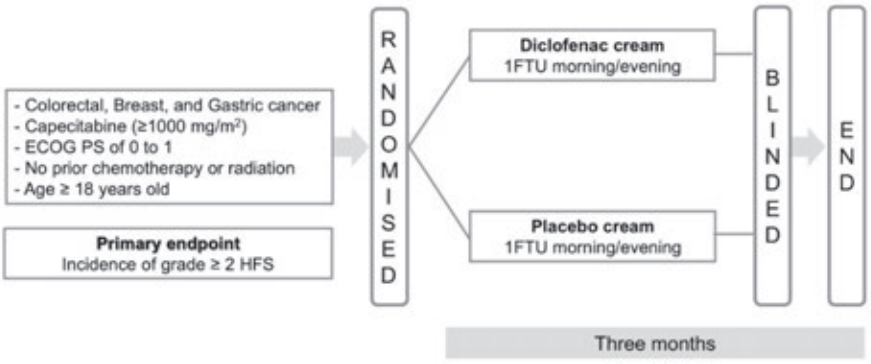
<Clinical Trial>

- Clinical trial for cancer supportive care led by pharmacist
  We are working on phase III study regarding to prevention of capecitabine-induced hand-foot syndrome (HFS). This project is supported by a Practical Research for Innovative Cancer Control project grant (No. 24ck0106978h0001 J-SUPPORT2401/ JORTC-SUP06 (J-DIRECT): a randomized, double blind, placebo-controlled Phase III study evaluating preventive effect of diclofenac cream for capecitabine related hand foot syndrome) from the Japan Agency for Medical Research (AMED). Primary investigator is Yohei Iimura. This trial is based on our phase II study (T-CRACC study, BMC Gastroenterol. 2022;22:341.). New evidence will be published in 2025 from our pharmacy.
- Collaboration with MASCC
  Yohei Iimura is the member of Multinational Association of Supportive Care in Cancer (MASCC) Oncodermatology Study Group. Skin toxicity guideline project is ongoing. MASCC guideline regarding to EGFR-I related skin toxicity and hand-foot syndrome and nail toxicity guideline will be published in 2025.
- Skin toxicity guideline project with JASCC
  Yohei Iimura is the board member of Japanese Association of Supportive Care in Cancer (JASCC) Oncodermatology Study Group. Skin toxicity guideline project for Asian

countries is ongoing. Several review regarding to skin



Preventive Effect of Diclofenac Topical for Capecitabine Related Hand Foot Syndrome a multicentre, randomised, double-blind, placebo-controlled, phase 3 trial: J-SUPPORT2401/JORTC-SUP06



toxicity for Asia will be published in 2025.

- Observational study

  Two following prospective observational study are ongoing.

  -Evaluation of clinical effects of a multidisciplinary-collaborated cancer support team for gastrointestinal cancer chemotherapy: prospective observational study; M-CAST study (BMC Gastroenterol. 2023;23:215.)

  -A prospective observational study to evaluate the efficacy of fosnetupitant for long-delayed chemotherapy-induced nausea and vomiting in patients receiving platinum- based chemotherapy (LODEC-N) (J Clin Trials. 13:532.)

We can establish new evidence with high quality clinical studies in various region.

IMSUT Hospital

Department of AIDS Vaccine Development
エイズワクチン開発担当

Invited Professor	Tetsuro Matano, M.D., D.M.Sc.		教授（委嘱）	博士（医学）	俣 野 哲 朗
Visiting Associate Professor	Ai Kawana-Tachikawa, D.M.Sc.		客員准教授	博士（医学）	立川（川名） 愛

We are working on Microbiology and Immunology to elucidate the immune mechanism for retroviral control in vivo. In particular, we are studying virus-host immune interaction and viral evolution using non-human primate models and human clinical samples derived from African and Asian countries as well as Japan. Furthermore, we are developing vaccines eliciting antibody and/or cytotoxic T lymphocyte responses targeting pathogens including HIV-1, HTLV-1, and SARS-CoV-2.

1. Longitudinal analysis of microbiome composition in Ghanaians living with HIV-1.

Lucky Ronald Runtuwene[1], Prince Kofi Parbie[1], Taketoshi Mizutani[2], Aya Ishizaka[3], Saori Matsuoka[1], Christopher Zaab-Yen Abana[4], Dennis Kushitor[4], Evelyn Yayra Bonney[4], Sampson Badu Ofori[5], Hiroshi Kiyono[6], Koichi Ishikawa[1], William Kwabena Ampofo, and Tetsuro Matano: [1]AIDS Research Center, National Institute of Infectious Diseases, Tokyo, Japan; [2]Department of Computational Biology and Medical Sciences, Graduate School of Frontier Sciences, University of Tokyo; [3]Division of Infectious Diseases, Institute of Medical Science, University of Tokyo; [4]Department of Virology, Noguchi Memorial Institute for Medical Research, University of Ghana; [5]Department of Internal Medicine, Eastern Regional Hospital Koforidua, Ghana Health Service; [6]Institute for Global Prominent Research, Graduate School of Medicine, Chiba University

HIV-1 infection is known to cause gut microbiota dysbiosis. The direct infection of HIV-1 in gut-resident $CD4^+$ T cells causes a cascade of phenomena resulting in the instability of the gut mucosa. The effect of HIV infection on gut microbiome dysbiosis remains unresolved despite antiretroviral therapy. In this study, we showed the results of a longitudinal study of microbiome analysis of people living with HIV (PLWH). We contrasted the diversity and composition of the microbiome of patients with HIV at the first and second time points (baseline_case and six months later follow-up_case, respectively) with those of healthy individuals (baseline_control). We found that despite low diversity indices in the follow-up_case, the abundance of some genera was recovered to be similar to baseline_control. Some genera were consistently in high abundance in HIV^+ samples. Furthermore, we found that the $CD4^+$ T-cell count and soluble CD14 level were significantly related to high and low diversity indices, respectively. We also found that the abundance of some genera was highly correlated with clinical features, especially with antiretroviral duration. This includes genera known to be correlated with worse HIV-1 progression (*Achromobacter* and *Stenotrophomonas*) and a genus associated with gut protection (*Akkermansia*). The fact that a protector of the gut and genera linked with a worse progression of HIV-1 are both enriched may signify that despite the improvement of clinical features, the gut mucosa remains compromised.

2. Prophylactic vaccination inducing anti-Env antibodies can result in protection against HTLV-1 challenge in macaques.

Midori Nakamura-Hoshi[1], Hiroshi Ishii[1], Takushi Nomura[1], Masako Nishizawa[1], Trang Thi Thu Hau[1], Nozomi Kuse[1], Midori Okazaki[1], Akira Ainai[7], Tadaki Suzuki[7], Hideki Hasegawa[8], Takeshi Yoshida[1], Kenzo Yonemitsu[9], Yuriko Suzaki[9], Yasushi Ami[9], Hiroyuki Yamamoto[1], and Tetsuro Matano: [7]Department of Pathology, National Institute of Infectious Diseases; [8]Center for Influenza and Respiratory Virus Research, National Institute of Infectious Diseases; [9]Management Department of Biosafety, Laboratory Animal, and Pathogen Bank, National Institute of Infectious Diseases

HTLV-1 infection occurs by cell-to-cell transmission and can induce fatal adult T-cell leukemia (ATL). Vaccine development is critical for the control of HTLV-1 transmission. However, determining whether vaccine-induced anti-Env antibodies can prevent cell-to-cell HTLV-1 transmission is challenging. In this study, we examined the protective efficacy of a vaccine inducing anti-Env antibodies against HTLV-1 challenge in cynomolgus macaques. Eight of ten vaccinated macaques produced anti-HTLV-1 neutralizing antibodies (NAbs) and were protected from an intravenous challenge with 10^8 HTLV-1-producing cells. In contrast, the two vaccinated macaques without NAb induction and ten unvaccinated controls showed HTLV-1 infection with detectable proviral load after challenge. Five of the eight protected macaques were administered with an anti-CD8 monoclonal antibody, but proviruses remained undetectable and no increase in anti-HTLV-1 antibodies was observed even after $CD8^+$ cell depletion in three of them. Analysis of Env-specific T cell responses did not suggest involvement of vaccine-induced Env-specific T cell responses in the protection. These results indicate that anti-Env antibody induction by vaccination can result in functionally sterile HTLV-1 protection, implying the rationale for strategies aimed at anti-Env antibody induction in prophylactic HTLV-1 vaccine development.

3. Characterization of the Proinflammatory Cytokine Profile during Acute SARS-CoV-2 Infection in People with Human Immunodeficiency Virus.

Alitzel Anzurez[1], Lucky Runtuwene[1], Thi Thu Thao Dang[1], Kaori Nakayama-Hosoya[1], Michiko Koga[3], Yukihiro Yoshimura[10], Hiroaki Sasaki[10], Nobuyuki Miyata[10], Kazuhito Miyazaki[10], Yoshimasa Talahashi[11], Tadaki Suzuki[7], Hiroshi Yotsuyanagi[3], Natsuo Tachikawa[10], Tetsuro Matano, and Ai Kawana-Tachikawa: [10]Department of Respiratory Medicine, Yokohama Municipal Citizens' Hospital; [11]Research Center for Drug and Vaccine Development, National Institute of Infectious Diseases

Persistent inflammation in chronic HIV infection may affect immune responses against SARS-CoV-2 infection. Plasma levels of multiple proinflammatory cytokines during acute SARS-CoV-2 infection were assessed in people with HIV (PWH) with effective cART. There were no significant differences in any of the tested cytokines between COVID-19 severity in PWH, while most of them were significantly higher in individuals with severe disease in HIV-uninfected individuals, suggesting that excess cytokines release by hyper-inflammatory responses does not occur in severe COVID-19 with HIV infection. The strong associations between the cytokines observed in HIV-uninfected individuals, especially between IFN-α/TNF-α and other cytokines, were lost in PWH. The steady state plasma levels of IP-10, ICAM-1, and CD62E were significantly higher in PWH, indicating that PWH are in an enhanced inflammatory state. Loss of the several inter-cytokine correlations were observed in *in vitro* LPS stimuli-driven cytokine production in PWH. These data suggest that inflammatory responses during SARS-CoV-2 infection in PWH distinct from those in HIV-uninfected individuals, partially due to the underlying inflammatory state and/or impairment of innate immune cells.

4. Virion-surface display of a chimeric immunoglobulin Fc domain facilitating uptake by antigen-presenting cells.

Sayuri Seki[1], Prince Kofi Parbie[1], Hiroyuki Yamamoto[1], and Tetsuro Matano

Antigen-presenting cells (APCs) play an important role in virus infection control by bridging innate and adaptive immune responses. Macrophages and dendritic cells (DCs) possess various surface receptors to recognize/internalize antigens, and antibody binding can enhance pathogen-opsonizing uptake by these APCs via interaction of antibody fragment crystallizable (Fc) domains with Fc receptors, evoking profound pathogen control in certain settings. In this study, we examined phagocytosis-enhancing potential of Fc domains directly oriented on a retroviral virion/virus-like particle (VLP) surface. We generated an expression vector coding a murine Fc fragment fused to the transmembrane region (TM) of a retroviral envelope protein, deriving expression of the Fc-TM fusion protein on the transfected cell surface and production of virions incorporating the chimeric Fc upon co-transfection. Incubation of Fc-displaying SIV with murine J774 macrophages and bone marrow-derived DCs derived Fc receptor-dependent enhanced uptake, being visualized by imaging cytometry. Alternative preparation of a MLV backbone-based Fc-displaying VLP loading an influenza virus HA antigen resulted in enhanced HA internalization by macrophages, stating antigen compatibility of the design. Results show that the Fc-TM fusion molecule can be displayed on certain viruses/VLPs and may be

utilized as a molecular adjuvant to facilitate APC antigen uptake.

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IMSUT Distinguished Professor Unit

Division of Virology
ウイルス感染部門

Project Professor　Yoshihiro Kawaoka, D.V.M., Ph.D.　特任教授　獣医学博士　河　岡　義　裕

Viruses can cause devastating diseases. The long-term goal of our research is to understand the molecular pathogenesis of viral diseases by using influenza virus, Ebola virus, and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections as models. Interactions between viral and host gene products during viral replication determine the consequences of infection (i.e., the characteristics of disease manifestation, whether limited or widespread); hence, our research has centered on such interactions during these viral infections.

1. Pathogenicity and transmissibility of bovine H5N1 influenza virus.

Eisfeld AJ[1], Biswas A[1], Guan L[1], Gu C[1], Maemura T[1], Trifkovic S[1], Wang T[1], Babujee L[1], Dahn R[1], Halfmann PJ[1], Barnhardt T[2], Neumann G[1], Suzuki Y[3], Thompson A[4], Swinford AK[5], Dimitrov KM[5], Poulsen K[6], Kawaoka Y. [1]Influenza Research Institute, Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI, USA. [2]Heritage Vet Partners, Johnson, KS, USA. [3]Department of Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan. [4]Texas A&M Veterinary Medical Diagnostic Laboratory, Canyon, TX, USA. [5]Texas A&M Veterinary Medical Diagnostic Laboratory, College Station, TX, USA. [6]Wisconsin Veterinary Diagnostic Laboratory, University of Wisconsin-Madison, Madison, WI, USA.

Highly pathogenic H5N1 avian influenza (HPAI H5N1) viruses occasionally infect, but typically do not transmit, in mammals. In the spring of 2024, an unprecedented outbreak of HPAI H5N1 in bovine herds occurred in the USA, with virus spread within and between herds, infections in poultry and cats, and spillover into humans, collectively indicating an increased public health risk. Here we characterize an HPAI H5N1 virus isolated from infected cow milk in mice and ferrets. Like other HPAI H5N1 viruses, the bovine H5N1 virus spread systemically, including to the mammary glands of both species, however, this tropism was also observed for an older HPAI H5N1 virus isolate. Bovine HPAI H5N1 virus bound to sialic acids expressed in human upper airways and inefficiently transmitted to exposed ferrets (one of four exposed ferrets seroconverted without virus detection). Bovine HPAI H5N1 virus thus possesses features that may facilitate infection and transmission in mammals.

2. A human isolate of bovine H5N1 is transmissible and lethal in animal models.

Gu C[1], Maemura T[1], Guan L[1], Eisfeld AJ[1], Biswas A[1], Kiso M, Uraki R, Ito M, Trifkovic S[1], Wang T[1], Babujee L[1], Presler R Jr[1], Dahn R[1], Suzuki Y[3], Halfmann PJ[1], Yamayoshi S, Neumann G[1], Kawaoka Y.

The outbreak of clade 2.3.4.4b highly pathogenic avian influenza viruses of the H5N1 subtype (HPAI H5N1) in dairy cattle in the USA has so far resulted in spillover infections of at least 14 farm workers, who presented with mild respiratory symptoms or conjunctivitis, and one individual with no known animal exposure who was hospitalized but recovered. Here we characterized A/Texas/37/2024 (huTX37-H5N1), a virus isolated from the eyes of an infected farm work-

er who developed conjunctivitis. huTX37-H5N1 replicated efficiently in primary human alveolar epithelial cells, but less efficiently in corneal epithelial cells. Despite causing mild disease in the infected worker, huTX37-H5N1 proved lethal in mice and ferrets and spread systemically, with high titres in both respiratory and non-respiratory organs. Importantly, in four independent experiments in ferrets, huTX37-H5N1 transmitted by respiratory droplets in 17-33% of transmission pairs, and five of six exposed ferrets that became infected died. PB2-631L (encoded by bovine isolates) promoted influenza polymerase activity in human cells, suggesting a role in mammalian adaptation similar to that of PB2-627K (encoded by huTX37-H5N1). In addition, bovine HPAI H5N1 virus was found to be susceptible to polymerase inhibitors both in vitro and in mice. Thus, HPAI H5N1 virus derived from dairy cattle transmits by respiratory droplets in mammals without previous adaptation and causes lethal disease in animal models.

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Social Cooperation Research Program

Project Division of RNA Medical Science
RNA 医科学社会連携研究部門

Project Associate Professor Kaku Goto, Ph.D.

特任准教授　博士（医学）　後　藤　　覚

RNA plays a crucial in biological science for its role in life, as illustrated by RNA interference, molecular mimicry, ribosome structure, RNA quality control, and noncoding RNAs. Taking advantage of such biomolecular properties of RNA, we aim to create artificial aptamers to target proteins of therapeutic interest. Aptamers, generated through systematic evolution of ligands by exponential enrichment, can be used as therapeutic agents, reagents for affinity purification, or biosensor elements.

1. SELEX targeting virus-like particle generates RNA aptamers inhibiting chikungunya virus

Kaku Goto, Ryo Amano, Akiko Ichinose[1], Akiya Michishita[1], Michiaki Hamada[1], Yoshikazu Nakamura[2], Masaki Takahashi[2]: [1]Graduate School of Advanced Science and Engineering, Waseda University, Tokyo 169-8555, Japan; [2]RIBOMIC Inc., Tokyo 108-0071, Japan

Nucleic acid aptamers are a promising drug modality, but it remains difficult to generate virus-neutralizing aptamers due to the lack of a robust production system. We report on a platform technology for the development of virus-neutralizing RNA aptamers using chikungunya virus (CHIKV) as a model target by adopting our SELEX method with virus-like particles (VLPs), VLP-SELEX. An identified aptamer raised against CHIKV-VLPs, Apt#1, and its truncated derivatives showed neutralizing activity with IC50 values in the nanomolar range in a CHIKV pseudoparticle (CHIKVpp) assay. An antiviral-based chemical genetic approach to explore the interaction domain of Apt#1 revealed additive effects of Apt#1 with most of the evaluated antivirals. Meanwhile, strong competition with suramin, a reported interactant with domain A of the E2 envelope protein (E2DA), was demonstrated in both CHIKVpp and surface plasmon resonance analyses, predicting E2DA to be the interface for Apt#1. In addition, Apt#1 was suggested to act as an attachment inhibitor targeting E2DA of CHIKV. These results suggest that VLP-SELEX may be a novel, viable option for exploring virus neutralizing agents targeting virus particles of interest.

2. A chimeric RNA consisting of siRNA and aptamer for inhibiting dengue virus replication

Ryo Amano, Masaki Takahashi[1], Kazumi Haga[2], Mizuki Yamamoto[3], Kaku Goto, Akiko Ichinose[4], Michiaki Hamada[4], Jin Gohda[3], Jun-ichiro Inoue[5], Yasushi Kawaguchi[3,5,6], Meng Ling Moi[2,5], Yoshikazu Nakamura[1]: [1]RIBOMIC Inc., 3-16-13, Shirokanedai, Minato-ku, Tokyo 108-0071, Japan; [2]Department of Developmental Medical Sciences, School of International Health, Graduate School of Medicine, The University of Tokyo, 7-3-1, Hongo, Bunkyo-ku, Tokyo 113-0033, Japan; [3]Research Center for Asian Infectious Diseases, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan; [4]Graduate School of Advanced Science and Engineering, Waseda University, 3-4-1, Okubo, Shinjuku-ku, Tokyo 169-8555, Japan; [5]The University of Tokyo Pandemic Preparedness, Infection and Advanced Research Center (UTOPIA), 4-6-1, Shirokanedai, Minato-ku,

Tokyo 108-8639, Japan; [6]Division of Molecular Virology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, 4-6-1, Shirokanedai, Minato-ku, Tokyo 108-8639, Japan

A chimeric RNA, small interfering RNAs (siRNAs) to viral RNAs, conjugated with RNA aptamers to viral envelope proteins, is a promising modality for viral diseases, although practical evaluations are lacking. Here, we present a chimeric RNA composed of siRNA and RNA aptamer, both targeting all four serotypes of dengue virus (DENV), for the suppression of DENV replication. The siRNA against consensus sequences in the 3′-UTR of all four DENV serotypes suppressed the expression of a reporter gene carrying the siRNA-targeted sequence of DENV-1 by 70%. The RNA aptamer generated by VLP-SELEX using DENV-1-VLPs as baits showed an affinity for DENV-VLPs of all four serotypes, presumably without interfering with the fusion process. After conjugation of each modality, the chimeric RNA significantly suppressed authentic DENV-1 and DENV-2 production. Our study demonstrates that chimeric RNAs are potentially effective antivirals.

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Social Cooperation Research Program

Project Division of Advanced Biopharmaceutical Science

先進的バイオ医薬品学社会連携研究部門

Project Professor	Kouhei Tsumoto, Ph.D.		特任教授	博士(工学)	津 本 浩 平
Project Associate Professor	Susana de Vega, Ph.D.		特任准教授	博士(生物学)	スサーナ デ ベガ

Various types of antibodies have been approved for therapeutic use and are currently being examined in clinical trials. In the later years, the development of new technologies for the discovery and optimization of high-potency antibodies has led to the ability to design specific and stable antibodies with desired biological properties. Biophysical analyses of therapeutic antibodies, particularly those of protein interaction and stability, are critical for the development of biopharmaceuticals, and an essential step to design next-generation antibodies. The development of analytical methods with quantitative and high-sensitive detection of antigen interaction, protein stability, and biological function of an antibody, therefore, has been challenging for pharmaceutical companies. In this division, we study the biophysical properties of various antibodies to propose a new strategy for the development of highly efficient next-generation antibodies.

1. Experimental modification in thermal stability of oligomers by alanine substitution and site saturation mutagenesis of interfacial residues

Hoya M, Matsunaga R, Nagatoishi S, Tsumoto K.

For certain industrial applications, the stability of protein oligomers is important. In this study, we demonstrated an efficient method to improve the thermal stability of oligomers using the trimeric protein chloramphenicol acetyltransferase (CAT) as the model. We substituted all interfacial residues of CAT with alanine to detect residues critical for oligomer stability. Mutation of six of the forty-nine interfacial residues enhanced oligomer thermal stability. Site saturation mutagenesis was performed on these six residues to optimize the side chains. About 15% of mutations enhanced thermal stability by more than 0.5 °C and most did not disrupt activity of CAT. Certain combinations of mutations further improved thermal stability and resistance against heat treatment. The quadruple mutant, H17V/N34S/F134A/D157C, retained the same activity as the wild-type after heat treatment at 9 °C higher temperature than the wild-type CAT. Furthermore, combinations with only alanine substitutions also improved thermal stability, suggesting the method we developed can be used for rapid modification of industrially important proteins.

2. IL-6 Reduces Spheroid Sizes of Osteophytic Cells Derived from Osteoarthritis Knee Joint via Induction of Apoptosis

Negishi Y, Adili Arepati, Susana de Vega, Momoeda M, Kaneko H, Mehmet Zeynel Cilek, Yoshinaga C, Takafuji K, Otsuka Y, Shimoda M, Negishi-Koga T, Ishijima M, Okada Y

Osteophytes in osteoarthritis (OA) joints contribute to restriction of joint movement, joint pain, and OA progression, but little is known about osteophyte

regulators. Examination of gene expression related to cartilage extracellular matrix, endochondral ossification, and growth factor signaling in articular cartilage and osteophytes obtained from OA knee joints showed that several genes such as COL1A1, VCAN, BGLAP, BMP8B, RUNX2, and SOST were overexpressed in osteophytes compared with articular cartilage. Ratios of mesenchymal stem/progenitor cells, which were characterized by co-expression of CD105 and CD166, were significantly higher in osteophytic cells than articular cells. A three-dimensional culture method for cartilage and osteophyte cells was developed by modification of cultures of self-assembled spheroid cell organoids (spheroids). These spheroids cultured in the media for mesenchymal stem cells containing transforming growth factor-β3 showed characteristic morphologies and gene expression profiles of articular cartilage and osteophytes, respectively. The effects of IL-1β, tumor necrosis factor-α, and IL-6 on the spheroids of articular and osteophytic cells were studied. To the best of our knowledge, they provide the first evidence that IL-6 suppresses the spheroid size of osteophytic cells by inducing apoptosis and reducing extracellular matrix molecules. These data show that IL-6 is the suppressor of osteophyte growth and suggest that IL-6 expression and/or activity are implicated in the regulation of osteophyte formation in pathologic joints.

3. Generation of antibodies to an extracellular region of the transporters Glut1/Glut4 by immunization with a designed antigen

Sumikawa T, Nakakido M, Matsunaga R, Kuroda D, Nagatoishi S, Tsumoto K.

Monoclonal antibodies are one of the fastest growing class of drugs. Nevertheless, relatively few biologics target multispanning membrane proteins because of technical challenges. To target relatively small extracellular regions of multiple membrane-spanning proteins, synthetic peptides, which are composed of amino acids corresponding to an extracellular region of a membrane protein, are often utilized in antibody discovery. However, antibodies to these peptides often do not recognize parental membrane proteins. In this study, we designed fusion proteins in which an extracellular helix of the membrane protein glucose transporter 1 (Glut1) was grafted onto the scaffold protein Adhiron. In the initial design, the grafted fragment did not form a helical conformation. Molecular dynamics simulations of full-length Glut1 suggested the importance of intramolecular interactions formed by surrounding residues in the formation of the helical conformation. A fusion protein designed to maintain such intramolecular interactions did form the desired helical conformation in the grafted region. We then immunized an alpaca with the designed fusion protein and obtained VHH (variable region of heavy-chain antibodies) using the phage display method. The binding of these VHH antibodies to the recombinant Glut1 protein was evaluated by surface plasmon resonance, and their binding to Glut1 on the cell membrane was further validated by flow cytometry. Furthermore, we also succeeded in the generation of a VHH against another integral membrane protein, glucose transporter 4 (Glut4) with the same strategy. These illustrates that our combined biochemical and computational approach can be applied to designing other novel fusion proteins for generating site-specific antibodies.

4. Unveiling the affinity-stability relationship in anti-measles virus antibodies: a computational approach for hotspots prediction

Paul R, Kasahara K, Sasaki J, Pérez JF, Matsunaga R, Hashiguchi T, Kuroda D, Tsumoto K.

Recent years have seen an uptick in the use of computational applications in antibody engineering. These tools have enhanced our ability to predict interactions with antigens and immunogenicity, facilitate humanization, and serve other critical functions. However, several studies highlight the concern of potential trade-offs between antibody affinity and stability in antibody engineering. In this study, we analyzed anti-measles virus antibodies as a case study, to examine the relationship between binding affinity and stability, upon identifying the binding hotspots. We leverage in silico tools like Rosetta and FoldX, along with molecular dynamics (MD) simulations, offering a cost-effective alternative to traditional in vitro mutagenesis. We introduced a pattern in identifying key residues in pairs, shedding light on hotspots identification. Experimental physicochemical analysis validated the predicted key residues by confirming significant decrease in binding affinity for the high-affinity antibodies to measles virus hemagglutinin. Through the nature of the identified pairs, which represented the relative hydropathy of amino acid side chain, a connection was proposed between affinity and stability. The findings of the study enhance our understanding of the interactions between antibody and measles virus hemagglutinin. Moreover, the implications of the observed correlation between binding affinity and stability extend beyond the field of anti-measles virus antibodies, thereby opening doors for advancements in antibody research.

5. THOUSAND-GRAIN WEIGHT 6, which is an IAA-glucose hydrolase, preferentially recognizes the structure of the indole ring

Akabane T, Suzuki N, Ikeda K, Yonezawa T, Nagatoishi S, Matsumura H, Yoshizawa T, Tsuchiya W, Kamino S, Tsumoto K, Ishimaru K, Katoh E, Hirotsu N.

An indole-3-acetic acid (IAA)-glucose hydrolase, THOUSAND-GRAIN WEIGHT 6 (TGW6), negatively regulates the grain weight in rice. TGW6 has been used as a target for breeding increased rice yield. Moreover, the activity of TGW6 has been thought to involve auxin homeostasis, yet the details of this putative TGW6 activity remain unclear. Here, we show the three-dimensional structure and substrate preference of TGW6 using X-ray crystallography, thermal shift assays and fluorine nuclear magnetic resonance (19F NMR). The crystal structure of TGW6 was determined at 2.6 Å resolution and exhibited a six-bladed β-propeller structure. Thermal shift assays revealed that TGW6 preferably interacted with indole compounds among the tested substrates, enzyme products and their analogs. Further analysis using 19F NMR with 1,134 fluorinated fragments emphasized the importance of indole fragments in recognition by TGW6. Finally, docking simulation analyses of the substrate and related fragments in the presence of TGW6 supported the interaction specificity for indole compounds. Herein, we describe the structure and substrate preference of TGW6 for interacting with indole fragments during substrate recognition. Uncovering the molecular details of TGW6 activity will stimulate the use of this enzyme for increasing crop yields and contributes to functional studies of IAA glycoconjugate hydrolases in auxin homeostasis.

6. Structural basis for the recognition of human hemoglobin by the heme-acquisition protein Shr from Streptococcus pyogenes

Senoo A, Hoshino M, Shiomi T, Nakakido M, Nagatoishi S, Kuroda D, Nakagawa I, Tame JRH, Caaveiro JMM, Tsumoto K.

In Gram-positive bacteria, sophisticated machineries to acquire the heme group of hemoglobin (Hb) have evolved to extract the precious iron atom contained in it. In the human pathogen Streptococcus pyogenes, the Shr protein is a key component of this machinery. Herein we present the crystal structure of hemoglobin-interacting domain 2 (HID2) of Shr bound to Hb. HID2 interacts with both, the protein and heme portions of Hb, explaining the specificity of HID2 for the heme-bound form of Hb, but not its heme-depleted form. Further mutational analysis shows little tolerance of HID2 to interfacial mutations, suggesting that its interaction surface with Hb could be a suitable candidate to develop efficient inhibitors abrogating the binding of Shr to Hb.

7. A high-resolution structural characterization and physicochemical study of how a peptoid binds to an oncoprotein MDM2

Yokomine M, Morimoto J, Fukuda Y, Ueda T, Takeuchi K, Umezawa K, Ago H, Matsuura H, Ueno G, Senoo A, Nagatoishi S, Tsumoto K, Sando S.

Peptoids are a promising drug modality targeting disease-related proteins, but how a peptoid engages in protein binding is poorly understood. This is primarily due to a lack of high-resolution peptoid-protein complex structures and systematic physicochemical studies. Here, we present the first crystal structure of a peptoid bound to a protein, providing high-resolution structural information about how a peptoid binds to a protein. We previously reported a rigid peptoid, oligo(N-substituted alanine) (oligo-NSA), and developed an oligo-NSA-type peptoid that binds to MDM2. X-ray crystallographic analysis of the peptoid bound to MDM2 showed that the peptoid recognizes the MDM2 surface predominantly through the interaction of the N-substituents, while the main chain acts as a scaffold. Additionally, conformational, thermodynamic, and kinetic analysis of the peptoid and its derivatives with a less rigid main chain revealed that rigidification of the peptoid main chain contributes to improving the protein binding affinity. This improvement is thermodynamically attributed to an increased magnitude of the binding enthalpy change, and kinetically to an increased association rate and decreased dissociation rate. This study provides invaluable insights into the design of protein-targeting peptoids.

8. Thermodynamic and molecular dynamic insights into how fusion influences peptide-tag recognition of an antibody

Miyanabe K, Yamashita T, Tsumoto K.

To understand the effect of protein fusion on the recognition of a peptide-tag by an antibody, we fused a CCR5-derived peptide-tag (pep1) to GFP and investigated its recognition by an anti-pep1 antibody, 4B08. First, to characterize the thermodynamic properties associated with the pep1-4B08 binding, isothermal titration calorimetry experiments were conducted. It was found that pep1 fused to the C-terminus of GFP (GFP-CT) enhanced the enthalpic gain by 2.1 kcal mol-1 and the entropic loss only by 0.9 kcal mol-1, resulting in an 8-fold increase in the binding affinity compared to the unfused pep1. On the other hand, pep1 fused to the N-terminus of GFP (GFP-NT) enhanced the enthalpic gain by 3.0 kcal mol-1 and the entropic loss by 3.2 kcal mol-1, leading to no significant enhancement of the binding affinity. To gain deeper insights, molecular dynamics simulations of GFP-NT, GFP-CT, and pep1 were performed. The results showed that the location of the fusion point sensitively affects the interaction energy, the solvent accessible surface area, and the fluctuation of pep1 in the unbound state, which explains the difference in the experimental thermodynamic properties.

9. Functional insights of Tyr37 in framework region 2 directly contributing to the binding affinities and dissociation kinetics in single-domain VHH antibodies

Yamamoto K, Nagatoishi S, Nakakido M, Kuroda D, Tsumoto K.

Single-domain VHH antibody is regarded as one of the promising antibody classes for therapeutic and diagnostic applications. VHH antibodies have amino acids in framework region 2 that are distinct from those in conventional antibodies, such as the Val-37Phe/Tyr (V37F/Y) substitution. Correlations between the residue type at position 37 and the conformation of the CDR3 in VHH antigen recognition have been previously reported. However, few studies focused on the meaning of harboring two residue types in position 37 of VHH antibodies, and the concrete roles of Y37 have been little to be elucidated. Here, we investigated the functional states of position 37 in co-crystal structures and performed analyses of three model antibodies with either F or Y at position 37. Our analysis indicates that Y at position 37 enhances the dissociation rate, which is highly correlated with drug efficacy. Our findings help to explain the molecular mechanisms that distinguish VHH antibodies from conventional antibodies.

10. Cryo-EM structures elucidate the multiligand receptor nature of megalin

Goto S, Tsutsumi A, Lee Y, Hosojima M, Kabasawa H, Komochi K, Nagatoishi S, Takemoto K, Tsumoto K, Nishizawa T, Kikkawa M, Saito A.

Megalin (low-density lipoprotein receptor-related protein 2) is a giant glycoprotein of about 600 kDa, mediating the endocytosis of more than 60 ligands, including those of proteins, peptides, and drug compounds [S. Goto, M. Hosojima, H. Kabasawa, A. Saito, Int. J. Biochem. Cell Biol. 157, 106393 (2023)]. It is expressed predominantly in renal proximal tubule epithelial cells, as well as in the brain, lungs, eyes, inner ear, thyroid gland, and placenta. Megalin is also known to mediate the endocytosis of toxic compounds, particularly those that cause renal and hearing disorders [Y. Hori et al., J. Am. Soc. Nephrol. 28, 1783-1791 (2017)]. Genetic megalin deficiency causes Donnai-Barrow syndrome/facio-oculo-acoustico-renal syndrome in humans. However, it is not known how megalin interacts with such a wide variety of ligands and plays pathological roles in various organs. In this study, we elucidated the dimeric architecture of megalin, purified from rat kidneys, using cryoelectron microscopy. The maps revealed the densities of endogenous ligands bound to various regions throughout the dimer, elucidating the multiligand receptor nature of megalin. We also determined the structure of megalin in complex with receptor-associated protein, a molecular chaperone for megalin. The results will facilitate further studies on the pathophysiology of megalin-dependent multiligand endocytic pathways in multiple organs and will also be useful for the development of megalin-targeted drugs for renal and hearing disorders, Alzheimer's disease [B. V. Zlokovic et al., Proc. Natl. Acad. Sci. U.S.A. 93, 4229-4234 (1996)], and other illnesses.

11. Next-Generation Anti-TNFα Agents: The Example of Ozoralizumab

Tsumoto K, Takeuchi T.

Biologic therapy involving anti-tumor necrosis factor-α (anti-TNFα) agents has fundamentally changed the management of patients with immune-mediated inflammatory diseases, including rheumatoid arthritis, thus benefiting many patients. Nevertheless, the inability of some patients to achieve low disease activity or clinical remission remains a major concern. To address such concerns, next-generation anti-TNFα agents that differ from the immunoglobulin G-format anti-TNFα agents that have been used to date are being developed using antibody-engineering technology. Their unique design employing novel molecular characteristics affords several advantages, such as early improvement of clinical symptoms, optimization of drug bioavailability, enhancement of tissue penetration, and a reduction in side effects. This holds promise for a new paradigm shift in biologic therapy via the use of next-generation anti-TNFα agents. Ozoralizumab, a next-generation anti-TNFα agent that was recently approved in Japan, comprises a variable region heavy-chain format. It has a completely different structure from conventional therapeutic antibodies, such as a small molecular size, an albumin-binding module, and a unique format that produces an avidity effect. Ozoralizumab exhibited rapid biodistribution into joints, provided attenuation of Fcγ receptor-mediated inflammatory responses, and had a high binding affinity to TNFα in non-clinical studies. In clinical trials, ozoralizumab yielded an early improvement in clinical symptoms, a sustained efficacy for up to 52 weeks, and an acceptable tolerability in patients with rheumatoid arthritis. This review focuses on the results of pre-clinical and clinical trials for ozoralizumab and outlines the progress in next-generation antibody development.

12. Characterization of a novel format scFv×VHH single-chain biparatopic antibody against metal binding protein MtsA

Asano R, Takeuchi M, Nakakido M, Ito S, Aikawa C, Yokoyama T, Senoo A, Ueno G, Nagatoishi S, Tanaka Y, Nakagawa I, Tsumoto K.

Biparatopic antibodies (bpAbs) are engineered antibodies that bind to multiple different epitopes within the same antigens. bpAbs comprise diverse formats, including fragment-based formats, and choosing the appropriate molecular format for a desired function against a target molecule is a challenging task. Moreover, optimizing the design of constructs requires selecting appropriate antibody modalities and adjusting linker length for individual bpAbs. Therefore, it is crucial to understand the characteristics of bpAbs at the molecular level. In this study, we first obtained single-chain variable fragments and camelid heavy-chain variable domains targeting distinct epitopes of the metal binding protein MtsA and then developed a novel format single-chain bpAb connecting these fragment antibodies with various linkers. The physicochemical properties, binding activities, complex formation states with antigen, and functions of the bpAb were analyzed using multiple approaches. Notably, we found that the assembly state of the complexes was controlled by a linker and that longer linkers tended to form more compact complexes. These observations provide detailed molecular information that should be considered in the design of bpAbs.

13. Crystal structures of human CD40 in complex with monoclonal antibodies dacetuzumab and bleselumab

Asano R, Nakakido M, Pérez JF, Ise T, Caaveiro JMM, Nagata S, Tsumoto K.

CD40 is a member of the tumor necrosis factor receptor superfamily, and it is widely expressed on immune and non-immune cell types. The interaction between CD40 and the CD40 ligand (CD40L) plays an essential function in signaling, and the CD40/CD40L complex works as an immune checkpoint molecule. CD40 has become a therapeutic target, and a variety of agonistic/antagonistic anti-CD40 monoclonal antibodies (mAbs) have been developed. To better understand the mode of action of anti-CD40 mAbs, we determined the X-ray crystal structures of dacetuzumab (agonist) and bleselumab (antagonist) in complex with the extracellular domain of human CD40, respectively. The structure reveals that dacetuzumab binds to CD40 on the top of cysteine-rich domain 1 (CRD1), which is the domain most distant from the cell surface, and it does not compete with CD40L binding. The binding interface of bleselumab spread between CRD2 and CRD1, overlapping with the binding surface of the ligand. Our results offer important insights for future structural and functional studies of CD40 and provide clues to understanding the mechanism of biological response. These data can be applied to developing new strategies for designing antibodies with more therapeutic efficacy.

14. High-throughput system for the thermostability analysis of proteins

Ito S, Matsunaga R, Nakakido M, Komura D, Katoh H, Ishikawa S, Tsumoto K.

Thermal stability of proteins is a primary metric for evaluating their physical properties. Although researchers attempted to predict it using machine learning frameworks, their performance has been dependent on the quality and quantity of published data. This is due to the technical limitation that thermodynamic characterization of protein denaturation by fluorescence or calorimetry in a high-throughput manner has been challenging. Obtaining a melting curve that derives solely from the target protein requires laborious purification, making it far from practical to prepare a hundred or more samples in a single workflow. Here, we aimed to overcome this throughput limitation by leveraging the high protein secretion efficacy of Brevibacillus and consecutive treatment with plate-scale purification methodologies. By handling the entire process of expression, purification, and analysis on a per-plate basis, we enabled the direct observation of protein denaturation in 384 samples within 4 days. To demonstrate a practical application of the system, we conducted a comprehensive analysis of 186 single mutants of a single-chain variable fragment of nivolumab, harvesting the melting temperature (Tm) ranging from -9.3 up to +10.8°C compared to the wild-type sequence. Our findings will allow for data-driven stabilization in protein design and streamlining the rational approaches.

15. Impact of single-residue mutations on protein thermal stability: The case of threonine 83 of BC2L-CN lectin

Hoya M, Matsunaga R, Nagatoishi S, Ide T, Kuroda D, Tsumoto K.

The thermal stability of trimeric lectin BC2L-CN was investigated and found to be considerably altered when mutating residue 83, originally a threonine, located at the fucose-binding loop. Mutants were analyzed using differential scanning calorimetry and isothermal microcalorimetry. Although most mutations decreased the affinity of the protein for oligosaccharide H type 1, six mutations increased the melting temperature (Tm) by >5 °C; one mutation, T83P, increased the Tm value by 18.2 °C(T83P, Tm = 96.3 °C). In molecular dynamic simulations, the investigated thermostable mutants, T83P, T83A, and T83S, had decreased fluctuations in the loop containing residue 83. In the T83S mutation, the side-chain hydroxyl group of serine formed a hydrogen bond with a nearby residue, suggesting that the restricted movement of the side-chain resulted in fewer fluctuations and enhanced thermal stability. Residue 83 is located

at the interface and near the upstream end of the equivalent loop in a different protomer; therefore, fluctuations by this residue likely propagate throughout the loop. Our study of the dramatic change in thermal stability by a single amino acid mutation provides useful insights into the rational design of protein structures, especially the structures of oligomeric proteins.

16. PDZD8-FKBP8 tethering complex at ER-mitochondria contact sites regulates mitochondrial complexity

Nakamura K, Aoyama-Ishiwatari S, Nagao T, Paaran M, Obara CJ, Sakurai-Saito Y, Johnston J, Du Y, Suga S, Tsuboi M, Nakakido M, Tsumoto K, Kishi Y, Gotoh Y, Kwak C, Rhee HW, Seo JK, Kosako H, Potter C, Carragher B, Lippincott-Schwartz J, Polleux F, Hirabayashi Y.

Mitochondria-ER membrane contact sites (MERCS) represent a fundamental ultrastructural feature underlying unique biochemistry and physiology in eukaryotic cells. The ER protein PDZD8 is required for the formation of MERCS in many cell types, however, its tethering partner on the outer mitochondrial membrane (OMM) is currently unknown. Here we identified the OMM protein FKBP8 as the tethering partner of PDZD8 using a combination of unbiased proximity proteomics, CRISPR-Cas9 endogenous protein tagging, Cryo-Electron Microscopy (Cryo-EM) tomography, and correlative light-EM (CLEM). Single molecule tracking revealed highly dynamic diffusion properties of PDZD8 along the ER membrane with significant pauses and capture at MERCS. Overexpression of FKBP8 was sufficient to narrow the ER-OMM distance, whereas independent versus combined deletions of these two proteins demonstrated their interdependence for MERCS formation. Furthermore, PDZD8 enhances mitochondrial complexity in a FKBP8-dependent manner. Our results identify a novel ER-mitochondria tethering complex that regulates mitochondrial morphology in mammalian cells.

17. Development of novel humanized VHH synthetic libraries based on physicochemical analyses

Nakakido M, Kinoshita S, Tsumoto K.

Due to the high affinity and specificity of antibodies toward antigens, various antibody-based applications have been developed. Recently, variable antigen-binding domains of heavy-chain antibodies (VHH) have become an attractive alternative to conventional fragment antibodies due to their unique molecular characteristics. As an antibody-generating strategy, synthetic VHH libraries (including humanized VHH libraries) have been developed using distinct strategies to constrain the diversity of amino acid sequences. In this study, we designed and constructed several novel synthetic humanized VHH libraries based on biophysical analyses conducted using the complementarity determining region-grafting method and comprehensive sequence analyses of VHHs deposited in the protein data bank. We obtained VHHs from the libraries, and hit clones exhibited considerable thermal stability. We also found that VHHs from distinct libraries tended to have different epitopes. Based on our results, we propose a strategy for generating humanized VHHs with distinct epitopes toward various antigens by utilizing our library combinations.

18. Systematic Preparation of a 66-IgG Library with Symmetric and Asymmetric Homogeneous Glycans and Their Functional Evaluation

Manabe S, Iwamoto S, Nagatoishi S, Hoshinoo A, Mitani A, Sumiyoshi W, Kinoshita T, Yamaguchi Y, Tsumoto K.

Immunoglobulin G (IgG) antibodies possess a conserved N-glycosylation site in the Fc domain. In FcγRIIIa affinity column chromatography, unglycosylated, hemiglycosylated, and fully glycosylated IgG retention times differ considerably. Using retention-time differences, 66 different trastuzumab antibodies with symmetric and asymmetric homogeneous glycans were prepared systematically, substantially expanding the scope of IgGs with homogeneous glycans. Using the prepared trastuzumab with homogeneous glycans, thermal stability and antibody-dependent cellular cytotoxicity were investigated. In some glycan series, a directly proportional relationship was observed between the thermal unfolding temperature (Tm) and the calorimetric unfolding heat (ΔHcal). Antibody function could be deduced from the combination of a pair of glycans in an intact form. Controlling glycan structure through the combination of a pair of glycans permits the precise tuning of stability and effector functions of IgG. Overall, our technology can be used to investigate the effects of glycans on antibody functions.

19. Triphenylphosphonium-modified cationers enhance in vivo mRNA delivery through stabilized polyion complexation

Norimatsu J, Mizuno HL, Watanabe T, Obara T, Nakakido M, Tsumoto K, Cabral H, Kuroda D, Anraku Y.

Nanocarriers based on cationic materials play a central role in the success of mRNA-based therapies. Traditionally, amine-bearing lipids and polymers have been successfully employed for creating mR-

NA-loaded nanocarriers, though they still present challenges, such as physical and biological instability, limiting both delivery efficiency and therapeutic potential. Non-amine cations could be a promising avenue in addressing these limitations. However, such alternatives remain notably underexplored. Herein, we introduced triphenylphosphonium (TPP) as an alternative cationic moiety for mRNA delivery, leveraging its advantageous properties for nucleic acid complexation. Through the modification of amine-bearing catiomers, we replaced traditional amine-based counterparts with TPP to create innovative polymeric micelles as mRNA nanocarriers. A comprehensive analysis, encompassing physicochemical, thermodynamic, and computational approaches, revealed that the TPP substitution significantly influenced polymer self-assembly, mRNA binding, and the overall stability of mRNA-loaded polymeric micelles. Upon intravenous injection, TPP-bearing micelles demonstrated a remarkable increase in mRNA bioavailability, facilitating efficient protein production in solid tumors. These findings provide a compelling rationale for substituting amines with TPP, emphasizing their potential for advancing mRNA therapeutics.

20. Unveiling the structural mechanisms behind high affinity and selectivity in phosphorylated epitope-specific rabbit antibodies

Kasahara K, Kawade R, Nakakido M, Matsunaga R, Akiba H, Entzminger KC, Maruyama T, Okumura SCJ, Caaveiro JMM, Kuroda D, Tsumoto K.

Protein phosphorylation is a crucial process in various cellular functions, and its irregularities have been implicated in several diseases, including cancer. Antibodies are commonly employed to detect protein phosphorylation in research. However, unlike the extensive studies on recognition mechanisms of the phosphate group by proteins such as kinases and phosphatases, only a few studies have explored antibody mechanisms. In this study, we produced and characterized two rabbit monoclonal antibodies that recognize a monophosphorylated Akt peptide. Through crystallography, thermodynamic mutational analyses, and molecular dynamics simulations, we investigated the unique recognition mechanism that enables higher binding affinity and selectivity of the antibodies compared to other generic proteins with lower binding affinity to phosphorylated epitopes. Our results demonstrate that molecular dynamics simulations provide novel insights into the dynamic aspects of molecular recognition of posttranslational modifications by proteins beyond static crystal structures, highlighting how specific atomic level interactions drive the exceptional affinity and selectivity of antibodies.

21. Structural basis for the ligand promiscuity of the hydroxamate siderophore binding protein FtsB from Streptococcus pyogenes

Fernandez-Perez J, Senoo A, Caaveiro JMM, Nakakido M, de Vega S, Nakagawa I, Tsumoto K.

Pathogenic bacteria must secure the uptake of nutritional metals such as iron for their growth, making their import systems attractive targets for the development of new antimicrobial modalities. In the pathogenic bacterium Streptococcus pyogenes, the iron uptake system FtsABCD transports iron encapsulated by siderophores of the hydroxamate class. However, the inability of S. pyogenes to produce these metabolites makes the biological and clinical relevance of this route unresolved. Herein, we demonstrated that the periplasmic binding protein FtsB recognizes not only the hydroxamate siderophore ferrichrome, as previously documented, but also ferrioxamine E (FOE), ferrioxamine B (FOB), and bisucaberin (BIS), each of them with high affinity (nM level). Up to seven aromatic residues in the binding pocket accommodate the variable backbones of the different siderophores through CH-π interactions, explaining ligand promiscuity. Collectively, our observations revealed how S. pyogenes exploits the diverse xenosiderophores produced by other microorganisms as iron sources to secure this precious nutrient.

22. Malaria parasites require a divergent heme oxygenase for apicoplast gene expression and biogenesis

Blackwell AM, Jami-Alahmadi Y, Nasamu AS, Kudo S, Senoo A, Slam C, Tsumoto K, Wohlschlegel JA, Manuel Martinez Caaveiro J, Goldberg DE, Sigala PA.

Malaria parasites have evolved unusual metabolic adaptations that specialize them for growth within heme-rich human erythrocytes. During blood-stage infection, Plasmodium falciparum parasites internalize and digest abundant host hemoglobin within the digestive vacuole. This massive catabolic process generates copious free heme, most of which is biomineralized into inert hemozoin. Parasites also express a divergent heme oxygenase (HO)-like protein (PfHO) that lacks key active-site residues and has lost canonical HO activity. The cellular role of this unusual protein that underpins its retention by parasites has been unknown. To unravel PfHO function, we first determined a 2.8 Å-resolution X-ray structure that revealed a highly α-helical fold indicative of distant HO homology. Localization studies unveiled PfHO targeting to the apicoplast organelle, where it is imported and undergoes N-terminal processing but retains most of the electropositive transit peptide. We observed that conditional knockdown of PfHO was le-

thal to parasites, which died from defective apicoplast biogenesis and impaired isoprenoid-precursor synthesis. Complementation and molecular-interaction studies revealed an essential role for the electropositive N-terminus of PfHO, which selectively associates with the apicoplast genome and enzymes involved in nucleic acid metabolism and gene expression. PfHO knockdown resulted in a specific deficiency in levels of apicoplast-encoded RNA but not DNA. These studies reveal an essential function for PfHO in apicoplast maintenance and suggest that Plasmodium repurposed the conserved HO scaffold from its canonical heme-degrading function in the ancestral chloroplast to fulfill a critical adaptive role in organelle gene expression.

23. Disrupted odontoblast differentiation and dentin dysplasia in Epiprofin-deficient mice

Jiménez-Rojo L, de Vega S, Ibarretxe G, Nakamura T, Unda FJ.

Tooth formation is a process tightly regulated by reciprocal interactions between epithelial and mesenchymal tissues. These epithelial-mesenchyme interactions regulate the expression of target genes via transcription factors. Among the regulatory elements governing this process, Epiprofin/Sp6 is a zinc finger transcription factor which is expressed in the embryonic dental epithelium and in differentiating pre-odontoblasts. Epiprofin knockout (Epfn-/-) mice present severe dental abnormalities, such as supernumerary teeth and enamel hypoplasia. Here, we describe dentin defects in molars and incisors of Epfn-/- mice. We observed that in the absence of Epfn, markers of early odontoblast differentiation, such as alkaline phosphatase activity, Dsp/Dpp expression, and Collagen Type I deposition, are downregulated. In addition, the expression of tight and gap junction proteins was severely impaired in the predontoblastic cell layer of developing Epfn-/- molars. Altogether, our data shows that Epfn is crucial for the proper differentiation of dental mesenchymal cells towards functional odontoblasts and subsequent dentin-matrix deposition.

24. Specific recognition mechanism of an antibody to sulfated tyrosine and its potential use in biological research

Ujiie K, Nakakido M, Kinoshita S, Jose Caaveiro M M, Entzminger, C J Okumura, Maruyama, Miyauchi k, Matano T, Tsumoto K

Post-translational modification of proteins is a crucial biological reaction that regulates protein functions by altering molecular properties. The specific detection of such modifications in proteins has made significant contributions to molecular biology research and holds potential for future drug development applications. In HIV research, for example, tyrosine sulfation at the N-terminus of C-C chemokine receptor type 5 (CCR5) is considered to significantly enhance HIV infection efficiency. However, antibodies specific to sulfated CCR5 still need to be developed. In this study, we successfully generated an antibody that specifically recognized the sulfated N-terminal peptide of CCR5 through rabbit immunization and panning via phage display using a CCR5 N-terminal peptide containing sulfate modification. We used various physicochemical methods in combination with molecular dynamics simulation to screen for residues that could be involved in recognition of the sulfated peptide by this antibody. We also confirmed that this antibody recognized the sulfated full-length CCR5 on the cell surface, which suggested it should be useful as a research tool that could lead to the development of novel therapeutics. Although the antibody binding did not inhibit HIV infection, it could be also described as sulfation site-specific binding, beyond sulfation-specific binding.

25. The pericellular function of Fibulin-7 in the adhesion of oligodendrocyte lineage cells to neuronal axons during CNS myelination

Yamada M, Sasaki B,Yamada N, Hayashi C, Tsumoto K, de Vega S, Suzuki N

Myelin is an electrical insulator that enables saltatory nerve conduction and is essential for proper functioning of the central nervous system (CNS). It is formed by oligodendrocytes (OLs) in the CNS, and during OL development various molecules, including extracellular matrix (ECM) proteins, regulate OL differentiation and myelination; however, the role of ECM proteins in these processes is not well understood. Our present work is centered on the analyses of the expression and function of fibulin-7 (Fbln7), an ECM protein of the fibulin family, in OL differentiation. In the expression analysis of Fbln7 in the CNS, we found that it was expressed at early postnatal stage and localized in the processes of OL precursor cells (OPCs), in the inner region of myelin, and in axons. The functional analysis using recombinant Fbln7 protein (rFbln7) revealed that rFbln7 promoted OPC attachment activity via β1 integrin and heparan sulfate receptors. Further, rFbln7 induced the adhesion to neurites and the differentiation of OLs. Altogether, our results show that Fbln7 promotes the adhesion between OLs and axons and OL differentiation.

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Social Cooperation Research Program

Project Division of Genomic Medicine and Disease Prevention

ゲノム予防医学社会連携研究部門

Project Professor　Toru Suzuki, M.D., Ph.D.

特任教授　博士（医学）　鈴　木　　亨

Diseases, including cancer and common/chronic conditions, develop/progress by the combination/interaction of genetic background, acquired environmental exposures, life-style factors and aging. Identification of risk factors at time of birth and later in life provides information on which approaches to disease prevention can be tailored. The Project Division of Genomic Medicine and Disease Prevention was started in July 2019 in cooperation with Nippon Telegram and Telephone Cooperation (NTT), with a goal to develop personalized/precision-based prevention of diseases by integrating genomic information, health records and life-style data. The Division has started its next term for another five years in April 2024 and will aim to conduct further research as well as implement outputs to healthcare.

The Project Division of Genetic Medicine and Disease Prevention was established in 2019 to obtain scientific evidence to enable disease prevention by integrating genetic information into healthcare-based information (eg health records), life-style data and age.

For this purpose, a collaborative project with NTT Life Science, Corp. was initiated in 2020 to undertake research to integrate genetic testing with healthcare data to identify disease risk. Consenting employees of NTT group companies who undertake regular/annual physical examinations were recruited to a comprehensive survey program of genetic testing using microarray analysis and healthcare data collection. The program aims to investigate use of polygenic risk scores to identify genetic risk of conditions, and to share information with participants to potentially improve/intervene through lifestyle modifications improvement in a manner that is compliant in terms of ethical, legal and social issues (ELSI). This project is being undertaken in collaboration with several hospitals, including the Center for Disease Prevention at the NTT Medical Center, Tokyo. Integration of genetic information into health records and re-evaluation of disease risks of individuals are also being examined.

In 2021, a grant from the Japanese Science and Technology Agency's JST-Mirai Program on Advanced Intelligent Information Society mission area (Human centric digital twins services) was awarded to investigate "Development of disease prevention systems by integrating multi-layered biomedical information". The prioritized theme targets individuals and organizations as components of society, using the premise of AI digital twin as its core, and aims to: (1) create new value for emerging needs and issues, and (2) propose and realize new concepts and services related to AI digital twin. Specifically, it aims at an optimal combination of technologies related to data collection, processing, conversion, and integration, which are the prerequisites for digital twinning, as well as data conversion technologies suitable for modern AI technology, intelligent integration of output results, etc., with an eye toward the future of services, in addition to the advancement of individual

core technologies.

In 2023, a grant from the Japanese Cabinet Office's Cross-ministerial Strategic Innovation Promotion (SIP) Program on Development of an Integrated Healthcare System was awarded to develop and implement a life-record-type digital twin. This project utilizes the serial healthcare/clinical data including biomedical information of the NTT working generation cohort to analyse and visualize disease onset and progression including risks.

In April 2024, the Division has started its next term for another five years and will aim to conduct further research with the aforementioned funded activities as well as implement outputs to healthcare.

Social Cooperation Research Program

Project Division of Clinical Precision Research Platform

臨床精密研究基盤社会連携研究部門

Project Professor Satoshi Takahashi, M.D., D.M.Sc.
Project Assistant Professor Kimihito Kawabata, M.D., D.M.Sc.

特任教授 博士(医学) 高橋 聡
特任助教 博士(医学) 川畑 公人

Since the opening of our new laboratory under a joint research agreement with Daiichi Sankyo (formerly Daiichi Sankyo RD Novare) Co., Ltd, we have been struggling to establish our experimental flow for drug susceptibility screening using primary tumor samples from patients with hematological malignancies. After years of efforts to optimize the platform, the novel platform for precision medicine projects combining Drug sensitivity screening (DSS) and comprehensive multi-omics analysis has actually started to work in the analysis of our samples. As we continue to add more primary tumor samples (PTS) from acute myeloid leukemia (AML) patients to our cohort, we have continuously sought to optimize our tissue culture methods for PTS from AML patients. Using clinical specimens kindly provided by the Department of Hematology and Oncology, IMSUT Hospital and our network hospitals, we were now able to culture PTSs ex vivo for 21 days with more efficient maintenance and expansion of premature leukemia fractions. After confirming its leukemic stemness after long-term ex vivo culture with other collaborators, we are expanding the flexibility of our experiments in ex vivo drug treatments of PTS. This may lead to the possibility of long-term drug exposure depending on the mechanism of action of the drug. While working on these cell processing procedures, we have also made progress in high-throughput DSS system with automation technologies. Our efforts are expected to provide us with tools to understand the pathogenesis of hematological malignancies and develop further therapeutics.

1. Clinical precision research for hematological malignancies

Kimihito Cojin Kawabata1, Hironobu Komori1,2, Hayato Tsuji1,2, Yoshiharu Takama1,2, Hideaki Kakinuma1,2, Maiko Morita1, Sanae Suzuki1, Tetsushi Oka2, Gen Kudo2, Yasuhito Nannya1, Satoshi Takahashi1.
1 IMSUT, 2 Daiichi Sankyo Co., Ltd.

The aim of this study is to perform a comprehensive multi-omics analysis including genetic and epigenetic alterations as well as gene expressions, which can be compared side-by-side with functional analyses such as ex vivo drug responses. All of these experiments use primary tumor sample (PTS) derived from patients with hematopoietic malignancies. Our current focus is on acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS).

In the aforementioned network with the Human Genome Center and the Division of Hematopoietic Disease Control in the IMSUT, we have performed whole genome or whole exome analyses on the PTS of patients with hematopoietic malignancies, together with RNA expression, transcriptome analyses. We further aim to organize these omics approaches with

higher resolution at the single cell level. These omics profiles can be directly compared to the clinical course of actual patients. The NGS data compared to the clinical profile are discussed in detail in regular tumor board meetings (the tumor board meets every two weeks).

Drug sensitivity screening (DSS) using PTS is another focus of the group's efforts. In this topic, several other groups have already published their data on similar aspects (Nature. 2018;562(7728):526-31, Cancer Discovery. 2022;12(2):388-401, etc.). However, the more samples and compounds we use at multiple doses, the more intensive work and time we need in these high-throughput procedures. In this sense, the field is still in its infancy. Therefore, we have installed more updated and modernized technologies in these wet experiments and subsequent analyses. One of them is a fully automated ex vivo drug screening system, which uses a robotic arm to integrate several machines that can automatically handle the entire process from cell seeding, drug injection, cell incubation, liquid handling to flow cytometry. After data acquisition, drug susceptibility profile data is also collected through an automated data processing pipeline. After installation, we have completed the optimization of the entire system so that every PTS we have in the lab is analyzed in a 384-well plate format. We are currently evaluating the significance of these results. For example, in our first series of samples evaluated in depth, an AML cell with a TP53 mutation shows higher resistance to several of the 30 drugs included in the DSS. When we further examined the sample at the high resolution level using a multicolor flow cytometry panel, the result appears to correlate well with the clinical course of the patients, who were resistant to chemotherapy including AraC and anthracycline, and partially sensitive to Venetoclax + Azacytidine combo therapy, but later relapsed. In addition, novel epigenetic modifiers such as OTX051 were shown to be effective in subclusters that were resistant to clinically available therapies. The correlation between the results of the more detailed fractions and clinical progression will be analyzed further.

To date, there is a great deal of variability in the methods used for ex vivo culture of PTS in the field of DSS. These methods need to be compared and ultimately further optimized to obtain drug sensitivity profiles using cells in a better state. To reiterate, the limited number of cells collected from patients and the use up all available frozen vials in initial screening experiments are two major problems in experiments using PTS. To expand the capabilities of this AML/MDS PTS for more advanced applications, we have conducted side-by-side comparisons of different ex vivo culture methods. At the Clinical Precision Research Platform, we have already collected 159 different PTSs, aliquoted in 1495 cryovials. They are continuously used either in the automated DSS experiments, OMICS analyses or in these ex vivo culture method development. We have found that 1. Short-term culture protocols (up to 7 days) optimized for the current DSS assays may be improved to the prolonged culture system by changing the concentrations of serum or other supplements to the culture media. 2. The use of stromal feeder cells is superior to other conventional stroma-free culture methods currently used for normal hematopoietic stem cells. Moreover, 3. Recently, we have installed the most updated culture methods to challenge for stroma-free, serum-free ex vivo culture system optimized for PTS. The idea for the updated culture media is derived from ongoing collaborative research projects on normal hematopoietic stem cell culture. Initial data on this topic continue to fuel our research motivation on a daily basis. Details of these findings will be updated as they become available.

2. Generation of antigen-specific T cells derived from cord blood

Maiko Morita [1], Kimihito Cojin Kawabata[1], Satoshi Yamazaki[1], Ai Tachikawa-Kawana[1,2], Satoshi Takahashi[1]
1 IMSUT, 2 National Institute of Infectious Diseases

The aim of this project is to generate and expand viral antigen-specific T cells from naive human cord blood (CB)-derived T cells for clinical application. In a previous study, we targeted adenovirus, cytomegalovirus, BK virus, Epstein-Barr virus, which are the most common causes of infection after HSCT, and succeeded in generating viral antigen-specific T cells (VSTs) against these viruses from CB-derived T cells using a type of STING ligand, cGAMP. Expanded CB VSTs recognise more than 3 of the 4 viruses used for expansion in each sample, and their target region is shown to be relatively diverse.

When cultured to 3 rd stimulus and compared to PB-VSTs, CB-VSTs are uniquely differentiated in the expression of CD45RA/CCR7 and CD27/CD28. Interestingly, it is shown that CB-VSTs are less likely to induce activation-induced cell death and it is demonstrated that CB-VST is a potent product of third party VST for the treatment of infection after HSCT. As a next step, we plan to perform multi-omics analysis including single-cell RNA-seq to investigate the more detailed difference between PB-VSTs and CB-VSTs and to identify the factors for efficient induction of CTLs from naive T cells.

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Social Cooperation Research Program

Project Division of Generative AI Utilization Aging Cells

生成 AI 活用加齢医学社会連携研究部門

Project Associate Professor　Teh-Wei Wang, Ph.D.　特任准教授　博士（理学）　王　　德　瑋

Aging and many age-related diseases are fundamentally linked to chronic inflammation. It is widely accepted that the accumulation of senescent cells in the body over time is one of the major contributors to chronic inflammation. However, our understanding of senescent cells in human tissues remains extremely limited. In our study, we utilized large-scale single-cell RNA sequencing data obtained from mouse models and integrated it with generative AI techniques to explore the characteristics of senescent cells. By applying these insights to human datasets, we aim to further elucidate the features of senescent cells in human tissues and identify potential strategies to target these cells, providing a clue for novel anti-aging therapeutic approaches.

1. Senescent hepatocytes promote liver fibrosis through activating LIFR pathway

Koji Nishikawa[1,2], Teh-Wei Wang, Satoshi Kawakami[1], Shota Tanimoto[1], Kiyoshi Yamaguchi[3], Taketomo Kido[4], Masamichi Kimura[2], Tsunekazu Hishima[5], Yuki T. Okamura[1], Satotaka Omori[6], Takumi Iritani[7], Toshikaze Chiba[8], Takehiro Jimbo[8], Michio Katano[7], Kansuporn Kamataki[7], Ryoichi Yokoyama[9], Eigo Shimizu[10], Kiminori Kimura[2], Satoshi Yamzaki[11], Seiya Imoto[10], Yoichi Furukawa[3], Atsushi Miyajima[4], Yoshikazu Johmura[12] and Makoto Nakanishi[1]

[1]Division of Cancer Cell Biology, IMSUT. [2]Department of Hepatology, Tokyo Metropolitan Cancer and Infectious Diseases Center, Komagome Hospital. [3]Division of Clinical Genome Research, IMSUT. [4]Laboratory of Cell Growth and Differentiation, Institute for Quantitative Biosciences, The University of Tokyo. [5]Department of Pathology, Tokyo Metropolitan Cancer and Infectious diseases Center Komagome Hospital. [6]Integrated Biosciences, Redwood City, CA, USA. [7]GMO Internet Group, Inc. [8]GMO Research Activity Support & Technology, Inc. [9]GMO Healthtech, Inc. [10]Division of Health Medical Intelligence, Human Genome Center, Center for Experimental Medicine and Systems Biology, IMSUT. [11]Division of Cell Regulation, Center of Experimental Medicine and Systems Biology, IMSUT. [12]Division of Cancer and Senescence Biology, Cancer Research Institute, Kanazawa University.

Liver fibrosis is a harmful outcome of the tissue repair process following chronic liver injury, predominantly thought to be initiated by hepatocytes. However, the specific hepatocyte subtypes and signaling pathways responsible for this activation remain poorly understood. Previous studies have demonstrated a strong correlation between the severity of fibrosis in cirrhotic patients and the prevalence of hepatocytes expressing high levels of $p16^{Ink4a}$ ($p16^{h}$ hepatocytes). Based on this observation, we hypothesized that $p16^{h}$ hepatocytes might play a key role in triggering fibrogenic responses upon liver injury.

In a long-term CCl_4-induced hepatitis model, a marked accumulation of $p16^{h}$ hepatocytes were ob-

served specifically in zone 3 of the liver. These cells displayed several hallmarks of cellular senescence, and their abundance was significantly associated with the extent of liver fibrosis. Remarkably, selective depletion of $p16^h$ hepatocytes alleviated CCl_4-induced liver fibrosis, likely by reducing the activation of hepatic stellate cells. Single-cell transcriptomic analysis of murine and human hepatocytes further identified the LIFR signaling pathway as a critical mediator linking $p16^h$ hepatocytes to the fibrogenic activation of hepatic stellate cells.

In addition, by using generative AI, we translated the gene signatures of $p16^h$ hepatocytes from mice into corresponding human genes, based on both gene sequences and protein sequences. Using these translated results, we investigated senescent hepatocytes in human cirrhotic samples. Our analysis revealed that the subset of hepatocytes with the highest similarity to the mouse $p16^h$ profile is likely associated with precancerous lesions.

Publication

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Social Cooperation Research Program

Project Division of International Healthcare Innovation Research

国際健康医療推進社会連携研究部門

Project Associate Professor　Koichiro Yuji, M.D., Ph.D.　特任准教授　博士（医学）　湯　地　晃一郎

The mission of the Project Division is to facilitate the social implementation, engagement with the public, and propagation of research findings in the healthcare field at international medical facilities through collaboration between the Institute of Medical Science, the University of Tokyo, and Tokyu Land Corporation.

Implementing advanced medical research at IMSUT

Yuji, K.

The Project Division was established in November 2024 as the successor to the former Project Division of International Advanced Medical Research. Our mission is to contribute to facilitate the social implementation, engagement with the public, and propagation of research findings in the healthcare field at international medical facilities through collaboration between the Institute of Medical Science, the University of Tokyo, and Tokyu Land Corporation. We conduct lectures at international medical facilities to share knowledge and raise public awareness of the latest advances in healthcare.

Medical DX and Clinical Laboratory Data

Yuji, K.

In the era of digital transformation (DX), the collection and utilization of big data in the healthcare field is an urgent issue. Healthcare big data is primarily comprised of electronic health records (EHR) and personal health records (PHR). Standardization, interoperability, and the establishment of a common platform for EHR/PHR are essential for medical DX. Clinical laboratory data account for a large portion of medical information, especially within EHRs, which reportedly comprise 93% of electronic medical records. This data includes various types of laboratory test results, such as blood tests and urine tests. PHRs, gaining attention in recent years, provide a mechanism for individuals and families to accurately understand and utilize health and medical data related to their daily lives as electronic records, including self-tracked measurements such as blood pressure, pulse rate, and blood glucose levels, as well as information from healthcare providers.

We actively contribute to the standardization, interoperability, and establishment of a common platform for EHR/PHR from the perspective of clinical laboratory data.

Publications

1. 湯地 晃一郎. 疾病の診断・治療を目的とした医療プログラム (SaMD; Software as a Medical Device) における臨床検査.. 日本臨床検査医学会誌. 72(4); 289-293, 2024.
2. 湯地 晃一郎. 医療DXにおける臨床検査の展望. 臨床化学. 53(4);222-228, 2024.

Corporate Sponsored Research Program

Project Division of Oncolytic Virus Development

ウイルス療法開発寄付研究部門

Project Professor Minoru Tanaka, M.D, Ph.D.

特任教授 博士(医学) 田 中 実

We have been conducting basic research and clinical projects to devise oncolytic virus therapies for solid cancers, including glioblastoma, olfactory neuroblastoma, and malignant pleural mesothelioma. We focus on oncolytic virus drug manufacturing processes, including scale-up, purification, filling, quality and stability testing, and characterization, as well as the development of next-generation oncolytic virus drugs to contribute to the advancement of oncolytic virus therapy in Japan.

Introduction

Our division was established as an endowed division by Denka Company Limited. We work in close conjunction with the laboratory of Innovative Cancer Therapy. Oncolytic viruses are genetically modified to replicate in and kill cancer cells while leaving normal tissues unharmed. The genetic modification of the viruses also grants them the ability to elicit anti-cancer immunity through multiple mechanisms of the patient's immune system. Genetically engineered, conditionally replicating herpes simplex viruses type 1 (HSV-1) are promising therapeutic agents for solid cancers. Our division focuses on process development and scale-up of oncolytic HSV-1 production.

A triple-mutated, third-generation oncolytic HSV-1, G47Δ, teserpaturev.

We developed a triple-mutated, third-generation oncolytic HSV-1, G47Δ, teserpaturev that has triple mutations within the viral genome. A phase II clinical trial of G47Δ was conducted since 2014 in patients with glioblastoma. In June 2021, G47Δ was approved as the world's first oncolytic virus drug for malignant gliomas. Upon commercial distribution, the oncolytic virus therapy using G47Δ (Delytact®) for patients with malignant glioma started at IMSUT hospital in November 2021. Clinical trials were also conducted for malignant pleural mesothelioma and olfactory neuroblastoma. In mesothelioma, the safety of G47Δ was confirmed, while for olfactory neuroblastoma, the recruitment of patients has been completed, and data analysis is currently ongoing.

Production of clinical-grade oncolytic HSV-1

We excel at producing master virus seed stocks (MVSS) and subsequent production of working virus seed stocks (WVSS): free of contamination, replication-competent (high titer), identity, purity, and stability. We begin with selecting cell lines for adherent or suspension culture growth, optimization of media and buffers, cell lysis, and purification of oncolytic HSV-1. We performed G47Δ genome structure analysis, stability tests, and preclinical safety evaluation. Clinical-grade G47Δ products were prepared at the Therapeutic Vectors Development Center, IMSUT hospital, with Good Manufacturing Practice (GMP). The Therapeutic Vectors Development Center has been maintained to meet the current GMP standard through regular validation of equipment and produc-

tion and an ISO9001:2015-certified quality management system. We continue to optimize oncolytic HSV-1 production to improve their safety, efficacy, and manufacturability for scale-up.

Clinical Sample Analysis and Mechanistic Insights

Through the analysis of clinical samples, including blood and pleural effusion from patients enrolled in our trials, we are investigating immune response dynamics using advanced genomic and transcriptomic approaches. These analyses focus on identifying patterns of change in the expression levels of immune-related genes before and after treatment, with particular interest in significant shifts, such as tenfold or greater changes. By continuously deepening our understanding through these studies, we aim to generate insights that will contribute to the development of novel oncolytic virus products incorporating additional immunomodulatory molecules to enhance antitumor immunity.

A recombinant herpes simplex type 1 with human IL-12 expression, T-hIL12

One of the advantages of HSV-1 is its capacity to incorporate large or multiple transgenes within the viral genome. Incorporating transgenes encoding immunomodulatory molecules into G47Δ can enhance its ability to trigger anti-cancer immunity. T-hIL12 is a G47Δ-based recombinant HSV-1 that expresses human interleukin-12 (IL-12). This IL-12-mediated anti-tumor immunity is thought to be T cell-mediated. We started a phase 1/2 clinical trial of T-hIL12 in patients with malignant melanoma in January 2020 jointly with Shinshu University. Phase 2 part of this trial is ongoing. In the ongoing phase II part, we are focusing on the collection of comprehensive data on efficacy and safety in patients with malignant melanoma. Early findings suggest that T-hIL12 effectively elicits a T cell-mediated immune response, underscoring its potential as an immunotherapeutic agent for solid tumors.

A recombinant herpes simplex type 1 with human bevacizumab expression, T-BV

Phase II trials of G47Δ in glioblastoma showed efficacy and safety, but cases of temporary brain edema were observed during the induction of anti-tumor immunity by G47Δ. To further improve the safety of viral therapy for brain tumors, we have developed T-BV, a G47Δ-based recombinant HSV-1 expressing bevacizumab that can reduce brain edema without systemic administration of bevacizumab. We have produced clinical-grade T-BV, and the protocol for the first-in-human (FIH) phase I clinical trial has been draft. We expect to start the clinical trial in the near future.

Future Directions

Building upon this year's progress, our division aims to advance ongoing clinical trials, including the completion of data analysis for olfactory neuroblastoma and the initiation of FIH trials for T-BV. We will also focus on further optimizing automated manufacturing processes and expanding the clinical applications of oncolytic HSV-1. These efforts align with our overarching goal of increasing the availability and accessibility of oncolytic virus therapies in Japan.

Consortium

Consortium for Gene Therapy and Regenerative Medicine

遺伝子治療・再生医療コンソーシアム

Professor	Atsushi Iwama, M.D., Ph.D.	教　授	博士(医学)	岩　間　厚　志
Professor	Tomoki Todo, M.D., Ph.D.	教　授	博士(医学)	藤　堂　具　紀
Professor	Kaori Muto, Ph.D.	教　授	博士(保健学)	武　藤　香　織
Professor	Takashi Okada, M.D., Ph.D.	教　授	博士(医学)	岡　田　尚　巳
Professor	Hideki Taniguchi, M.D., Ph.D.	教　授	博士(医学)	谷　口　英　樹
Professor	Fumitaka Nagamura, M.D., Ph.D.	教　授	博士(医学)	長　村　文　孝
Professor	Tomoji Mashimo, Ph.D.	教　授	博士(人間・環境学)	真　下　知　二
Professor	Satoshi Yamazaki, Ph.D.	教　授	博士(生命科学)	山　崎　　聡
Associate Professor	Tokiko Nagamura-lnoue, M.D., D.M.Sc.	准教授	博士(医学)	長　村　登紀子

In recent years, as gene and cell therapy and regenerative medicine have been actively researched and developed, the relationship between these fields is becoming closer both scientifically and in terms of clinical practice. Many of the diseases they target are common, and many common technologies exist. Based on this background, researchers of gene and cell therapy and regenerative medicine working at IMSUT have worked closely together to create a consortium to build an international base for gene and cell therapy and regenerative medicine. We will bring together IMSUT's human resources and technologies, which include not only gene and cell therapy and regenerative medicine, but also research on ELSI (ethical, legal, and social issues) and regulatory science, to advance cutting-edge research. As part of these activities, four research support projects (viral vector manufacturing/provision infrastructure development project, test manufacturing support project/vector, test manufacturing support project/cell, and commercialization strategy support) were selected as representatives of the AMED Acceleration Program of R&D and Implementation for Regenerative Medicine and Cell and Gene Therapy, and support began in FY2023. We will provide various forms of support to help realize gene and cell therapy, and regenerative medicine in Japan.

Dean's Office

Project Coordination Office
プロジェクトコーディネーター室

Professor Mutsuhiro Takekawa, M.D., Ph.D.

教 授 博士(医学) 武 川 睦 寛

Our major missions are to coordinate institutional projects and to enhance the mutual cooperation and alliance among teaching and research staff, administration staff, and technical staff in order to execute the activities in our institute effectively. For these purposes, we carry out several tasks such as planning for new institutional research programs and symposiums, fundraising, supporting international students and researchers, outreach activities, providing academic advice to administration staff, and other projects directed by the Dean. In May 2023, the Project Coordination Office merged with the former International Affairs Office, which was responsible for public relations and language support, to strengthen its functions. On the public relations front, the Office continues to focus on an effective PR strategy including publishing new information about a variety of scientific research conducted by IMSUT on its official website and social media. The Office is also working to increase IMSUT's international presence through press releases in both Japanese and English, and IMSUT's PR magazines. On the language support side, the Office provides translation support from Japanese to English to create a more inclusive working environment for all IMSUT members.

1. Support for the management of institutional projects

Kiyomi Nakagawa, Yoko Udagawa, Ayako Miyake

We served as a secretariat for institutional projects implemented by the Institute of Medical Science, the University of Tokyo (IMSUT) and supported their management. The projects we supported are as follows:
- "Studies to Control Emerging, Re-emerging and Imported Infectious Diseases to Be Conducted in International Collaboration Sites in China" supported by Japan Program of Infectious Diseases Research and Infrastructure from Japan Agency for Medical Research and Development (AMED)
- "World-leading Innovative Graduate Study Program for Life Science and Technology (WINGS-LST)" supported by the Doctoral Program for World-leading Innovative & Smart Education from Japan Society for the Promotion of Science (JSPS)
- On-campus job project for student financial support FY 2024

2. International Joint Usage/Research Center Program of MEXT (International Joint Usage/Research Center Office)

Kaori Inoue

Accredited by MEXT in 2010 as a Joint Usage/Research Center and in 2018 as an International Joint Usage/Research Center (IJURC), IMSUT serves as a global hub for universities and research institutions, promoting international collaborative research to advance both basic and applied medical science and realize cutting-edge medical care.

The IJURC Office, functioning as the management

office for this initiative, undertakes the following responsibilities in partnership with the Research Promotion Team:

- Managing calls for joint research proposals
- Preparing evaluation materials for MEXT
- Hosting seminars led by IMSUT Joint Research Project (JRP) Researchers
- Creating presentation materials to introduce IJURC's activities
- Organizing academic events for students and young researchers
- Contributing articles on IJURC's joint research to the IMSUT newspaper

The IJURC Office compiles these activities and achievements into an annual report submitted to MEXT. In 2024, IMSUT's efforts and achievements, aligned with the goals of this initiative, received high recognition, earning the highest "S" rating in the interim evaluation of the 4th Mid-term Goals period (from FY2022 to FY2027) and maintaining its top rating from the previous evaluation at the end of the 3rd term.

3. Data acquisition about research and educational activities of IMSUT

Kiyomi Nakagawa

We collected and stocked data using an original format to construct a data system available any time for evaluation, submission of various reports, public relation activities, and basic data for application of external funds

4. Publication of Press Releases on IMSUT research results

Kiyomi Nakagawa, Asako Shimizu

In cooperation with Ms. Reiko Suzuki, who is in charge of research public relations in IMSUT's Technical Office, we issued press releases on various new findings from IMSUT and distributed Japanese press releases to media institutions and science journalists strategically. The Office also disseminated English press releases to the global community of science journalists through the official website, social media such as Twitter, along with the international public relations website "Eurek Alert!".

5. Publication of the Public Relations magazine

Kiyomi Nakagawa, Asako Shimizu

In cooperation with Ms. Reiko Suzuki, the Office worked closely with the faculty members who belong to the public relations magazine working group and published the PR magazine "PLATINUM STREET TIMES" in June and December 2024, featuring the Medical Science Museum, and the history and research results of hematological studies at IMSUT.

6. Language support

Kazuyo Ohara

In 2024, the Office worked closely with the administrative staff to communicate important notices and updates in English to the international members of IMSUT. This included the translation of the Dean's messages, correspondence with overseas organizations, IMSUT prescribed forms and various materials related to the promotion of the welfare of IMSUT members. The Office also vigorously undertook a wide range of translation and proofreading work, aiming to create a more inclusive working environment for all IMSUT members by offering tailored language services.

7. Others

Kiyomi Nakagawa, Ayako Miyake, Yoko Udagawa

a. Educational activities:
- Support for the call for applications and the selection of the Outstanding Student Publication Award of IMSUT

b. International activities:
- Support for the conclusion and renewal of MOUs
- Support for the delegation and management of international exchange events, "East Asia Joint Symposium on Biomedical Research" and "International Symposium of the Institute Network for Biomedical Sciences"
- Translation of documents and manuscripts
- Support for foreign researchers by providing assistance in English
- Support for reception of overseas visitors
- Support for management of the University of Tokyo New York Office, Inc. and its event organization
- Planning and running of get-together party for international students and foreign researchers

c. Public relations:
- Support for updating information on the IMSUT website
- Editing of brochures of IMSUT (Japanese and English versions) and support for edition of the Annual Report

d. Support for evaluation work:
- National university corporation evaluation
- Self-review and self-evaluation of IMSUT - External review of IMSUT

Dean's Office

Research Platform Office
学術研究基盤支援室

Chair and Professor	Mutsuhiro Takekawa, M.D., Ph.D.	教授・室長	博士(医学)	武 川 睦 寛
Advisor and Project Professor	Jun-ichiro Inoue, Ph.D.	特任教授・アドバイザー	薬学博士	井 上 純一郎
Project Professor	Yataro Daigo, M.D., Ph.D.	特任教授	博士(医学)	醍 醐 弥太郎
Project Associate Professor	Atsushi Takano, M.D., Ph.D.	特任准教授	博士(医学)	高 野 淳

The "Platforms for Advanced Technologies and Research Resources" (square.umin.ac.jp/platform) was launched in fiscal year (FY) 2022 under the new framework of the Grant-in-Aid for Transformative Research Areas (A) by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT). It consists of six platforms, four of which support life science research. These platforms have been developed and strengthened based on previous programs: "Support Programs for Three Fields in Life Sciences (Cancer, Genome, and Brain Sciences)" (FY 2010–2015) and "Platforms for Advanced Technologies and Research Resources" (FY 2016–2021).

The initiative aims to establish academic research support platforms that effectively address the diverse needs of researchers funded by grants-in-aid for Scientific Research (KAKENHI). It also strives to promote close cooperation with relevant core institutions including Inter-University Research Institutes and Joint Usage/Research Centers. Our office primarily serves as the representative secretariat for the "Committee on Promoting Collaboration in Life Sciences", an academic collaborative foundation, and coordinates with the four platforms mentioned above. The goal is to contribute to the continuous progress of academic research in Japan by providing cutting-edge technologies and biological resources to individual researchers receiving KAKENHI.

We also aim to strengthen cooperation among researchers across different support functions and disciplines, while promoting the development of human resources. To achieve this, the General Management Group was established to facilitate close cooperation among the four platforms, which include 54 universities and 21 research institutions nationwide, and provide more than 70 support functions. In 2016, our office was established within this institute as the Dean's Office to enable flexible management. Furthermore, we hold several management board meetings with 22 members, including representatives from the four platforms and 18 board members. This helps build a cooperative system that facilitates cross-functional support and provides technical assistance to universities and research institutions nationwide.

Management of the "Committee on Promoting Collaboration in Life Sciences" and the two plat- forms: Advanced Animal Model Support (AdAMS) and Cohort Study and Biospecimen Analysis (CoBiA):

Jun Saito, Tomoko Fujita, Yuko Sonoda, Atsuko Ishizaki, Miyuki Kawakami, Atsushi Takano, Yataro Daigo, Jun-ichiro Inoue, and Mutsuhiro Takekawa

The following activities were carried out under the management of our office in 2024.

1. Planning and organizing budget allocations.
2. Providing a one-stop service to applicants through our website.
3. Organizing events to foster the development of young scientists and promote interdisciplinary research.
4. Holding public symposiums on the intersection of life sciences and society.
5. Conducting informational sessions for target applicants and posting videos of the sessions on our website.
6. Carrying out public relations activities, including organizing luncheon seminars, participating in scientific exhibitions, and posting updates on social media platforms such as X, Instagram, and Facebook.
7. Facilitating collaboration between our platforms and other domestic and international organizations supporting life science research.

Dean's Office

BioBank Japan
バイオバンク・ジャパン

Professor	Makoto Nakanishi, M.D., Ph.D.	教　授	医学博士	中　西　　真
Project Professor	Koichi Matsuda, M.D., Ph.D.	特任教授	博士(医学)	松　田　浩　一
Project Professor	Yoichiro Kamatani, M.D., Ph.D.	特任教授	博士(医科学)	鎌　谷　洋一郎
Visiting Professor	Takayuki Morisaki, M.D., Ph.D.	客員教授	医学博士	森　崎　隆　幸

In 2003, BioBank Japan (BBJ) started establishing one of the world's largest disease biobanks, creating a foundation for genomic and clinical research. From a total of 267,000 patients representing 440,000 cases of 51 primarily multifactorial diseases, BBJ has collected DNA, serum, medical records. BBJ is promoting the utilization of the registered samples and data acquired over the years, resulting in important research findings contributing to the realization of genomic medicine.

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SCIENTIFIC MEETINGS & SEMINARS

51st IMSUT Founding Commemorative Symposium Frontier of Gastrointestinal Cancer

本研究所では伝染病研究所から医科学研究所への改組を記念して創立記念シンポジウムを毎年開催している。

本年は「Frontier of Gastrointestinal Cancer」というテーマで講演を行った。

日　時：令和 6 年 5 月 31 日（金）　13:00 ～ 17:00
会　場：医科学研究所 1 号館講堂

Hiroyoshi Nishikawa（Department of Immunology, Nagoya University Graduate School of Medicine/ Chief Division of Cancer Immunology, Research Institute/Exploratory Oncology Research and Clinical Trial Center（EPOC), National Cancer Center Japan）
Immuno-genomic analysis of immunosuppressive mechanisms in the tumor microenvironment

Dai Shida（Division of Frontier Surgery, The Institute of Medical Science, IMSUT）
Robotic Surgery for Colorectal Cancer: Beyond the Era of Laparoscopic Surgery

Koshi Mimori（Department of Surgery, Kyushu University Beppu Hospital）
Cellular Society Comprising Cancer Microenvironment Impacts on Diverse Colorectal Cancer Evolution

Nobuyuki Kakiuchi（The Hakubi Center for Advanced Research, Kyoto University）
Clonal expansion in non-cancer digestive epithelium

Narikazu Boku（Department of Oncology and General Medicine, IMSUT Hospital, IMSUT）
Recent progress in systemic chemotherapy for gastrointestinal cancer

学友会セミナー

（令和６年１月～令和６年 12 月）

１月11日　演題：　The characteristics of in vivo senescent somatic cells in aging and cancer
　　演者：　Wang, Teh-Wei

１月12日　演題：　Perturbations in epitope-specific T cells and their receptors following influenza and SARS-CoV-2 infection and vaccination
　　演者：　Louise Rowntree

１月16日　演題：　単球の異常増殖がサルコイドーシスにおける肉芽腫形成を誘導する
　　演者：　平沼　亮祐

１月16日　演題：　臍帯由来間葉系細胞の持つ造血支持能の解析と実用化に向けた課題
　　演者：　須藤　和寛

１月29日　演題：　頂底極性の制御因子 EHBP1L1 はマウス赤芽球・筋細胞核の極性化に必須の役割を担う
　　演者：　Wu Ji（ウー　ジー）

２月21日　演題：　多能性はシグナル伝達の選択的翻訳制御によって維持されている
　　演者：　大久保　周子

２月28日　演題：　造血器腫瘍予後改善に向けた基礎的・臨床的取り組み
　　演者：　神保　光児

２月28日　演題：　Representation Learning in Single Cell Analysis
　　演者：　Heryanto, Yusri Dwi

３月４日　演題：　New Insights in Plasmodium vivax biology
　　演者：　Laurent RENIA

３月 13 日　演題：　神経膠芽腫治療のための薬剤開発と細胞モデル：ターゲティングとデリバリー
　　演者：　Susana de Vega（スサーナ　デ　ベガ）

３月 19 日　演題：　Genomic surveillance of emerging viral pathogens: Lessons from the Past Outbreaks.
　　演者：　Tommy tsan Yuk Lam

４月３日　演題：　加齢や神経疾患に伴い蓄積する p16 高発現神経細胞
　　演者：　川上　聖司

４月５日　演題：　Beyond the spike: viral targets of the antibody response to SARS-CoV-2
　　演者：　Niloufar KAVIAN-TESSLER

４月12日　演題：　病原体センサーを介した病態発症メカニズムの解明
　　演者：　柴田　琢磨

５月17日　演題：　The role of endogenous “non-self” elements in the chronic inflammation and aging 内在的「非自己」の慢性炎症および老化における役割
　　演者：　高橋　岳浩

５月23日　演題：　先天性 PD-1・PD-L1 欠損症例から学ぶヒト免疫系の調節機構
　　演者：　大岸　誠人
６月７日　演題：　Digging for Meaning in the Big Data of Mass Spectrometry-based Proteomics
　　演者：　Tzong-Yi Lee
６月10日　演題：　研究倫理コンサルテーションの実践と医事法の視点での研究倫理研究
　　演者：　遠矢　和希
６月21日　演題：　The roles of cell proteins that interact with Human papillomavirus during virus entry
　　演者：　Yuka TAKEO
６月21日　演題：　Innate lymphoid cells: development, differentiation, dynamics
　　演者：　ジム　ディサント
７月１日　演題：　種々の原虫病に対する制御法の開発に関する基盤研究
　　演者：　玄　学南
７月５日　演題：　Unveiling the protective role of ribosomal RNA against auto-immunity
　　演者：　Michael P. Gantier
７月８日　演題：　Causal Modelling of Spatio-Temporal Epidemiological and Clinical Data
　　演者：　Marco Scutari
８月６日　演題：　脳腫瘍における遺伝子異常と起源細胞の解明
　　演者：　鈴木　啓道
８月６日　演題：　ゲノム DNA の安定維持機構に異常を示す遺伝性難病の原因究明と分子病態理解
　　演者：　荻　朋男
８月７日　演題：　Current Status and Future Strategies of Poultry Vaccines
　　演者：　HYUNG-KWAN JANG
８月20日　演題：　生体分子凝集体と遺伝子制御：スーパーエンハンサーから染色体外 DNA へ
Biomolecular Condensate and Gene Regulation: from Super-enhancer to Extrachromosomal
　　演者：　鈴木　洋
９月６日　演題：　劇症型 NK 細胞白血病細胞の動態解析および治療標的候補分子の同定
　　演者：　宮竹　佑治
９月13日　演題：　Vaccine and antibody discovery targeting influenza virus neuraminidase
　　演者：　Masaru Kanekiyo
10月11日　演題：　Mitochondria as drug target : From parasites to virus
　　演者：　北　潔
10月17日　演題：　Can we prevent and/or treat flavivirus infections?
　　演者：　David I Watkins
11月７日　演題：　３次元イメージングシステムを用いた肺動脈性肺高血圧症の病態解明
　　演者：　武田　憲文

11月11日　演題：　自己および家族の遺伝学的リスクを早期に知ることに関する医療社会学―球脊髄性筋萎縮症（SBMA）と認知症を事例に

演者：　木矢　幸孝

11月14日　演題：　大腸癌脳転移の予後と治療の進展

演者：　今泉　潤

11月28日　演題：　抗ヒト血管内皮増殖因子（VEGF）抗体発現型単純ヘルペスウイルスⅠ型（T-BV）の臨床開発

演者：　伊藤　博崇

12月６日　演題：　Adjuvants strategies for injectable and mucosal subunit vaccines

演者：　Ed Lavelle

12月６日　演題：　The ACOD1-itaconate pathway in intracellular bacterial infection: an immune-metabolic swiss army-knife to kill invaders and balance macrophage activation?

演者：　Roland Lang

12月６日　演題：　Defining Spike Correlates of Coronavirus Emergence

演者：　Vineet D. Menachery

12月６日　演題：　Host-Focused Approaches to Investigating Virus Emergence

演者：　Angela L. Rasmussen

12月６日　演題：　Models and Insights into Long COVID

演者：　Ralph S. Baric

12月13日　演題：　Publishing your work: advice from a scientific editor

演者：　Cheri Sirois

12月23日　演題：　大腸癌における手術治療の進歩と抗がん剤治療の有効性の評価

演者：　柵山　尚紀

EDUCATION

大学院セミナー

医科学研究所では、毎年テーマを決めて大学院生を対象としたセミナーを開いている。各々の年の決定されたテーマに関していろいろな視点から最先端の研究を展開しておられる方々に講師をお願いし、現在どのような研究が進められていて、どこまで明らかにされているかが幅広く理解できるように計画が立てられている。2024 年度は「プロテオスタシスの理解と創薬」というテーマの下で次のようなセミナーが行われた。

プロテオスタシスの理解と創薬

	月　日	講師名		演　題
1	4月8日	佐伯　泰	東京大学医科学研究所 タンパク質代謝制御分野 教授	プロテオスタシス概論
2	4月15日	伊藤　拓水	東京医科大学 医学総合研究所 客員准教授	タンパク質分解誘導剤についてサリドマイドを中心に
3	4月22日	鐘巻　将人	国立遺伝学研究所 教授	オーキシンデグロン技術開発と染色体維持研究への利用
4	5月13日	田中　元雅	国立研究開発法人理化学研究所 チームリーダー	アミロイド生物学の理解と創薬
5	5月20日	本田　一文	日本医科大学 大学院医学研究科 大学院教授	タンパク質翻訳後修飾を利用した病態診断バイオマーカーの探索と臨床開発
6	5月27日	三原田賢一	熊本大学 国際先端医学研究機構 特別招聘教授	母胎代謝物による胎児プロテオスタシス制御
7	10月7日	中戸川　仁	東京工業大学 科学技術創成研究院 教授	オートファジーの分子基盤と生理的意義
8	10月21日	内藤　幹彦	東京大学大学院薬学系研究科 特任教授	Targeted Protein Degradation and Drug Discovery
9	10月28日	岩崎　未央	京都大学 iPS 細胞研究所 講師	多能性幹細胞における mRNA とタンパク質の量の違いを紐解く
10	11月11日	徳永　文稔	大阪公立大学 大学院医学研究科 教授	ユビキチン修飾による細胞生死制御と創薬
11	11月18日	小林　妙子	東京大学医科学研究所 タンパク質代謝制御分野 准教授	成体神経幹細胞におけるプロテオスタシス制御
12	11月25日	大竹　史明	星薬科大学 先端生命科学研究所 特任准教授	ユビキチン修飾の多様性と創薬応用
13	12月2日	村田　茂穂	東京大学大学院薬学系研究科 教授	細胞内分解装置プロテアソームのバイオロジーと疾患・創薬

学術フロンティア講義

医科学研究所では、教養学部前期課程の学生を対象に、「医科学研究最前線」として、平成２７年度から学術フロンティア講義を開講している。研究所を構成する６つの基幹部門・施設から選出された講師が、それぞれの研究分野の最新の動向をわかりやすく講義した。

日　時：令和 6 年 12 月 14 日（土）9：15 ～ 16：40
　　令和 6 年 12 月 15 日（日）9：30 ～ 16：40
場　所：医科学研究所 1 号館講堂

教員および題目

12 月 14 日（土）

講　師　名		題　　目
武川　睦寛	基礎医科学部門 分子シグナル制御分野	医科研紹介
井元　清哉	ヒトゲノム解析センター 健康医療インテリジェンス分野	共生微生物メタゲノム解析が創る新しいヘルス・メディカルケア
昆　　彩奈	先端医療研究センター 血液・腫瘍生物学分野	血液がんのゲノム解析から分子病態解明・創薬への展開
小檜山康司	感染・免疫部門 ワクチン科学分野	補助に終わらないアジュバント
西山　敦哉	癌・細胞増殖部門 癌防御シグナル分野	DNA メチル化制御の分子機構

12 月 15 日（日）

講　師　名		題　　目
小林　妙子	基礎医科学部門 タンパク質代謝制御分野	神経幹細胞の機能制御
田中　洋介	システム疾患モデル研究センター 細胞制御研究分野	DNA バーコード技術の最前線
加藤　哲久	感染・免疫部門 ウイルス病態制御学分野	ヘルペス脳炎を司る分子機構
山口貴世志	先端医療研究センター 臨床ゲノム腫瘍学分野	ゲノム解析技術の進展と遺伝子診断

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