Corporate Sponsored Research Program

Project Division of Molecular and Developmental Biology

再生基礎医科学国際研究拠点寄付研究部門(ロート製薬, 慈照会, VICX, トミー)

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Our long-term goal is to understand the molecular mechanisms which coordinately regulate growth and differentiation of stem cells and differentiated cells with emphasis on intracellular signal transduction. For this purpose we are using models ranging from iPS and various culture cells, zebrafish, mouse, to clinical samples. Based on our research background on the area of cytokine signals, we now focus on the analysis of development and regeneration of neural retina.
The neural retina is a part of the central nervous system (CNS), and regeneration of the retina from retinal stem cells or other sources by transplantation is a critical issue from both clinical and neurobiological points of view. Although reports of successful regeneration of the CNS from neural stem cells (NSC) have appeared in the literature, such has not been the case for the vertebrate neural retina. Furthermore, the nature of retinal stem cells has not been clarified, making it difficult to attempt regeneration of the retina. Based on the techniques and knowledge that have been accumulated through work on of haematopoietic systems in our laboratory, we attempt to identify mammalian retinal stem cells and following developmental processes by revealing the expression pattern of cell surface proteins. We found that various CD antigens mark spatiotemporally distinct populations of retinal cells, and genes specifically expressed in such populations has been revealed by microarray analyses. Various signaling molecules and transcriptional factors are under investigation for their roles on retinal development. For developmental biological analyses, we use zebrafish in addition to mouse as model animals. We also work on molecular analysis of glioma causative genes using mouse model. Projects, which gave major findings during 2014 are as follows.

1. BMP signaling participates in late phase differentiation of the retina, partly via upregulation of *Hey2*

Hiroshi Kuribayashi, Yukihiro Baba, Sumiko Watanabe

Bone morphogenetic protein (BMP) plays pivotal roles in early retinal development. However, its roles in the late phase of retinal development remain unclear. We found that BMP receptors and ligands were expressed in the postnatal mouse retina. Furthermore, immunostaining revealed that phosphorylated Smads were enriched in various cells types in the inner nuclear layer postnatally. However, phosphorylated Smads were not detected in photoreceptors, suggesting that BMP may play roles in retinal cells in the inner nuclear layer. Forced expression of constitutively active BMP receptors during retinal development resulted in an increased number of bipolar cells and Müller glia and a decreased number of rod photoreceptors; however, proliferation was not perturbed. The expression of dominant negative BMP receptors re-

sulted in a decreased number of Müller glia and bipolar cells. In addition, inhibiting BMP signaling in retinal monolayer cultures abrogated Müller glial process extension, suggesting that BMP signaling also plays a role in the maturation of Müller glia. The expression of the basic helix-loop-helix transcription factor Hey2 was induced by BMP signaling in retinas. The co-expression of sh-Hey2 with constitutively active BMP receptors suggested that the effects of BMP signaling on retinal differentiation could be attributed partly to the induction of Hey2 by BMP. We propose that BMP signaling plays pivotal roles in the differentiation of retinal progenitor cells into late differentiating retinal cell types and in the maturation of Müller glia; these effects were mediated, at least in part, by Hey2.

2. Use of cell type-specific transcriptome to identify genes specifically involved in Müller glia differentiation during retinal development

Yujin Mochizuki[1], Atsumi Iida, Hiromitsu Nakauchi[2], Akira Murakami[1], Sumiko Watanabe: [1]Department of Ophthalmology, Juntendo University School of Medicine, [2]Division of Stem Cell Therapy, Center for Stem Cell Biology and Regenerative Medicine, Institute of Medical Science, University of Tokyo

Retinal progenitor cells alter their properties over the course of development, and sequentially produce different subpopulations of retinal cells. We had previously found that early and late retinal progenitor cell populations can be distinguished by their surface antigens, SSEA-1 and c-kit, respectively. Using DNA microarray analysis, we examined the transcriptomes of SSEA-1 positive cells at E14, and c-kit positive and c-kit negative cells at P1. By comparing data, we identified genes specifically expressed in c-kit positive late retinal progenitor cells. The previous literature suggests that most of the c-kit positive cell-specific genes are related to glia differentiation in brain or are expressed in Müller glia. Since Notch signaling promotes Müller glia differentiation in retina, we examined the effects of gain- and loss-of-Notch signaling on expression of these genes and found that all the genes were positively affected by Notch signaling. Finally, we screened the genes for their function in retinal development by shRNA-based suppression in retinal explants. In about half the genes, Müller glia differentiation was perturbed when their expression was suppressed. Taken together, these results show that at P1, c-kit positive retinal progenitor cells, which include Müller glia precursor cells, are enriched for genes related to glial differentiation. We propose analysis of purified subsets of retinal cells as a powerful tool to elucidate the molecular basis of retinal development.

3. Roles Histone H3K27 tri-methylase Ezh2 in retinal proliferation and differentiation

Atsumi Iida, Toshiro Iwagawa, Yukihiro Baba, Hiromitsu Nakauchi[2], Sumiko Watanabe

The histone modification H3K27me3 regulates transcription negatively, and Jmjd3 and Ezh2 demethylate and methylate H3K27me3 and H3K27, respectively. We demonstrated previously that Jmjd 3 plays pivotal roles in the differentiation of subsets of bipolar cells by regulating H3K27me3 levels at the *Bhlhb4* and *Vsx1* loci, both of which are transcription factors essential for the maturation of bipolar cell subsets. In this study, we examined the role of Ezh2 in retinal development using retina-specific Ezh2 conditional knockout mice (Ezh2-CKO). The eyes of the Ezh2-CKO mice were microphtalemic, and the proliferation of retinal cells was diminished postnatally in Ezh2-CKO. Differentiation of all examined retinal subsets was observed with higher proportion of bipolar cell subsets, which was determined by immunostaining using specific retinal markers. The onsets of Müller glia and rod photoreceptor differentiation were accelerated. The expression of *Bhlhb4* was increased in postnatal retinas, which was accompanied by the loss of H3K27me3 modifications at these genetic loci. Decreased expression of pro-neural genes in postnatal stage was observed. As reported previously in other Ezh2-KO tissues, increased expression of Arf/Ink4a was observed in the Ezh2-CKO retinas. The ectopic expression of Arf or Ink4a in the retina suppressed proliferation and increased apoptosis. In addition, earlier onset of Müller glia differentiation was observed in Ink4a-expressing cells. These results support an important role for histone H3K27me3 modification in regulating the proliferation and maturation of certain subsets of interneurons in the retina.

4. Critical Roles of DNase1l3l in Lens Nuclear Degeneration in Zebrafish

Atsumi Iida, Yukihiro Baba, Sumiko Watanabe

The vertebrate lens undergoes organelle and nuclear degradation during lens development, allowing the lens to become transparent. DNase2b is an enzyme responsible for nuclear degradation in the mouse lens; however, *dnase2b* expression in zebrafish showed a distribution pattern that differed from that in mice. No zebrafish *dnase2b* was detected by reverse-transcription polymerase chain reaction until around 120 h postfertilization (hpf), suggesting that *dnase2b* is not expressed in the critical period for lens nuclear degradation, which corresponds to 56-74 hpf. However, public database searches have indicated that *dnase1l3l* is strongly

and specifically expressed in embryonic zebrafish lens. Whole mount *in situ* hybridization showed that *dnase1l3l* expression began around 36 hpf and was found exclusively in the lens until the adult stage. Morpholino (MO)-dependent downregulation of *dnase1l3l* expression during early development in zebrafish led to the failure of nuclear degradation in the lens. Immunostaining of lens sections showed that expression of Pax6, Prox1 and β-catenin was comparable to the control in the early stage of development in dnase1l3l-MO injected embryos. However, downregulation of expression of these genes in lens was not observed in dnase1l3l-MO-treated zebrafish at 72 hpf, suggesting that the lens development was halted. Taken together, we showed that dnase1l3l plays major roles in nuclear degradation in zebrafish lens development. No homologous gene was found in other species in public databases, suggesting that dnase1l3l developed and acquired its function specifically in zebrafish.

5. Functional analysis of candidate cancer genes that have been identified through transposon mutagenesis screens

Hideto Koso, Hirofusa Yi, Sumiko Watanabe

The *Sleeping Beauty* (*SB*) transposon mutagenesis is an unbiased and high-throughput method to profile the landscape of driver genes in a mouse model system. Glioblastoma multiforme (GBM) is the most common form of malignant brain cancer in adults. Patients with GBM have a uniformly poor prognosis, with a mean survival of one year. Thus, advances on all fronts, both basic and applied, are needed in order to combat this deadly disease. To better understand genes and signaling pathways that are able to transform neural stem cells into glioma-initiating cells, we have performed a transposon mutagenesis screen in mice. An RNA-binding protein has been identified as a novel tumor suppressor gene in glioma, and we have performed functional analysis of this gene. Expression of the RNA-binding protein was decreased in glioma compared to normal neural stem cells. Consistent with this, gene silencing by shRNA in primary astrocytes promoted tumor outgrowth in xenograft mouse models. Moreover, overexpression of this gene in glioma cell lines suppressed proliferation *in vitro* by inducing mitotic arrest and apoptosis. We are currently investigating molecular mechanisms responsible for the growth inhibitory effects of the RNA-binding protein in glioma.

6. DNA methylation attenuates the expression of miR-142-3p in fibroblasts and allows its expression in induced pluripotent stem cells

Siti Razila Abdul Razak, Yukihiro Baba, Hiromitsu Nakauchi[2], Makoto Otsu[2], Sumiko Watanabe

We determined that miR-142-3p is expressed at higher levels in induced pluripotent stem (iPS) cells relative to fibroblasts in mice. Loss-of-function analyses of miR-142-3p revealed that it plays roles in the proliferation and differentiation of iPS cells. Moreover, CpG motifs were found in the 5′ genomic region of the *miR-142-3p* gene; they were highly methylated in fibroblasts, but not in immature iPS cells. Treating fibroblasts with 5-aza-2′-deoxycytidine increased the expression of miR-142-3p significantly and reduced methylation at the CpG sites, suggesting that the expression of miR-142-3p is suppressed by DNA methylation in fibroblasts. Luciferase analysis using various lengths of the 5′ genomic region of miR142-3p showed that CpGs in the proximal enhancer region play pivotal roles in suppressing the expression of miR-142-3p in fibroblasts.

7. Ablation of Kcnj10 expression in retinal explants revealed pivotal roles for Kcnj10 in the proliferation and development of Müller glia

Eisuke Arai[1], Yukihiro Baba, Toshiro Iwagawa, Hiroshi Kuribayashi, Yujin Mochizuki[1], Akira Murakami[1], Sumiko Watanabe

We previously found that Kcnj10, an inwardly-rectifying potassium channel, is one of genes expressed in c-kit-positive retinal progenitor cells on postnatal day 1. The shRNA-mediated screening of functions of the genes for retinal development in retinal explant culture suggested that a role for Kcnj10 in the differentiation of Müller glia. In the present study, we extended the work and focused to analyze roles of Kcnj10 in retinal development. The shRNA-mediated down regulation of Kcnj10 in retinal explant and in vivo mouse retina at P1 stage was done. Differentiation and proliferation of retina were examined by immunohistochemistry. Effect of treatment of barium (Ba^{2+}), which inhibits potassium currents by blocking potassium channels, for retinal development was examined. We found that when Kcnj10 was down-regulated at E18, cellular proliferation and morphological differentiation were perturbed; in particular, a decreased number of Müller glial cells with abnormal morphological maturation was observed. The overexpression of Kcnj10 in retinal progenitors did not result in gross abnormality during retinal development, but resuced the abnormal differentiation induced by sh-Kcnj10. The presence of barium (Ba^{2+}), in retinal explants medium lead to a phenotype similar to that seen with sh-Kcnj10. Ba^{2+} exerts its effects mainly during late retinal development, and sh-Kcnj10 in P 1 retina affected Müller glia maturation, suggesting that Kcnj10 plays a pivotal role in the maturation of

retinal cell subsets. A previous study of Kcnj10-knockout mice showed no obvious abnormality in retinal differentiation, especially of Müller glia. We examined the effects of the down-regulation of Kcnj10 by the *in vivo* electroporation of sh-Kcnj10 into P1 retina. Retinal differentiation was perturbed, as seen following the *in vitro* down-regulation of Kcnj10, suggesting that compensatory gene expression and/or signaling occurred in the Kcnj10 knockout in retina, leading to normal eye development. Kcnj10 plays roles for Müller glia maturation during retinal development probably through its ionic-channel activities.

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Social Coorperation Research Program

Project Division of Social Communication System for Advanced Clinical Research

先端医療社会コミュニケーションシステム社会連携研究部門

Project Professor　Masahiro Kami, M.D., Ph.D.

特任教授　医学博士　　上　　昌　広

The aim of our division is to establish and popularize state-of-art medicine and to promote translational research. We investigate ideal medical governance and methodology to develop national consensus in healthcare, especially through the media.
We also perform individual case studies on vaccine development, research misconduct, and medical support for disaster-stricken area by the Great East Japan Earthquake on March 11, 2011. In each case, we also study the system of management, information circulation, and network.

【Medical Governance】

Yasuhiro Mizuno[1], Hiroto Narimatsu[2], Yuko Kodama, Tomoko Matsumura, Masahiro Kami, Koichiro Yuji[3]: [1]Maru Clinic, [2]Graduate School of Medicine/Cohort Management Unit, Yamagata University, [3]Center for Antibody and Vaccine Therapy, Institute of Medical Science, the University of Tokyo

In next 20 years, Japan will face an unprecedented situation, with fewer children and an aging society. We simulated the insufficiency of medical staff, and discuss the necessity of new medical school in Japan.

【Research misconduct】

Tetsuya Tanimoto[4], Masahiro Kami, Kenji Shibuya[5]: [4]Navitas Clinic, [5]Department of Global Health Policy, Graduate School of Medicine, The University of Tokyo

We studied the situation of research misconducts in Japan. We propose structural reform, such as more transparent relations between academia and the pharmaceutical industry, righteous management for whistleblowers' allegation, and introduction of fair penalty system.

【Medical support for disaster-stricken area】

Masaharu Tsubokura, Tomohiro Morita, Yuko Kodama, Masahiro Kami, Tomoyoshi Oikawa[6], Kaoru Hayashi[6], Michiko Takano[6], Mayumi Nagano[6], Katsuko Onoda[6], Toshiharu Yoshida[6], Akemi Takada[6], Tatsuo Hanai[6], Shunji Shimada[6], Satoko Shimada[6], Yasuyuki Nishiuchi[6], Syuichi Onoda[6], Kazuo Monma[6], Tomoko Matsumura, Yukio Kanazawa[6], Tomohiro Nagatsuka[7], Takeo Hirata[7], Kazuo Hara[8], Amina Sugimoto[9], Shuhei Nomura[10], Masamitsu Hinata[11], Kenji Shibuya[5], Takeaki Ishii[12], Shigeaki Kato[12], Stuart Gilmour[5], Yoshitaka Nishikawa[12], Yasutoshi Saito[12], Masahiko Nihei[13], Yu Sakuma[13], Tetsuya Tanimoto[4], Tsuyoshi Nemoto[6], Ryugo S Hayano[14], Tomoyuki Furutani[15], Daisuke Yoneoka[5]: [6]Minamisoma Municipal General Hospital, Fukushima, [7]Graduate School of Sport Sciences, Waseda University, [8]Department of Metabolic Disease, Graduate School

of Medicine, The University of Tokyo, [9]London school of hygiene and tropical medicine, London, [10] Imperial College London, London, [11]Ichiyokai Hospital, Fukushima, [12]Soma Central Hospital, Fukushima, [13]Hirata Central Hospital, Fukushima, [14]Department of Physics, The University of Tokyo, [15]Faculty of Policy Management, Keio University

After Great East Japan Earthquake and subsequent Fukushima Daiichi nuclear power plant disaster, our team continues to provide the medical care for residents in the disaster-stricken area, especially in Soma and Minamisoma cities. Collaborating with medical staff who work in the areas and support physicians, we conducted researches regarding the levels of internal and external radiation exposure, and gave medical advice to the local people through radiation seminars. We also examined the impacts of the nuclear disaster on public health other than cancer development by radiation exposure. These include deterioration of chronic diseases, decreased strength and psycho-social impacts. These results were widely published in newspapers and popular magazines.

【Vaccine development in Japan】

Tetsuya Tanimoto[4], Eiji Kusumi[4], Kazutaka Hosoda[4], Kaduki Kouno[4], Tamae Hamaki[4], Masahiro Kami, Haruka Nakada, Koichiro Yuji[3], Masaharu Tsubokura, Yukio Ohsawa[16], Masahiro Kami: [16]Department of Systems Innovation, School of Engineering, The University of Tokyo

Vaccines are sometimes useful to prevent the disease development; however, they have potential adverse effects, and they are not always effective for every patient despite of extremely high cost. We studied problems about approval, development, and usage of vaccines in Japan.

【Clinically Oriented Research】

Natsuko Watanabe[17], Hiroto Narimatsu[2], Kazuhiko Kobayashi[18], Yoshitaka Nishikawa[12], Masahiro Kami, Tomohiro Morita, Tetsuya Tanimoto[4], Masayishi Nagata[19], Masahiro Kami, Jun Aoki[20], Kenji Tsuda[21]: [17]Ito Hospital, [18]Teikyo University Medical Center, [19]National Center for Global Health and Medicine Center Hospital, [20]Komagome Hospital, [21]Teikyo University Chiba Medical Center

We studied effective treatment for thyroid, prostate, and hematological cancers. These results were published in scientific journals.

【Economic Burden of Health Care on Patients】

Yuko Kodama, Ryoko Morozumi[22], Akihiko Matsui[23], Masahiro Kami: [22]Faculty of Economics, University of Toyama, [23]Faculty of Economics, The University of Tokyo

We conducted a research about high medical expenses of long term patients with economists. This research will change the government's plan for the burden of high medical expenses of the patients. The economic burden on patients or the government with prevailing advanced medical care including anticancer drugs is an important issue, we continue further investigation.

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Corporate Sponsored Research Program

Project Division of Antibody, Vaccine & Experimental Therapy
抗体・ワクチン治療寄付研究部門

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Tumors contain a small population of putative cancer stem cells (CSC), which possess unique self-renewal properties, and survive in a quiescent state for many years after remission and result in later relapse and metastasis. Therefore, it is conceivable that targeting CSCs will eradicate tumor-initiating cells, whereas conventional chemotherapies will only eradicate the bulk of a tumor.
Cancer stem cells and normal tissue stem cells utilize the same self-renewal pathway. However, researchers characterize some of changes, which occur in cancer stem cells, not in normal tissue stem cells. The design of new therapeutic agents should be aimed at targeting these unique molecular changes.
We have currently focused on studying these unique molecular changes, which occur in cancer stem cells, not in normal tissue stem cells. This could be a new therapeutic target against solid tumors.

A) Zinc-finger-containing transcriptional factor, Kruppel-like factor 2 (KLF2)

The Kruppel-like factor (KLF) proteins are multitasked transcriptional regulators with an expanding tumor suppressor function. KLF2 is a member of the KLF family of zinc-finger transcription factors and is involved in maintaining T-cell quiescence, regulating preadipocyte differentiation, endothelial cell function, lung development and the self-renewal of ES cells. Furthermore, KLF2 is one of the prominent members of the family because of its diminished expression in malignancies and its growth-inhibitory, pro-apoptotic and anti-angiogenic roles.

We indicate that epigenetic silencing of KLF2 occurs in cancer cells through direct transcriptional repression mediated by the Polycomb group protein Enhancer of Zeste Homolog 2 (EZH2). Binding of EZH2 to the 5′-end of KLF2 is also associated with a gain of trimethylated lysine 27 histone H3 and a depletion of phosphorylated serine 2 of RNA polymerase.

Upon depletion of EZH2 by RNA interference, short hairpin RNA or use of the small molecule 3-Deazaneplanocin A, the expression of KLF2 is restored. The transfection of KLF2 in cells with EZH2-associated silencing showed a significant anti-tumoral effect, both in culture and in xenografted nude mice.

In this last setting, KLF2 transfection was also associated with decreased dissemination and lower mortality rate. In EZH2-depleted cells, which characteristically have lower tumorigenicity, the induction of KLF2 depletion 'rescued' partially the oncogenic phenotype, suggesting that KLF2 repression has an important role in EZH2 oncogenesis.

Most importantly, the translation of the described results to human primary samples demonstrated that patients with prostate or breast tumors with low levels of KLF2 and high expression of EZH2 had a shorter overall survival.

B) PR domain-containing protein, PRDM14

PRDM have been linked to human cancers. To explore the role of the PR domain family genes in breast carcinogenesis, we examined the expression profiles of 16 members of the PRDM gene family in a panel of breast cancer cell lines and primary breast cancer specimens using semiquantitative real-time PCR.

We found that PRDM14 mRNA is overexpressed in about two thirds of breast cancers. Moreover, immunohistochemical analysis showed that expression of PRDM14 protein is also up-regulated. PRDM14 are known as a key transcription factor required for the maintenance of hESC identity and the reacquisition of pluripotency in human somatic cells.

Introduction of PRDM14 into cancer cells reduced their sensitivity to chemotherapeutic drugs. Conversely, knockdown of PRDM14 by siRNA induced apoptosis in breast cancer cells and increased their sensitivity to chemotherapeutic drugs. Moreover, PRDM14 regulated cancer metastasis, angiogenesis, and stemness of cancer cells.

That little or no expression of PRDM14 is seen in noncancerous tissues suggests that PRDM14 could be an ideal therapeutic target for the treatment of breast cancer. Now, we also develop new methodology with nuclear acid medicine and modified antibody drug against PRDM14.

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Please refer to Medical Proteomics Laboratory.

Social Coorperation Research Program

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RNA no longer stands behind DNA or protein but stands in front of DNA and protein. Recent achievements and discovery in biological science clearly emphasize the importance of RNA in life; the discovery of RNA interference, molecular mimicry between protein and RNA, ribosome structure at atomic resolution, and RNA/polypeptide quality control triggered by aberrant mRNAs. Moreover, the completed human genome project revealed, to our great surprise, the existence of a large amount of protein-noncoding RNAs (ncRNAs). These ncRNAs can be classified into two types: one, like antisense and microRNA, those function with the sequence complementarity to the target mRNA or DNA, while the other, like aptamer, those function independent of the sequence complementarity. In our laboratory, we aim to: 1) uncover the molecular mechanism underlying the mRNA surveillance and aberrant product clearance; and 2) create artificial aptamers to target proteins of therapeutic interest.

1. Nonstop mRNA and Protein Degradation in *Drosophila melanogaster* Cells

Isao Kashima, Yoshifumi Hashimoto, Masaki Takahashi, Eri Sakota, Yoshikazu Nakamura, Toshifumi Inada[1]: [1]Graduate School of Pharmaceutical Sciences, Tohoku University

When ribosomes encounter mRNAs lacking stop codons, two quality-control machineries, NSD for nonstop mRNA decay and ribosome quality control (RQC) for co-translational degradation of the nonstop protein by the proteasome, are triggered to eliminate aberrant molecules. In yeast, it is known that Dom34 (a homolog of eRF1) and Ltn1 (an E3 ubiquitin ligase) play crucial roles in NSD and RQC, respectively, by triggering ribosome rescue at the 3' end of nonstop mRNAs and proteasome-dependent polypeptide degradation. The molecular basis of NSD is well clarified in yeast, but NSD in higher eukaryote is not fully understood. Therefore, we investigated the molecular mechanism of NSD using *Drosophila melanogaster* cells as a model of higher eukaryote by knocking down relevant factors by double stranded RNA interference (RNAi).

Here we confirmed the essential role of Ltn1 in RQC for nonstop products in *Drosophila* cells as found in yeast, and further uncovered a functional role of ABCE1, a eukaryotic ribosome recycling factor, in NSD in *Drosophila* cells.

In yeast, it is known that Ski-exosome complex (3'-to-5' endoribonuclease) and Xrn1 (5'-to-3' endoribonuclease), together with the release factor homologs Dom34/Hbs1, play crucial roles in NSD. However, in *Drosophila* cells, Xrn1 is not appreciably involved in NSD. A near complete disruption of NSD can be accomplished in *Drosophila* cells by knocking down either two factors of Dom34, Hbs1 and ABCE1, giving rise to a remarkable accumulation of poly-ribosomes associated with nonstop mRNAs. This finding might provide us with an opportunity to discover natural mRNA substrates for NSD.

2. Therapeutic RNA Discovery

a. Selection of RNA aptamer against integrin alpha V-beta 6

Masaki Takahashi, Eri Sakota, Yoshikazu Nakamura

Integrins are heterodimeric transmembrane receptors composed of alpha and beta subunits, and are closely associated with a variety of physiological and pathological processes. Among various combinations of heterodimer, integrin alpha V-beta 6 complex (ITGAV-B6), receptor to fibronectin, is recently identified as a novel druggable target for pulmonary fibrosis. By screening of a large library of nuclease-resistant RNA oligonucleotides by SELEX, we selected RNA aptamers that bind human ITGAV-B6. Currently, stable cell lines over-expressing ITGAV-B6 are being established for further functional analysis of anti-ITGAV-B6 aptamers at the cellular levels.

b. A novel measurement of allele discrimination for assessment of allele-specific silencing by RNA interference

Masaki Takahashi, Hirohiko Hohjoh[1]: [1]Department of Molecular Pharmacology, National Institute of Neuroscience, NCNP

Allele-specific silencing by RNA interference (ASP-RNAi) is an atypical RNAi that is capable of discriminating target alleles from non-target alleles, and may be therapeutically useful for specific inhibition of disease-causing alleles without affecting their corresponding normal alleles. However, it is difficult to design and select small interfering RNA (siRNAs) that confer ASP-RNAi. A major problem is that there are few appropriate measures in determining optimal allele-specific siRNAs. Here we show two novel formulas for calculating a new measure of allele-discrimination, named "ASP-score". The formulas and ASP-score allow for an unbiased determination of optimal siRNAs, and may contribute to characterizing such allele-specific siRNAs.

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特任准教授 理学博士 金 玟 秀
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Many pathogenic bacteria, including Shigella, enteropathogenic Escherichia coli (EPEC), and enterohemorrhagic E.coli (EHEC), are associated with diarrheal diseases and are an important cause of death in many countries. Our current interest is to understand the complex interactions among pathogenic bacteria, the gastrointestinal epithelium and microbiota during pathogenic bacteria infection. The main goal of our research is to develop new therapeutic tools or vaccines that will target these bacterial infections.

1. Exploitation of the host ubiquitin system by human bacterial pathogens.

Ashida H, Kim M, Sasakawa C[1,2]: [1]Nippon Institute for Biological Science, Shinmachi, Ome, Tokyo, Japan; [2]Medical Mycology Research Center, Chiba University, Chiba, Japan.

Ubiquitylation is a crucial post-translational protein modification that regulates several cellular processes in eukaryotes, including inflammatory responses, endocytic trafficking and the cell cycle. Importantly, ubiquitylation also has a central role in modulating eukaryotic defence systems; however, accumulating evidence shows that many bacterial pathogens exploit host ubiquitin systems for their own benefit. In this Review, we highlight the ways in which human bacterial pathogens target ubiquitylation to subvert and manipulate host defence systems, with a focus on the role of molecular mimicry and secreted bacterial effector proteins. These strategies enable bacterial pathogens to maximize effector function and obtain nutrients, thereby promoting bacterial proliferation.

2. *Shigella* IpaH7.8 E3 ubiquitin ligase targets glomulin and activates inflammasomes to demolish macrophages.

Suzuki S[1,3], Mimuro H[1,2], Kim M, Ogawa M[1], Ashida H, Toyotome T[1], Franchi L[3], Suzuki M[1], Sanada T[1,2], Suzuki T[1,4], Tsutsui H[5], Núñez G[3], Sasakawa C[1,6,7]: [1]Department of Microbiology and Immunology, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [2]Division of Bacteriology, Department of Infectious Disease Control, International Research Center for Infectious Diseases, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [3]Department of Pathology and Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, MI 48109, U.S.A; [4]Department of Microbiology and Immunology, Department of Molecular Bacteriology and Immunology, Graduate School of Medicine, University of the Ryukyus, Okinawa 903-0125, Japan; [5]Department of Microbiology, Hyogo College of Medicine, Hyogo 663-8501, Japan; [6]Nippon Institute for Biological Science, Shinmachi, Ome, Tokyo, Japan; [7]Medical Mycology Research Center, Chiba University, Chiba, Japan.

When nucleotide-binding oligomerization domain-like receptors (NLRs) sense cytosolic-invading bacteria, they induce the formation of inflammasomes and initiate an innate immune response. Recent studies indicated that *Shigella* deploy multiple mechanisms to stimulate NLR inflammasomes

through type III secretion during infection. Here, we show that *Shigella* induces rapid macrophage cell death by delivering the invasion plasmid antigen H7.8 (IpaH7.8) enzyme 3 (E3) ubiquitin ligase effector via the type III secretion system, thereby activating the NLR family pyrin domain-containing 3 (NLRP3) and NLR family CARD domain-containing 4 (NLRC4) inflammasomes and caspase-1 and leading to macrophage cell death in an IpaH7.8 E3 ligase-dependent manner. Mice infected with *Shigella* possessing IpaH7.8, but not with *Shigella* possessing an IpaH7.8 E3 ligase-null mutant, exhibited enhanced bacterial multiplication. We defined glomulin/flagellar-associated protein 68 (GLMN) as an IpaH7.8 target involved in IpaH7.8 E3 ligase-dependent inflammasome activation. Modifying GLMN levels through overexpression or knockdown led to reduced or augmented inflammasome activation, respectively. Macrophages stimulated with lipopolysaccharide/ATP induced GLMN puncta that localized with the active form of caspase-1. Macrophages from GLMN(+/−) mice were more responsive to inflammasome activation than those from GLMN(+/+) mice. Together, these results highlight a unique bacterial adaptation that hijacks inflammasome activation via interactions between IpaH7.8 and GLMN.

3. *Shigella* type III secretion protein MxiI is recognized by Naip2 to induce Nlrc4 inflammasome activation independently of Pkcδ.

Suzuki S[1], Franchi L[1,2], He Y[1], Muñoz-Planillo R[1], Mimuro H[3], Suzuki T[4], Sasakawa C[5], Núñez G[1]: [1]Department of Pathology and Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, Michigan, U.S.A; [2]Lycera Corp., Ann Arbor, Michigan, U.S.A; [3]Division of Bacteriology, Department of Infectious Disease Control, International Research Center for Infectious Diseases, Institute of Medical Science, The University of Tokyo, Tokyo, Japan; [4]Department of Microbiology and Immunology, Department of Molecular Bacteriology and Immunology, Graduate School of Medicine, University of the Ryukyus, Okinawa 903-0125, Japan; [5]Nippon Institute for Biological Science, Shinmachi, Ome, Tokyo, Japan

Recognition of intracellular pathogenic bacteria by members of the nucleotide-binding domain and leucine-rich repeat containing (NLR) family triggers immune responses against bacterial infection. A major response induced by several Gram-negative bacteria is the activation of caspase-1 via the Nlrc4 inflammasome. Upon activation, caspase-1 regulates the processing of proIL-1β and proIL-18 leading to the release of mature IL-1β and IL-18, and induction of pyroptosis. The activation of the Nlrc4 inflammasome requires the presence of an intact type III or IV secretion system that mediates the translocation of small amounts of flagellin or PrgJ-like rod proteins into the host cytosol to induce Nlrc4 activation. Here, we show that Naip2 recognizes the *Shigella* T3SS inner rod protein MxiI and induces Nlrc4 inflammasome activation. The expression of MxiI in primary macrophages was sufficient to induce pyroptosis and IL-1β release, which were prevented in macrophages deficient in Nlrc4. In the presence of MxiI or *Shigella* infection, MxiI associated with Naip2, and Naip2 interacted with Nlrc4. siRNA-mediated knockdown of Naip2, but not Naip5, inhibited *Shigella*-induced caspase-1 activation, IL-1β maturation and Asc pyroptosome formation. Notably, the Pkcδ kinase was dispensable for caspase-1 activation and secretion of IL-1β induced by *Shigella* or *Salmonella* infection. These results indicate that activation of caspase-1 by *Shigella* is triggered by the rod protein MxiI that interacts with Naip2 to induce activation of the Nlrc4 inflammasome independently of the Pkcδ kinase.

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The immune system in mammals consists of many types of cells. They interact with each other and construct a complex network to maintain homeostasis and protect from pathogens. Our goal is to investigate the function of each immune cell from various points of view and analyze the multicellular event by using bioinformatics technique. Furthermore, we will apply the knowledge from our research for the discovery of novel drug or method for the treatment of immune diseases.

1. Systems biological analysis of macrophage in small intestinal lamina propria (LP)

Yasumasa Kimura[1], Yasuo Ouchi[2], Naoki Takemura[2], Kouta Matsunaga[2], Shintaro Sato[3], Shizuo Akira[4], Hiroshi Kiyono[3], Satoshi Uematsu[2], Takeshi Satoh[1]: [1]Division of Systems Immunology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [2]Division of Innate Immune regulation, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [3]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, [4]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University.

In the intestinal organ, there is a variety of immune cell. Especially, intestinal lamina propria (LP) is an important tissue for immune responses. Uematsu et al. recently showed the functional analysis of macrophages in intestinal LP. $CD11c^{int}CD11b^{int}$ cells in small intestinal LP are resident macrophages and interestingly they strongly induce $FoxP3^{+}$ regulatory T cells in the presence of TGF-β. We are analyzing the regulatory mechanisms of $CD11c^{int}CD11b^{int}$ macrophages with regards to TLR stimulation and difference from other tissue macrophages. Using their gene expression data, together with public open data of expression and transcription factor binding site profiles from other tissue-macrophages, we identified some putative key regulatory genes. We will perform further analyses in conjunction with other public data sets, such as protein interaction data.

2. Systems biological analysis of dendritic cells (DCs) in small intestinal LP

Yasumasa Kimura[1], Yasuo Ouchi[2], Naoki Takemura[2], Kouta Matsunaga[2], Kouji Kobiyama[3], Taiki Aoshi[3], Ken J Ishii[3], Shintaro Sato[4], Shizuo Akira[5], Hiroshi Kiyono[4], Satoshi Uematsu[2], Takeshi Satoh[1]: [1]Division of Systems Immunology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [2]Division of Innate Immune regulation, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [3]Laboratory of Adjuvant Innovation, National Institute of Biomedical Innovation, [4]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Sci-

ence, The University of Tokyo, [5]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University.

Dendritic cell is a key player of governing immune responses. In intestinal LP, there are several populations of DCs distinguished by cell surface marker expression. $CD11c^{hi}CD11b^{hi}$ dendritic cells (DCs), which are $CD103^{+}$, specifically express Toll-like receptor (TLR) 5 and induce the differentiation of naïve B cells into IgA^{+} plasma cells. Intraperitoneal injection of activated antigen(Ag)-loaded $CD11c^{hi}CD11b^{hi}$ DCs induced Ag-specific IgG in serum and IgA in stool samples. Thus, $CD11c^{hi}$ $CD11b^{hi}$ DCs are suitable targets for oral vaccines in the intestine. We are analyzing the regulatory mechanisms of $CD11c^{hi}CD11b^{hi}$ DCs with regards to TLR stimulation and difference from other tissue DCs. We combined their gene expression data with other tissue-DC expression and transcription factor binding site data from public source, and are analyzing the genes for their regulatory function. We will also perform further analyses in conjunction with other public data sets, such as protein interaction data.

3. Meta-genome analysis of gut microbiota

Yasumasa Kimura[1], Naoki Takemura[2], Shintaro Sato[3], Shuji Suzuki[4], Masanori Kakuta[4], Hiroshi Kiyono[3], Rui Yamaguchi[5], Seiya Imoto[5], Yasushi Akiyama[4], Satoru Miyano[5], Satoshi Uematsu[2], Takeshi Satoh[1]: [1]Division of Systems Immunology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [2]Division of Innate Immune regulation, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [3]Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, [4]Department of Computer Science, Graduate School of Information Science and Engineering, Tokyo Institute of Technology, [5]Human Genome Center, The Institute of Medical Science, The University of Tokyo.

There are huge numbers of intestinal commensal bacteria (more than 100 trillions in human) mutually interacting host organism and regulating host immune system. We initiated collaboration with Human Genome Center in our institute and Tokyo Institute of Technology for high performance computing to analyze meta-genome sequence produced by next generation sequencers. We are preparing samples from several knock-out (KO) mice and will analyze the effect of KO to colonization of commensal bacteria.

Publications

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Our major goal is to develop the regenerative medicine and cure diseases with various stem cells, including mesenchymal stem cells (MSC), and pluripotent stem cells (PSC), such as embryonic stem cells and induced pluripotent stem cells (ESC and iPSC, respectively). Currently our efforts are directed toward 1) characterizing the capabilities of MSC derived from various sources (adipose tissue, umbilical cord, bone marrow, and PSC) to apply for regenerative medicine, 2) the establishment of novel therapeutic application using PSC, and the analysis of pathogenesis of a variety of disorders based on disease-specific iPSC.

Basic research for developing cell-based therapy using human mesenchymal stem/stromal cells

Hidenori Nonaka, Tsuyoshi Ishii, Tetsumasa Yamada, TokikoNagamura-Inoue[1], Arinobu Tojo[2], Hiromitsu Nakauchi[3], Yasuhiro Ebihara: [1]Department of Cell Processing and Transfusion, Research Hospital, [2]Division of Molecular Therapy, Advanced Clinical Research Center, [3]Division of Stem Cell Therapy, Center for Stem Cell Therapy and Regenerative Medicine

The aim of this project is to understand the basic properties of human mesenchymal stem/stromal cells (MSCs) for use in a cell-based therapy. Stem cells have great potential for use in a regenerative medicine to treat an intractable disease where small molecule drugs had not been successfully developed. Because of their immunomodulatory properties, differentiation capability and less tumorigenic potential than ES and iPS cells, MSCs are one of the most promising tools for cell therapy. To develop a stem cell drug, it is necessary to establish cell manufacturing processes, to guarantee the quality and safety of the cells and to understand the mode of action against target diseases. In this project, we will focus on MSCs and study their basic properties to explore manufacturing technologies and therapeutic applications.

Establishment of human BM-derived MSC for the treatment of hemophilic arthropathy

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Hemophilia is a congenital disease with a lack of coagulation factors. Approximately one third of the patients need the mobility assistance because of hemophilic arthropathy. Although the pathogenesis of hemophilic arthropathy (HA) still has not been precisely clarified, the destruction of articular cartilage is the most prominent event in HA. Most surgical treatments for HA, such as synovectomy or total joint arthroplasty, are performed by Department of Joint Surgery in our hospital. So far, however, the efficacy of the treatment has been insufficient. Recently it has been shown that BM contains MSC, which can differentiate into various mesenchymal

tissue cells, such as osteocytes, adipocytes and chondrocytes. The environment surrounding MSC plays an important role in the commitment in addition to MSC differentiation. We are then preparing for the clinical trial of the transplantation of autologous culture-expanded BM-derived MSC into the articular cartilage defect in the HA patients.

Induction of hematopoiesis from human pluripotent stem cells in culture without animal serum or cells

Yasuhiro Ebihara, Kiyoko Izawa, Shinji Mochizuki[7], Emiko Matsuzaka[7], Makoto Otsu[7], Yuji Zaike[8], Hiromitsu Nakauchi[3], Kohichiro Tsuji[6]: [7]Division of Stem Cell Processing, Center for Stem Cell Biology and Regenerative Medicine, [8]Department of Laboratory Medicine, Research Hospital

It is important to establish an *in vitro* culture method for the induction of hPSC, such as hESC or hiPSC, to differentiate into mature blood cells without animal serum and cells. To achieve this, we first induced hPSC to differentiate into mesenchymal stem cells (MSC). When human ES or iPS cells cultured on murine embryonic fibroblast (MEF) feeder cells were recultured on gelatin-coated culture dishes with platelet lysate (PL)-containing media in the absence of MEF feeder cells. Cells were passaged several times with PL containing media, and then MSC were induced after 6 to 8 weeks. The MSC were spindle-like shaped, revealed a phenotype of CD45−, CD34−, CD14−, CD105+, CD166+, CD31−, and SEA-4−, and had the ability to differentiate into mesenchymal tissues such as bone, cartilage and fat in vitro. Murine MEF and undifferentiated hPSC were undetectable in the hPSC-derived MSC by reverse transcription polymerase chain reaction analysis. We then cocultured hPSC with MSC derived from hPSC themselves under serum-free condition. Two weeks later, a number of hematopoietic progenitor cells (HPC) appeared in the coculture. These HPC were cultured in hematopoietic colony assay using human serum. In result, hPSC-derived HPC produced various hematopoietic colonies, such as myeloid, erythroid and multilineage colonies, including all types of blood cells. The novel culture method must be useful for the clinical application of hPSC-derived MSC and blood cells.

Generation of disease-specific human iPS cells

Yasuhiro Ebihara, Kiyoko Izawa, Shinji Mochizuki[7], Emiko Matsuzaka[7], Makoto Otsu[7], Hiromitsu Nakauchi[3], Kohichiro Tsuji[6]

We have generated some disease-specific iPSC. We have analyzed their pathophysiology using iPS cells derived from 1) Down syndrome, 2) severe congenital neutropenia, and 3) acute myeloid leukemia (AML) developed from 8p11 myeloproliferative syndrome (EMS). Apart from these iPSC, we are focusing iPSC derived from the patient with juvenile myelomonocytic leukemia (JMML). We employed iPSC derived from patients with JMML (JMML-iPSC). 12 JMML-iPSC lines were all created from bone marrow cells and reprogrammed by the defined 4 reprogramming factors (OCT3/4, KLF4, SOX2, and c-MYC). We generated blood cells from JMML-iPSC with coculture system using AGMS-3 cells. Myelopoiesis was quite facilitated in cells derived from JMML-iPSC, and the response to granulocyte macrophage-colony stimulating factor (GM-CSF) for hematopoietic colony formation was highly promoted like the recipient BM cells. These results indicated that JMML-iPSC might reflect the pathophysiology of JMML.

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Social Coorperation Research Program

Project Division of International Advanced Medical Research

国際先端医療社会連携研究部門

Project Associate Professor　Koichiro Yuji, M.D., Ph.D.

特任准教授　医学博士　　湯　地　晃一郎

The mission of the Project Division is to apply changes in advanced medical research at the Institute of Medical Science at the University of Tokyo (IMSUT). Our activities include field research in which innovative medicine will be implemented; cross-disciplinary education of physicians, researchers, and professionals; collaboration in innovative projects in the Coastal Area Life Innovation Comprehensive Special Zone for International Competitiveness Development; and establishing projections of the future healthcare system of Japan, which will be the first fully fledged aged society.

Implementing advanced medical research at IMSUT

Yuji K.

The Project Division was established in November 2014. Our mission is to contribute to the progress of advanced medical research at IMSUT; to perform field research in which innovative medicine will be implemented; and to further the cross-disciplinary education of physicians, researchers, and professionals. Our future plans include collaboration in innovative projects in the Coastal Area Life Innovation Comprehensive Special Zone for International Competitiveness Development.

Leakage of Japanese genome information

Yuji K, Tanimoto T, and Oshima Y.

The leakage of Japanese genome data to outside Japan is a major concern. Once raw genetic data on an individual have been determined from a DNA sample, they can be moved, in digital form, across international borders. For example, in Japan, blood samples from Japanese mothers undergoing noninvasive prenatal testing (NIPT) are sent to an American company for analysis, and the findings are then sent back to Japan. Any regulatory framework for genome-based industry should therefore be based not solely on domestic laws. International harmonization is required.

Projections on the future healthcare system in Japan, the first fully fledged aged society

Yuji K, Imoto S, Yamaguchi R, Miyano S, Kami M, and Imai K.

Japan is rapidly becoming a fully fledged aged society, and the increasing dependence of the elderly population is a significant concern. We have simulated both the supply and demand features of Japan's future healthcare system.

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