Advanced Clinical Research Center

Division of Molecular Therapy
分子療法分野

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The main theme of our research is toward the development of novel therapeutic options against intractable malignant disorders including leukemia, lymphoma and various cancers. For this purpose, we are making every effort to master the mechanisms of normal and neoplastic stem cells on the basis of molecular and cellular biology as well as medical informatics. We also try to develop novel therapies in the field of regenerative medicine using bone marrow-derived mesenchymal stromal cells.

(1) Molecular and cellular analysis of hematological malignancies:
Tumor-specific genetic alterations often result in dysregulation of transcription factors and constitutive activation of tyrosine kinases, which appear to be the primary cause of those tumors. We are studying the molecular and cellular aspects of hematological malignancies as a model system. Furthermore, we performed clinical sequencing in tight collaboration with Human Genome Center and Health Intelligence Center to establish a platform for precision medicine.

(2) Development of anti-cancer therapy using recombinant vaccinia virus:
Vaccinia virus is a promising vector for oncolytic immunovirotherapy of cancer. For cancer specificity and safety, we introduced some genetic modifications into the viral genome by recombinant DNA technique. These include miRNA-regulated expression of B5R, an essential component for virus spreading, and deletion of thymidine kinase. We are now trying to apply MDVV (miRNA-regulated and thymidine kinase-deleted vaccinia virus) to a preclinical model of multiple myeloma.

(3) Investigation of cancer stem cells and search for molecular targets for their elimination:
We are focusing on cancer, stem cells, and cancer stem cells. We aim to elucidate molecular mechanisms how growth factor signaling regulates tumorigenesis and maintenance of stem cells and cancer stem cells. Moreover, by taking not only molecular biology but also new bioinformatics approaches, we aim to identify novel cancer biomarkers and molecular targets for cancer therapy. Our ultimate goal is to translate them into clinic.

(4) Clinical study of clonal evolution of HTLV-1-infected T cells into leukemia:
Adult T-cell leukemia is a T cell malignancy which develops in HTLV-1 infected individuals after long latency period. HTLV-1 infected cells are regarded to transform through multi-step oncogenesis process. We are analyzing HTLV-1 infected cells in different stages of transformation whose phenotypes such as CD7 and CADM1 expression vary in each stage by sorting them using flow cytometer. These analyses will provide useful information regarding molecular

mechanism to develop ATL.

1. Efficacy and safety of doubly-regulated recombinant vaccinia virus in a mouse xenograft model of multiple myeloma

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Multiple myeloma (MM) is an incurable hematological malignancy characterized by proliferation of plasma cells in the bone marrow (BM). Virotherapy is a potentially promising modality to treat refractory cancers. Vaccinia virus (VV) is a member of the poxvirus family and has several advantages over other oncolytic viruses, such as a short life cycle, potent tissue penetration and robust lytic activity. Myeloma cells are exceptionally susceptible to VV among hematological malignancies. To render VV more myeloma-specific, we generated let-7a-regulated and thymidine kinase-deleted VV (MDVV) in which the essential viral gene B5R is regulated by let-7a. Let-7a is a microRNA highly expressed in normal human cells but downregulated in myeloma cells. Intravenous injection of MDVV efficiently infected and killed MM cells with minimal adverse effects on normal cells and tissues in SCID mice carrying a subcutaneous xenograft. However, in another xenograft model carrying BM myeloma lesions, MDVV failed to reduce tumor burden probably due to its poor penetrance to the BM. For an alternative strategy, we tested the feasibility of a cell-based virus delivery targeting BM lesions, using a human myeloma cell line KPMM2 which can engraft in the BM of SCID mice. MDVV-infected KPMM2 cells successfully transferred MDVV to target cells and reduced viability of the target cells *in vitro*, but intravenous injection of the MDVV-infected myeloma cells did not inhibit tumor propagation in the BM myeloma model. For better viral delivery and anti-tumor effect, we are currently modifying tropism of MDVV to the BM by introducing a bone-targeting peptide daca-aspartate (D10). We are also modifying MDVV to stimulate anti-tumor immunity via natural killer T cells by CD1d, and cytotoxic T cells by tumor-associated antigens TRP2 and EphA2.

2. Proportion of $CD4^+CADM1^+$ population predicts clinical progression in HTLV-1 asymptomatic carrier and indolent ATL

Seiichiro Kobayashi[1,2], Eri Watanabe[2], Arinobu Tojo[1,2], and Kaoru Uchimaru[3]: [1]Division of Molecular Therapy, The Advanced Clinical Research Center, [2]IMSUT clinical flow cytometry laboratory, [3]Graduate School of Frontier Sciences, The University of Tokyo

In HTLV-1-infected patient samples, $CD4^+$ population includes CADM1-$CD7^{pos}$(P), $CADM1^+CD7^{dim}$ (D) and $CADM^+CD7^{neg}$(N) subpopulations. HTLV-1 infected cells and clones are efficiently enriched in the $CADM1^+$ subpopulations (D and N)(Kobayashi et al, Clinical Cancer Research 2014). In HTLV-1 asypmptomatic carrier (AC) and indolent ATL phase, disease progression are reflected in increase of the D + N(%). In aggressive ATL phase, loss of CD7 occurs in many cases and $CD4^+$ population is occupied by the $CADM^+CD7^{neg}$(N). From 2011 Jun to 2014 Sep, we analysed 60 AC / indolent ATL cases using this flow cytometry combined with HTLV-1 clonality and clinical data (abnormal lymphocytes (%), proviral load (PVL), etc)(Kobayashi et al, Cancer Science 2015). We categorised these cases into the following groups. G1(D + N≤10%) includes low PVL ACs. G2(10%<D + N≤25%) mainly includes ACs with oligoclonal HTLV-1 clones. G3 (25%<D + N≤50%) mainly includes ACs and smoldering ATLs. Many of these cases had major clones. G4(50%<D + N) mainly includes smoldering and chronic ATLs. We are following these cases to see how the flow cytometric profile (D + N(%)) predicts future risk. In this study, follow-up clinical and flow cytometric data (CADM1 vs CD7 plot in $CD4^+$ cells) were obtained in the previously analysed 60 ACs and indolent ATLs.

In cases in G1(D + N≤10%) and G2(10%<D + N≤ 25%), apparent clinical and flow cytometric progression were not observed in 5.5 years follow-up (at the time of this writing). In G3(25%<D + N≤50 %), two cases progressed from AC to smoldering ATL. In another smoldering ATL case, increase in abnormal lymphocytes and D + N(%) in flow cytometry were observed. The other 12 (of 15 in total G3) cases were stable in clinical and flow cytometric data. In G4(50%<D + N), only 4 (of 15 in total G4) cases were clinically stable. Other 4 cases were registered in clinical trial (JCOG1111). Remaining 7 cases resulted in disease progression/chemotherapy. In this group, in all evaluable cases except one, progression of flow cyometric profile was observed.

In conclusion, proportion of the $CD4^+CADM1^+$ population (D + N(%)) predicts clinical progression

in HTLV-1 ACs and indolent ATLs. Cases in G1(D+N≤10%) and G2(10%<D+N≤25%), including ACs, are stable and considered low-risk. Although many cases in G3(25%<D+N≤50%) are considered stable in this observation period (max 5.5 years), three cases clinically progressed. Cases in this group, including advanced ACs and smoldering ATLs in Shimoyama criteria, are therefore considered to form an intermediate-risk group. Cases in G 4(50%<D+N), mainly including indolent ATLs, are unstable and high-risk. Clinical intervention should be considered in this group.

3. Clinical sequencing of malignant as well as rare and undiagnosed hematological disorders with the aid of artificial intelligence

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Next-generation sequencing (NGS) is an attractive tool for prospective use in the field of clinical oncology. However, for this purpose, further innovations are necessary including medical informatics which links somatic mutations to clinical intervention. This process is currently labor-intensive, involving experienced curators who pick up the relevant evidence among a growing body of knowledge and translate it into medical practice. We organized a clinical sequencing team, called as IMSUT Tumor Board, and have been integrating clinical and genomic information in hematological malignancies with the aid of cognitive computing or artificial intelligence (AI). Genomic DNA was prepared from malignant cell fractions and normal tissues in each patient, and subjected to comparative NGS, mainly targeted deep sequencing with ready-made panels and, on demand, whole exome sequencing and or RNA sequencing. Sequence data was analyzed using a pipeline of in-house semi-automated medical informatics. AI was used to identify candidate driver mutations and pathways in each patient, from which pathogenic information as well as applicable drug information was deduced. A summary of NGS data was reported and discussed in IMSUT Tumor Board to deliberate upon potentially actionable findings. Until Nov. 2016, we performed NGS analysis on 113 patients with AML, MDS, MPN, et al., among which actionable findings could be obtained in 27 patients. Eight patients actually received treatments motivated in IMSUT Tumor Board. Our preliminary results indicate that AI can be well suited to clinical sequencing.

4. Phenotype-based gene analysis allowed successful diagnosis of X-linked neutropenia associated with a novel WASP mutation in a Japanese adult patient

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X-linked neutropenia (XLN) is a congenital neutropenia caused by gain-of-function mutation in Wiskott-Aldrich syndrome protein (WASP). To our knowledge, only 3 cases of XLN have been described thus far. Here we report a first Japanese case of XLN, who was successfully diagnosed by next generation sequencing (NGS). A 32-year-old male had repeated bacterial infections during his childhood and adolescence, and was clinically diagnosed as severe congenital neutropenia (SCN) when 6-year-old. Since adulthood, he had been free from regular medical follow-up with much less infectious episodes until when 29-year-old, he was admitted to our hospital for febrile neutropenia. His family history showed no significant evidence for inheritance of SCN. His peripheral blood showed severe neutropenia (ANC<200) and slight thrombocytopenia. Bone marrow examination revealed marked myeloid hypoplasia with a slight dysplastic change. *ELANE* gene mutation, a major cause of SCN, was kindly examined by the Department of Pediatrics at Hiroshima University School of Medicine, but was negative. Then, we performed NGS analysis of genomic DNA from the patient and his mother. Sequence data was subjected to medical informatics using in-house pipeline as well as phenotype-based gene analyzer (Yang H, et al. Nat Methods 12:841-3, 2015), resulting in identification of a missense mutation in exon 9 of *WAS* gene (c.T869 C, p.I290T), and his mother was a heterozygous carrier. This mutation is located in the GTPase binding domain of WASP like other mutations in 3 XLN patients (L270P, S272P, I294T). Based on this close similarity, we tentatively conclude that this is the 4th case of XLN caused by a novel WASP mutation. The pathogenesis of XLN is still to be elucidated.

5. An autocrine/paracrine circuit of growth differentiation factor (GDF) 15 has a role for maintenance of breast cancer stem-like cells

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Cancer stem cells are thought to be responsible for tumor growth, recurrence, and resistance to conventional cancer therapy. However, it is still unclear how they are maintained in tumor tissues. Here, we show that the growth differentiation factor 15 (GDF15), a member of the TGFβ family, may maintain cancer stem-like cells in breast cancer tissues by inducing its own expression in an autocrine/paracrine manner. We found that GDF15, but not TGFβ, increased tumor sphere formation in several breast cancer cell lines and patient-derived primary breast cancer cells. As expected, TGFβ strongly stimulated the phosphorylation of Smad2. GDF15 also stimulated the phosphorylation of Smad2, but the GDF15-induced tumor sphere forming efficiency was not significantly affected by treatment with SB431542, an inhibitor of the TGFβ signaling. Although TGFβ transiently activated ERK 1/2, GDF15 induced prolonged activation of ERK1/2. Treatment with U0126, an inhibitor of the MEK-ERK1/2 signaling, greatly inhibited the GDF15-induced tumor sphere formation. Moreover, cytokine array experiments revealed that GDF15, but not TGFβ, is able to induce its own expression; furthermore, it appears to form an autocrine circuit to continuously produce GDF15. In addition, we found heterogeneous expression levels of GDF15 among cancer cells and in human breast cancer tissues using immunohistochemistry. This may reflect a heterogeneous cancer cell population, including cancer stem-like cells and other cancer cells. Our findings suggest that GDF15 induces tumor sphere formation through GDF15-ERK1/2-GDF15 circuits, leading to maintenance of $GDF15^{high}$ cancer stem-like cells. Targeting GDF15 to break these circuits should contribute to the eradication of tumors.

6. Maintenance of stemness and niche environment of breast cancer cells by proteinα, a feedback inhibitor for HER2-ERK pathway, during mammary tumorigenesis

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Inflammatory microenvironment contributes to tumorigenesis. Although it is thought that breast cancer stem cells (CSCs) appear and grow in the inflammatory microenvironment that is called CSC niche, it remains largely unclear how it occurs. Here, we uncovered proteinα, a feed-back inhibitor of ERK activity in progenitor cells, plays critical roles for various cytokine production to increase progenitor cell- and CSC- state, and create the CSC niche. Expression levels of proteinα were increased in mammary tumors of MMTV-ErbB2 mice than in normal mammary tissues. Deficiency of proteinα greatly delayed mammary tumorigenesis, decreased mammosphere-forming ability and tumor stroma, a component of the CSC niche. Expression levels of various cytokines, including IGF1 and CXCL12, stemness- and stroma-inducing cytokines, respectively, were reduced in proteinα-mutant mammospheres, pre-cancerous mammary and tumor tissues. NFκB which regulates expression of IGF1 and CXCL12 was inactivated in proteinα-mutant mammary tissues. In vivo, transplanted CSCs of proteinα couldn't grow without proteinα in mammary tissues. Thus the mechanisms to maintain progenitor cell-state by prevention of excess ERK activity may permit production of various cytokines by activated NFκB to create CSC niche, and allow growth of CSCs. These results suggest that treatment with MEK inhibitors may allow survival of CSCs, raising the risk of recurrence. We provide a rationale of combination therapy targeting both proteinα and MEK inhibitors to eradicate tumors.

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Advanced Clinical Research Center

Division of Cellular Therapy
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Our major projects are (1) Co-ordinate control of cell division and differentiation by a crosstalk between JAK/STAT and small GTPases, (2) Molecular targeted therapies, and (3) Elucidation of molecular basis of leukemia, hematological malignancioes.

1. Co-ordinate control of cell division and cell differentiation of by the Rho family small GTPases.

Takeshi Fukushima, Yosuke Tanaka, Toshihiko Oki, Toshiyuki Kawashima, Kohtaro Nishimura, Susumu Goyama, and Toshio Kitamura

In search for key molecules that prevent murine M1 leukemic cells from undergoing IL-6-induced differentiation into macrophages, we previously isolated an antisense cDNA that encodes full-length mouse MgcRacGAP through functional cloning. In human HL-60 leukemic cells, overexpression of the human MgcRacGAP induced differentiation to macrophage. Interestingly, MgcRacGAP localized to the nucleus in interphase, accumulated to the mitotic spindle in metaphase, and was condensed in the midbody during cytokinesis. Moreover, the GAP activity of MgcRacGAP was required for completion of cytokinesis. We also found that MgcRacGAP is phosphorylated by Aurora B at the midbody. Intriguingly, this phosphorylation induced the Rho-GAP activity of MgcRacGAP, which was critical for completion of cytokinesis. We identified S387 as a phosphorylation site responsible for the acquirement of Rho-GAP activity during cytokinesis at the midbody. On the other hand, MgcRacGAP mainly localizes in the nucleus in the interphase. We demonstrated that MgcRacGAP directly bound transcription factors STAT3 and STAT5, and enhanced transcriptional activation of STAT proteins as a Rac GAP. MgcRacGAP was found to harbor functional NLS and works as a nuclear chaperon together with Rac1.

We found using an MgcRacGAP-GFP fusion protein that MgcRacGAP expression increased in the early G1 phase in parallel with or even earlier than Geminin, suggesting that MgcRacGAP may play roles in G1 check point. MgcRacGAP accumulates to the midbody during cytokinesis, and the midbody is included in one of the daughter cells after cell division. It was suggested by some researchers that the midbody is frequently released from the cells in stem cells. We therefore hypothesized that the cells with midbody tend to differentiate and the cells without midbody tend to self-renew or enter G0 phase. To test this hypothesis, we have recently generated a transgenic mouse expressing the MgcRacGAP-mVenus fusion protein in hematopoietic stem cells and/or progenitors.

2. Molecular targeting therapies using small molecule compounds

Akiho Tsuchiya, Reina Nagase, Toshiyuki Kawashima, Yukinori Minoshima, Susumu Goyama, and Toshio Kitamura

STAT3 is frequently activated in many cancers and leukemias, and is required for transformation of NIH3T3 cells. Therefore, we have started searching for STAT3 inhibitors. We established an efficient screening protocol for identification of STAT3 inhibitors. Through the screening of a library of small molecule compounds, we found the compounds RJSI-1 and RJSI-2 that inhibited STAT3 activation. RJSI-2 also inhibited activation of STAT1, STAT5, JAK1 and JAK2. On the other hand, RJSI-1 inhibited nuclear transport of phosphorylated STAT proteins, implicating a novel mechanism in inhibiting STAT proteins. We have also shown that these compounds are effective in a tumor-burden mouse model. In addition, we collaborated with a US-based biotech company in modification of RSJI-1 for optimization to develop anti-cancer drugs, and have developed JP1156 that kills the tumor cells more efficiently both in vitro and in vivo with much lower IC50.

In addition to STAT3 inhibitors, we have recently started a new project to develop STAT5 inhibitors in collaboration with a pharmaceutical company. To this end, we have developed a screening method to search for STAT5 inhibitors. In addition to STAT3/5 inhibitors, we have started several collaborations with several domestic and global pharmaceutical companies to evaluate the efficacies of a variety of molecular targeted therapies in our established mouse MDS/AML/MPN models.

3. Molecular basis of acute leukemia, myelodysplastic syndromes (MDS), MDS overt leukemia, and myeloproliferative neoplasms (MPN).

Daichi Inoue, Reina Nagase, Takeshi Fujino, Yasutaka Hayashi, Shuji Asada, Reina Takeda, Kojin C Kawabata, Naoko Watanabe, Makoto Saika, Yukiko Komeno, Naoko Kato, Yutaka Enomoto, Toshihiko Oki, Yuka Harada[1], Hironori Harada[2], Tetsuya Nosaka[3], Jiro Kitaura[4], Yosuke Tanaka, Tomofusa Fukuyama, Susumu, Goyama, and Toshio Kitamura: [1]Department of Clinical Laboratory Medicine, Bunkyo Gakuin University, Laboratory of Oncology, Department of Medical Science Tokyo University of Pharmacy and Life Sciences, [3]Mie University School of Medicine, and [4]Allergy Center, Juntendo University.

Recent progress using high-speed sequencing has identified mutations in genes that are not categorized to class I and class II mutations, including epigenetic factors, and splicing factors. We have recently established two MDS models induced by ASXL1 mutations and EZH2 mutations; mice transplanted with bone marrow cells expressing C-terminal truncating mutants of ASXL1 or EZH2 derived from MDS patients developed MDS-like diseases in a year or two. Concerning the molecular mechanisms, the ASXL1 mutant (ASXL1-MT) suppressed PRC2 functions, leading to the derepression of posterior HoxA genes and miR125a via inhibition of H 3K27 trimethylation. While expression of posterior HoxAs is known to contribute transformation of hematopoietic cells, miR125a is a well-known oncogenic micro RNA, in particular for hematological malignancies. In addition to known target genes of miR125a, we have identified Clec5a/MDL1. We have also found that Clec5a is required for granulocytic differentiation of 32D cells, implicating its downregulation in the pathogenesis of MDS. ASXL 1 mutations are frequently associated with SETBP1 mutations (SETBP1-MT) that stabilize SETBP1 and SET oncoprotein, leading to activation of the PI3K/Akt pathway. In the BMT model, combination of ASXL1-MT and SETBP1-MT induced AML with much shorter latencies. GSEA indicated that the TGF beta pathway was profoundly inhibited, implying the inhibition of the TGF beta pathway in leukemic transformation of MDS. Further experiment is now under way to clarify the molecular mechanisms by which the TGF beta pathway was inhibited.

We have recently established Rosa26-knock-in mice for ASXL1-MT and the EZH2 mutan. These KI mice did not develop MDS in a year, but presented disturbed differentiation of erythroid cells and mild macrocytic anemia.

4. Molecular pathogenesis of AML1-ETO and MLL-fusion leukemias

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Using human and mouse models for AML1-ETO and MLL-fusion leukemias, we have elucidated new molecular aspects in pathogenesis and progression of acute myeloid leukemia (AML). AML1-ETO leukemia is the most common cytogenetic subtype of acute myeloid leukemia, defined by the presence of t(8;21). In addition to the full-length of *AML1-ETO* that contains AML1 exons 1-5 and ETO exons 2-11, several alternatively spliced isoforms of the *AML1-ETO* transcript have been identified in t (8;21) patients. Among them, *AML1-ETO9a* encodes a C-terminally truncated AML1-ETO protein, and has been shown to possess strong leukemogenic potential in a mouse retroviral transduction-transplan-

tation model. However, AML1-ETO9a is barely expressed in patients' blasts or cell lines with t(8;21) which raises question if AML1-ETO9a takes important role in the clinical setting. We have identified another splicing variant that seems abundant at least in cell lines with t(8;21) and has significant ability to induce leukemia in a mouse model. Mechanisms of leukemogenesis by the newly identified alternative splicing variant is currently under investigation.

MLL-fusion leukemia is an aggressive form of leukemia carrying chimeric fusion of the *MLL* gene. We previously showed that the combined loss of *Runx1*/*Cbfb* inhibited the development of MLL-AF9-induced leukemia. However, c-Kit+/Gr-1-cells remained viable in Runx1/Cbfb-deleted cells, indicating that suppressing the RUNX activity may not eradicate the most immature LSCs. We found upregulation of several hemostasis-related genes, including the thrombin-activatable receptor PAR-1 (protease-activated receptor-1), in Runx1/Cbfb-deleted MLL-AF9 cells. Similar to the effect of Runx1/Cbfb deletion, PAR-1 overexpression induced CDKN1A/p21 expression and attenuated proliferation in MLL-AF9 cells. To our surprise, PAR-1 deficiency also prevented leukemia development induced by a small number of MLL-AF9 leukemia stem cells (LSCs) in vivo. PAR-1 deficiency also reduced leukemogenicity of AML1-ETO-induced leukemia. Re-expression of PAR-1 in PAR-1-deficient cells combined with a limiting-dilution transplantation assay demonstrated the cell-dose-dependent role of PAR-1 in MLL-AF9 leukemia: PAR-1 inhibited rapid leukemic proliferation when there were a large number of LSCs, while a small number of LSCs required PAR-1 for their efficient growth. Mechanistically, PAR-1 increased the adherence properties of MLL-AF9 cells and promoted their engraftment to bone marrow. Taken together, these data revealed a multifaceted role for PAR-1 in leukemogenesis, and highlight this receptor as a potential target to eradicate primitive LSCs in AML.

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Advanced Clinical Research Center

Division of Infectious Disease
感染症分野

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Our overall goal is medical sciences on infectious diseases in two directions, from clinic to bench and from bench to clinic. Our current main subject is immunopathogenesis of HIV-1 infection. We are focusing on how cellular immune responses fight against to HIV-1 and how immune system is disrupted and develops AIDS. We are also working on viral pathogenesis in HIV-infected patients. We work together with the staffs in the Department of Infectious Diseases and Applied Immunology in the IMSUT hospital and apply the research results to the people living with HIV-1/AIDS. We are extending our research project to other viral diseases including viral hepatitis and associated morbidities.

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

Michiko Koga, Tomohiko Koibuchi[1], Eisuke Adachi[1], Tadashi Kikuchi, Hiroshi Yotsuyanagi, Yasumasa Iwatani[2], Kazuhisa Yoshimura[3]: [1]Department of Infectious Diseases and Applied Immunology, IMSUT hospital, IMSUT, [2]Clinical Research Center, National Nagoya Medical Center, [3]Nathional institute of infectious diseases

Progress in antiretroviral treatment has led to fewer virological failure cases, but 10%-20% 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%, ranging from 5.2% in 2004 to 11.8% in 2010. The prevalence of RS was significantly higher among cases who were male, Japanese, and men who have sex with men. Common mutations in both groups were M46I/L and T215 revertants. Furthermore, sequences with these mutations, K103N and D30N/N 88D formed clusters on phylogenetic trees. It was suggested that HIV with these mutations have become circulating strains.

2. Short Intracellular HIV-1 Transcripts as Biomarkers of Residual Immune Activation in Patients on Antiretroviral Therapy.

Aya Ishizaka[1], Hidenori Sato[2], Michiko Koga, Tadashi Kikuchi, Eisuke Adachi[3], Tomohiko Koibuchi[3], Ai Kawana-Tachikawa[2], A, Mizutani T[1]: [1]Institute of Microbial Chemistry (BIKAKEN), [2]AIDS Research Center, National Institute of Infectious Diseases, [3]Department of Infectious Diseases and Applied Immunology, IMSUT hospital, IMSUT.

HIV-1 patients continue to remain at an abnormal immune status despite prolonged combination antiretroviral therapy (cART), which results in an increased risk of non-AIDS-related diseases. Given the growing recognition of the importance of un-

derstanding and controlling the residual virus in patients, additional virological markers to monitor infected cells are required. However, viral replication in circulating cells is much poorer than that in lymph nodes, which results in the absence of markers to distinguish these cells from uninfected cells in the blood. In this study, we identified prematurely terminated short HIV-1 transcripts (STs) in peripheral blood mononuclear cells (PBMCs) as an efficient intracellular biomarker to monitor viral activation and immune status in patients with cART-mediated full viral suppression in plasma. STs were detected in PBMCs obtained from both treated and untreated patients. ST levels in untreated patients generally increased with disease progression and decreased after treatment initiation. However, some patients exhibited sustained high levels of ST and low CD4(+) cell counts despite full viral suppression by treatment. The levels of STs strongly reflected chronic immune activation defined by coexpression of HLA-DR and CD38 on CD8(+) T cells, rather than circulating proviral load. These observations represent evidence for a relationship between viral persistence and host immune activation, which in turn results in the suboptimal increase in CD4(+) cells despite suppressive antiretroviral therapy. This cell-based measurement of viral persistence contributes to an improved understanding of the dynamics of viral persistence in cART patients and will guide therapeutic approaches targeting viral reservoirs.

3. Identification of two unique naturally occurring Vpr sequence polymorphisms associated with clinical parameters in HIV-1 chronic infection.

Doreen Kamori[1], Ai Kawana-Tachikawa[2], Michiko Koga, Tadashi Kikuchi, Eisuke Adachi[3], Tomohiko Koibuchi[3], Takamasa Ueno[1]: [1]Kumamoto University, [2]AIDS Research Center, National Institute of Infectious Diseases, [3]Department of Infectious Diseases and Applied Immunology, IMSUT hospital, IMSUT.

HIV-1 viral protein R (Vpr) plays important roles in HIV-1 replication. Despite the identification of a number of HLA class I-associated immune escape mutations; it is yet known whether immune-driven Vpr polymorphisms are associated with disease outcome. Hereby, we comprehensively analyzed Vpr sequence polymorphisms and their association with disease outcome and host HLA genotypes, by using plasma viral RNA isolated from 444 HLA-typed, treatment-naïve, chronically HIV-1 infected individuals. Vpr amino acid residues at positions 13, 37, 45, 55, 63, 77, 84, 85, 86, and 93 were significantly associated with patients' plasma viral load and/or CD4 count. Further analysis revealed Ala at position 55 was significantly associated with lower plasma viral load; and Thr at position 63 was significantly associated with lower plasma viral load and higher CD4 count. Also, the number of amino acid residues at the two positions, located in a functionally important α-helical domain, correlated inversely with plasma viral load and positively with CD4 count. Moreover, a phylogenetically corrected method revealed residues at positions 55 and 63 are associated with patients' HLA genotypes. Taken together, our results suggest that Vpr polymorphisms at functionally important and immune-reactive sites may contribute, at least in part, to viral replication and disease outcome in vivo.

4. Delineation of autoantibody repertoire through differential proteogenomics in hepatitis C virus-induced cryoglobulinemia.

Masato Ogushi[1], Hiroshi Yotsuyanagi[1,2], Kyoji Moriya[3], Kazuhiko Koike[4]: [1]Department of Infectious Diseases, Graduate School of Medicine, The University of Tokyo, [2]Department of Infectious Diseases and Applied Immunology, IMSUT hospital, IMSUT, [3]Department of Infectious Control and Prevention, Graduate School of Medicine, The University of Tokyo, [4]Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo.

Antibodies cross-reactive to pathogens and autoantigens are considered pivotal in both infection control and accompanying autoimmunity. However, the pathogenic roles of autoantibodies largely remain elusive without a priori knowledge of disease-specific autoantigens. Here, through a novel quantitative proteogenomics approach, we demonstrated a successful identification of immunoglobulin variable heavy chain (VH) sequences highly enriched in pathological immune complex from clinical specimens obtained from a patient with hepatitis C virus-induced cryoglobulinemia (HCV-CG). Reconstructed single-domain antibodies were reactive to both HCV antigens and potentially liver-derived human proteins. Moreover, over the course of antiviral therapy, a substantial "de-evolution" of a distinct sub-repertoire was discovered, to which proteomically identified cryoprecipitation-prone autoantibodies belonged. This sub-repertoire was characterized by IGHJ6*03-derived, long, hydrophobic complementarity determining region (CDR-H3). This study provides a proof-of-concept of de novo mining of autoantibodies and corresponding autoantigen candidates in a disease-specific context in human, thus facilitating future reverse-translational research for the discovery of novel biomarkers and the development of antigen-specific immunotherapy against various autoantibody-related disorders.

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Advanced Clinical Research Center

Division of Bioengineering
臓器細胞工学分野

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Our division has been conducting basic research projects for development of innovative cancer therapy using immunologic and gene therapy approaches. The reagents, modalities, and concepts developed in this division have been clinically applied as translational research projects. We believe that bidirectional information exchange between the bench and the bedside would be one of the most important requirements for the successful development of novel and effective therapies.

I. Clinical development of anti-programmed death 1 (PD-1) Ab in melanoma patients

Hideaki Tahara

Check-point blockades, which block the regulatory pathways of CTL activation with antagonistic antibodies to promote immunological responses, have been shown to be effective for various types of cancer in the clinical trials. Among them, anti-PD-1 antibodies have been particularly drawing attention of the oncologists.

Nivolumab (ONO-4538/BMS-936558/MDX-1106) is a fully human monoclonal IgG4 antibody (HuMAb) against PD-1 which has high affinity for PD-1 (Kd 2.6 nM) and block cross-linkage to both PD-L 1 (B7-H1) and L2. Based on good safety profiles and promising anti-tumor effects in phase I trial for recurrent solid tumor patients, we initiated phase II study of nivolumab as a pharmaceutical-supported trial to treat melanoma patients in Japan. The results of such trial have shown the significant anti-tumor effects and manageable side-effects, and nivolumab has become the first government-approved drug in the world as a PD-1 related drug. We are now analyzing the immunological parameters to further develop this powerful agent.

II. Augmentation of immune checkpoint blockade therapy with IL-18

Zhifeng Ma[1,2], Hideaki Tahara, and Haruki Okamura[1]: [1]Laboratory of Tumor Immunology and Immunotherapy, [2]Department of Orthopaedic Surgery, Hyogo College of Medicine, Nishinomiya, Hyogo, 663-8501, Japan.

Immune responses that lead to anti-tumor effects require both activation of effector cells and reduction of inhibitory elements of the immune system. Although animal models and recent clinical trials demonstrated that immune checkpoint blockade enhanced effector cell responses and tumor rejection, further development and improvement of cancer immunotherapy is necessary to achieve more favorable objective responses. In this study, we examined the effect of IL-18 on the treatment of peritoneal dissemination of CT-26 mouse colon carcinoma cell line harnessing anti-programmed death ligand-1 (PD-L1) monoclonal antibody (mAb) (αPD-L1) and/or anti-cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) mAb (αCTLA-4). The treatment of mice with αPD-L1 and/or αCTLA-4 significantly enhanced their survival. However, when combined with IL-18, αPD-L1 and/or αCTLA-4 provided much greater therapeutic benefits. IL-18 allowed ac-

cumulation of precursor of mature natural killer (pre-mNK) cells in the peritoneal cavity, when injected with these check-point inhibitors. The results strongly suggested that IL-18 promoted therapeutic effects of immune checkpoint blockade against peritoneal dissemination of carcinoma through accumulation of pre-mNK cells, memory-type CD8+ T cells, and deletion of CD4+CD25+ T cells. Our study provides a basis for cancer immunotherapy that would involve combination of cytokines activating innate immunity and immune checkpoint blockade.

III. Development of cancer immunotherapy using the blockade of MFG-E8

Yu Mizote, Mika Uematsu-Hamada, Hiroaki Uchida, Hideaki Tahara

The secreted protein, milk fat globule-EGF factor 8 (MFG-E8), stimulates disease progression through coordinated αvβ3 integrin signaling in tumor and host cells. MFG-E8 enhances tumor cell survival, invasion, and angiogenesis, and contributes to local immune suppression.

We have shown that systemic MFG-E8 blockade cooperates with cytotoxic chemotherapy, molecularly targeted therapy, and radiation therapy to induce destruction of various types of established mouse tumors. The combination treatments evoke extensive tumor cell apoptosis that is coupled to efficient dendritic cell cross-presentation of dying tumor cells. Our previous findings suggest that systemic MFG-E8 blockade might intensify the antitumor activities of existing therapeutic regimens through coordinated cell-autonomous and immune-mediated mechanisms also in human. In order to apply these findings to treat cancer patients, we have developed antibodies specific to the human MFG-E8. These antibodies include the one with blocking activity on MFG-E8 functions and the one suitable for immune-staining of human tissue. We are currently investigating the human situations related to MFG-E8 and have found that strong expression of MFG-E8 in the tumor cells has significant impact on the survival of certain types of cancer patients (manuscript in preparation). Furthermore, we are now in the process of developing this agent for clinical application.

IV. Development of novel gene and cell therapy against cancer via T-cell immune checkpoint blockade.

Yoshihiro Hayakawa, Hideo Yagita, Hideaki Tahara

We have reported for anti-tumor effects and mechanisms of IL-23, which is a cytokine secreted by dendritic cells, and have been trying to develop novel and effective cancer immunotherapy. Recently, we have been focused on T-cell suppressing pathway of immune responses against cancer including CTLA-4, PD-1, and TIM-3. These immune checkpoints have been blocked using antagonistic antibodies against them to enhance the anti-tumor immune response of gene therapy using cytokines with or without dendritic cell administration. At the same time, the mechanisms of such combination therapies have been investigated.

V. IL-17-producing NK1.1$^-$ CD27$^-$ γδT cells promote tumor malignant progression by inducing inflammatory microenvironment.

Yoshihiro Hayakawa[3], Yoshitaka Kimura[4], Hideaki Tahara: [3]Institute natural Medicine, University of Toyama, [4]The University of Tokyo.

Inflammatory microenvironment is an essential component of tumors and important for carcinogenesis and metastasis of tumor cells, however, the precise details of inflammatory immune responses to promote tumor malignant progression are still unclear. To characterize such tumor-promoting inflammatory immune responses, we employ a unique in vivo model in which low tumorigenic cell line QR-32 acquires high malignant phenotype after exposure to host inflammatory responses induced by an inflammation initiator. By using this model, we investigated the role of inflammatory cytokines IL-17 and IFNγ in tumor malignant progression process. We demonstrated that IL-17 and IFNγ played positive and negative roles, respectively, in the malignant progression of tumor cells and IL-17 played a predominant role in this process. Adoptive transfer of inflammatory cells from wild-type mice into IL-17-deficient mice recovered in vivo progression of QR-32 cells and the exact source of IL-17 within such inflammatory cells was determined as NK1.1$^-$ CD27$^-$ γδT cells. Furthermore, CD 11b$^+$ Ly-6G$^+$ neutrophils infiltrated into the inflammatory site primed by IL-17-producing NK1.1$^-$ CD 27$^-$ γδT cells in the presence of QR-32 and IL-17 played an important role for maintaining such tumor-associated inflammatory microenvironment. Collectively, our data clearly implicate that the inflammatory tumor microenvironment triggered by IL-17-producing NK1.1$^-$ CD27$^-$ γδT cells is important for tumor malignant progression. We are now further characterizing γδT cells in the inflammatory microenvironment promoting tumor malignant progression and exploring the components for downstream inflammatory immune responses triggered by IL-17.

VI. Treatment of malignant pleural mesothelioma using replication-defective recombinant adenoviral vector expressing the suppressor of cytokine signaling 3 (SOCS3). (Manufacture of the viral vector for preclinical studies in non-human primates)

Tetsuji Naka[5], Hiroyuki Mizuguchi[6], Takafumi Nakamura[7], Hisako Katano[8], Hiroaki Uchida, Takuma Suzuki, Hideaki Tahara: [5]Laboratory for Immune Signal, National Institute of Biomedical Innovation, Osaka, Japan [6]Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University, [7]Tottori University, 8University of Tokyo.

In collaboration with the research team, we have prepared the replication-defective recombinant adenoviral vector expressing the suppressor of cytokine signaling 3 (SOCS3), AdSOCS3 for treatment of malignant pleural mesothelioma. We have supported the vector production using Vector Facility in IMSUT utilizing the master and working cell banks of 293 cells, which we established previously. The purified final products have been used for preclinical study in monkey. We are also carrying out the safety and biodistribution studies for AdSOCS3 in the context of intrapleural or intravenous administration in a mouse model. Based on the results of these studies, we are in the phase of preparing the phase I study for the patients with malignant pleural mesothelioma using this strategy.

VII. Development of fully retargeted herpes simplex virus (HSV) vectors for oncolytic virotherapy

Hiroaki Uchida, Yu Okubo, Tomoko Shibata, Takuma Suzuki, Hitomi Ikeda, Shiori Inuzuka, Yukinari Kato[9], Hideaki Tahara: [9]Department of Regional Innovation, Tohoku University Graduate School of Medicine

Herpes simplex virus (HSV) vectors are promising agents for oncolytic virotherapy. Uchida established a fully retargeted HSV platform that mediates virus entry exclusively via tumor-associated antigens in the lab of Prof. Joseph Glorioso at the University of Pittsburgh. Entry of HSV is initiated by the binding of glycoprotein D (gD) to one of its receptors, herpesvirus entry mediator (HVEM) or nectin-1. This interaction results in a conformational change in gD, triggering sequential activation of gH and gB to execute fusion between the viral envelope and cell membranes. We inserted single-chain antibodies (scFv) against a number of different cell surface molecules such as epidermal growth factor receptor (EGFR), carcinoembryonic antigen (CEA), and epithelial cell adhesion molecule (EpCAM), into the retargeted HSV platform that encodes a gD ablated for binding to natural receptors and a gB containing entry-enhancing mutations we previously identified. As a result, we observed specific virus entry into cells expressing the cognate target antigen for each of the retargeted constructs. Our results indicate the adaptability of our system to different targeting ligands, leading to a new generation of broadly applicable and effective oncolytic HSV vectors. Furthermore, we introduced syncytial mutations into the gB and/or gK genes of gD-retargeted HSVs and found that gD retargeting does not abolish the hyperfusogenic activity of syncytial mutations and that these mutations do not eliminate the dependence of HSV entry and spread on a specific gD-receptor interaction. These observations suggest that syncytial mutations may be valuable for increasing the tumor-specific spreading of retargeted oncolytic HSV vectors.

VIII. Establishment of highly functional monoclonal antibodies through novel screening methods for targeted cancer therapy

Hiroaki Uchida, Miki Yamaguchi[10], Hitomi Ikeda, Yu Okubo, Hideaki Tahara: [10]Department of Molecular Medicine, Research Institute for Frontier Medicine, Sapporo Medical University School of Medicine

Monoclonal antibodies (mAbs) have become an established therapeutic modality in clinical oncology. In order to identify cell-surface molecules that may be useful for targeting various types of cancers, our group established a unique screening approach that employs an adenoviral vector harboring fiber proteins engineered to bind antibodies, Adv-FZ33. This approach led to the successful identification of an array of potential target molecules for cancer treatment. Immunotoxins (antibody-drug conjugates; ADC) are a promising class of cancer therapeutics composed of a cytotoxic agent linked covalently to a cancer-targeted antibody. To systematically hunt for cell-surface molecules that may be efficiently targeted by immunotoxins, our group created another method for screening highly functional cancer-targeted mAbs and cognate antigens. The receptor-binding domain of the Diphtheria toxin (DT) was replaced with the antibody-binding domain (3C) derived from the Streptococcal protein G. The resultant mutated toxin protein (DT-3C) was used for selection of mAbs for specific cell killing activity as components of immunotoxins. Our novel screening system is advantageous in that the selected antibodies bind to intact cancer cells and get internalized efficiently, which has been critically required for therapeutic applications but elusive thus far. Furthermore, we have successfully taken advantage of some of these in-house monoclonal anti-

bodies for development of novel fully retargeted HSV vectors.

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Advanced Clinical Research Center

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

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Research Projects
We have been working on the following five projects, 1) development of novel therapeutic strategies of human cancer, 2) discovery of molecular targeted anti-cancer drugs through a screening of large-scale chemical libraries, 3) establishment and investigation of mouse models of human cancer, 4) elucidation of genetic characteristics of human tumors and mechanisms of their development, and 5) clinical sequencing for the implementation of genomic medicine. These projects are aimed to develop strategies for better diagnosis, effective treatment, and prevention of human cancer.

1. Identification of novel molecular targets for the treatment of human cancers

Kiyoshi Yamaguchi, Yoichi Furukawa, Rui Yamaguchi[1], Seiya Imoto[2], and Satoru Miyano[1]: [1]Laboratory of DNA Information Analysis, Human Genome Center, [2]Division of Health Medical Data Science, Health Intelligence Center, IMSUT.

Epigenetic modifications such as DNA methylation and histone modification change gene expression with global dynamics of chromatin structure. Accumulated evidence has demonstrated that aberrant epigenetic modifications are implicated in carcinogenesis. Bromodomain has been known as a protein interaction module that recognizes acetylated lysine residues within histone and non-histone proteins. Through this interaction, protein containing a bromodomain(s) conducts 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. Integrative analysis of large-scale gene expression and ChIP-seq data identified a set of genes that were directly regulated by BRD8. Functional analysis of these target genes will give us a clue to understand the biological role of BRD8 in cancer cells. Since bromodomain proteins have emerged as druggable targets, BRD8 may also be an effective target for the treatment of colorectal cancer.

2. Cancer drug discovery through a large chemical library screening

Kiyoshi Yamaguchi, Yoichi Furukawa.

Establishment of well-designed high-throughput screening (HTS) system is an essential for the identification of small molecules that inhibit a signaling pathway or a molecule of interest. We recently developed a cell-based reporter assay system, named as a bidirectional dual reporter assay, to screen effectively and specifically small molecules that affect the transcriptional activity of the target molecule or signal transduction pathway. Applying this assay system, we performed an HTS of Wnt inhibitors using a chemical library containing 20,000 compounds. As a result, we have identified several can-

didate small molecules. We are now trying to determine the mechanism of their action using approaches of chemical biology. In addition, we are expanding our screening of chemical libraries with the help of Drug Discovery Initiative in the University of Tokyo, the National Institute of Advanced Industrial Science and Technology, and the Institute of Microbial Chemistry.

3. Establishment and investigation of novel mouse models of human cancer

Tsuneo Ikenoue, Yoichi Furukawa.

Genetically engineered mice are useful tools for studying human diseases, including cancer. In this project, we have successfully established a mouse model of intrahepatic cholangiocarcinoma (ICC) by liver-specific *Kras* activation and *Pten* deletion. Using a lineage tracing system, we have demonstrated that ICCs originate from cholangiocytes, but not from hepatocytes in this model. We are now studying the molecular mechanisms how *Kras* activation and *Pten* deletion induce ICC.

We have also generated a mouse strain carrying a conditional knockin allele of the *Fbxw*7 gene, which is frequently mutated in human colon and bile duct cancer. Phenotypes of the knockin mice are now under investigation. Intensive analysis of these models should provide better understanding of their carcinogenesis and facilitate the development of new therapies to these cancers.

4. Elucidation of genetic characteristics of human tumors and mechanisms of their development

Rei Noguchi, Kiyoshi Yamaguchi, Tsuneo Ikenoue, Yoichi Furukawa.

Pseudomyxoma peritonei (PMP) is a rare disorder, and characterized by the accumulation of abundant mucinous or gelatinous fluid that is produced from tumourous cells disseminated in the abdominal cavity and pelvis. We previously analyzed 18 PMPs containing 10 low-grade tumors and 8 high-grade tumors using targeted gene sequencing panels. These data suggested that *KRAS* and/or *GNAS* mutations are common genetic features of PMP, and that mutations in *TP53* and/or genes related to the PI3K-AKT pathway may render malignant properties to PMP. To comprehensively understand genetic alterations in PMP, we further applied whole genome sequencing and RNA sequencing to the DNA/RNA of the PMP tumors and matched normal colonic mucosa. Integrative analysis of whole genome and transcriptome data will provide the better understanding of tumor characteristics, and facilitate the development of personalized medicine for PMP.

5. Clinical sequencing for the implementation of genomic medicine

Kiyoshi Yamaguchi, Tsuneo Ikenoue, Yoichi Furukawa, Mitsuhiro Komura[1], Eigo Shimizu[1], Rui Yamaguchi[1], Tetsuo Shibuya[2], Satoru Miyano[1,2], Takanori Hasegawa[3], Seiya Imoto[3], Masayuki Kobayashi[4], Kazuaki Yokoyama[4], Arinobu Tojyo[4], Koichiro Yuji[5]: [1]Laboratory of DNA Information Analysis, [2]Laboratory of Sequence Analysis, Human Genome Center, [3]Division of Health Medical Data Science, Health Intelligence Center, [4]Division of Molecular Therapy, [5]Division of International Advanced Medical Research, Advanced Clinical Research Center.

Next-generation sequencing (NGS) has enabled us to analyze the comprehensive human genome, and facilitated the identification of germline changes responsible for hereditary diseases and somatic alterations in human neoplasms. In collaboration with Human Genome Center, Health Intelligence Center, and Advanced Clinical Research Center, we have been working on the following projects; 1) the determination of germline mutations in patients suspected of hereditary colon tumor, 2) application of a cognitive computing system, namely IBM Watson Genomic Analytics (WGA), for the personalized medicine. These projects are aimed to use the information of personal genome and/or cancer genome in clinic, and apply the data for their diagnosis and treatment.

In the first project, we applied NGS technology for patients with multiple adenomatous polyps in the colon. In the patients, we previously failed to identify pathological mutations within the 5' two-thirds region of the *APC* gene by Sanger sequencing. However, NGS successfully identified pathological mutations in three of the patients; two were somatic mosaic mutations of *APC*, and the other was a very rare mutation in the 3' terminal region of *APC*. In addition, whole genome sequencing identified a promoter deletion of ~10 kb encompassing promoter 1B and exon1B of the *APC* gene. These data have corroborated the usefulness of NGS in clinical diagnosis of cancer.

In the second project, we generated a pipeline to apply genomic data to IBM WGA. After written informed consent was obtained from the patients with pseudomyxoma peritonei (PMP), they were enrolled in this study. Genetic alterations in their tumor were determined by NGS and the data were subsequently analyzed by WGA. The results of WGA including predicted driver mutations and suggestion of actionable drugs were discussed in Tumor Board meeting of this project, which is held 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	医学博士	藤　堂　具　紀
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Project Associate Professor	Minoru Tanaka, M.D., Ph.D.	特任准教授	医学博士	田　中　　実
Senior Assistant Professor	Hiroyuki Momota, M.D., Ph.D.	講　師	医学博士	百　田　洋　之

The major research topic of our laboratory is to develop oncolytic virus therapies for various malignant tumors. Oncolytic viruses are designed so that they can infect, replicate selectively within, and destroy tumor cells. G47Δ, a recombinant, triple-mutated oncolytic herpes simplex virus type 1 (HSV-1), exhibits potent anti-tumor efficacy while maintaining safety. Two clinical trials using G47Δ are currently being conducted at IMSUT Hospital.

Creation of novel recombinant oncolytic HSV-1

The use of genetically-engineered oncolytic viruses is a novel therapeutic strategy for cancer. Various kinds of virus have been studied worldwide as oncolytic viruses, but genetically engineered HSV-1 is particularly useful because of following favorable characteristics: (1) It shows little toxicity to normal tissues, and there exist theoretical backgrounds for tumor cell selectivity. (2) The viral genome is stable. (3) It can efficiently infect wide range of tumor types and exhibits a potent oncolytic activity. (4) Cell-to-cell spread is minimally affected by circulating antiviral antibodies. (5) Inflammatory reactions to the virus are generally mild and repeated administrations are possible. (6) There are antiviral drugs available to terminate viral replication when undesired events occur. (7) Antitumor immune responses are elicited in the course of oncolytic activities by the virus. (8) The large size of HSV-1 genome (～152kb) allows insertion of large or multiple foreign genes.

Conventional homologous recombination techniques had required time-consuming processes to create new recombinant oncolytic HSV-1. We have established an innovative recombinant HSV-1 construction system using bacterial artificial chromosome and two sets of recombinases (Cre/loxP and FLP/FRT). This system allows rapid generation of multiple new recombinant HSV-1 with desired sequences inserted into a specific locus.

Application of oclolytic HSV-1 for malignant glioma is a major study interest in our laboratory. In addition, *in vitro* and *in vivo* tumor models of other cancers, including renal cancer, prostate cancer, bladder cancer, malignant mesothelioma, tongue cancer, esophageal cancer, colon cancer, lung cancer, breast cancer, nasopharyngeal cancer, cholangiocarcinoma, hepatic cancer, pancreatic cancer, malignant melanoma, and malignant lymphoma have also been used for testing efficacy and safety.

Studies using glioma-derived cancer stem cells

There exists a small population of tumor-initiating, stem-like cells within the tumor. Because cancer stem-like cells (CSC) are reported to be resistant to current therapies and responsible for recurrence, a novel approach that can eliminate CSCs is needed to cure the disease. We currently use glioma-derived CSCs to study new therapeutic approaches

including oncolytic virus therapy using genetically engineered HSV-1. G47Δ has been shown to kill CSCs efficiently. Novel oncolytic HSV-1 that exhibit high efficacy for tumors rich in CSCs have been created and are being evaluated.

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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　医学博士	長　村　文　孝
Associate Professor	Masanori Nojima, M.D., Ph.D., M.P.H.	准教授　医学博士	野　島　正　寛

Division of Advanced Medicine Promotion was established in 2011. Our mission is to assist the clinical development and the conduct of clinical trials, especially for translational researches. For this purpose, it is critical to discover the new "seeds" and to eradicate many blockades until the clinical utilization. In this sense, our role is the translation from the results of basic science of our Institute to the conduct of clinical trials in the IMSUT Hospital. In IMSUT Hospital, we work together with staffs of Center for Translational Research. Concurrently, for the reduction of blockades during translational researches, we engage in research on Regulatory Science.

1. Assistance of Clinical Trials/TRs at Research Hospital

Masanori Nojima, Fumitaka Nagamura

In Research Hospital, we work together with 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 results of the collaboration between Division of Advanced Medicine Promotion and Center for Translational Research. In 2016, we supported 4 investigator-initiated investigational new drug application (IND) clinical trials and 1 non-IND clinical studies.

2. Scholastic Program for the Graduate Students of Nurses in the Area of Translational Research.

Minako Kouno, Riyo Owada, Fumitaka Nagamura

TR is the early phase of clinical trials, which applied the developments of basic researches for patients with incurable and/or life-threatening diseases. Highly educated nurses are indispensable for the conducts of TRs in terms of the protection of participants in TRs and the conducts of scientifically appropriate TRs. We developed the scholastic program for the graduate students of nurses in the area of TR. We planned and implemented the one-week program to foster the expert research nurse aimed at the graduate students. It consists of the lectures on the feature points of TR (e.g. ethical considerations of TR, and the role of research nurse), role-plays of Institutional Review Board and obtaining Informed Consent, case conference, and the experience of the actual operations. We evaluated the reports and the questionnaires from the students to explore the degree of their understandings and satisfactions for this program. These reports and questionnaires were analyzed. Generally, our program meets the demands of the students, however, the improvement of the content on the experience of the actual operations is the next issue.

3. Management of "Translational Research Network Program" of Japan Agency for Medical Research and Development.

Hiroshi Yasui, Masanori Nojima, Fumitaka Naga-

mura

Ministry of Education, Culture, Sports, Science and Technology launched "Translational Research Network Program" to promote translational researches based on the results of basic science at academia. This grogram 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 early stage of clinical trial. In 2016, we supported 24 basic researches, 17 preclinical studies, and 6 clinical studies. The number of studies we assist has been increasing year by year. Organization reinforcement is the urgent problem.

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

Masanori Nojima

Epigenome and multi-omics research using clinical samples in collaborative study or public database of comprehensive omics-analysis. We are now focusing on the multi-omics approach integrating DNA methylation, mRNA expression, and miRNA, and building statistical models to assess functional linkage.

5. Statistical consulting for basic research

Masanori Nojima

For basic researchers belonging to IMSUT and other institutes, we suggest exploratory statistical approach and molecular epidemiological approach.

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Advanced Clinical Research Center

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

Associate Professor Naoya Kato, M.D., Ph.D.
Project Assistant Professor Ryosuke Muroyama, M.D., Ph.D.

准教授 医学博士 加藤直也
特任助教 医学博士 室山良介

Our major goal is to establish personalized medicine for patients with infectious diseases or cancers, especially those of gastrointestinal and hepatic fields, based on human or pathogenic microbe genome information.

1. Fusion HBx from HBV integrant might alter ER stress response and contribute to hepatocarcinogenesis

Ryosuke Muroyama, Kaku Goto, Yasuo Matsubara, Ryo Nakagawa, Jun Arai, Sayuri Morimoto, Yoshimi Kaise, Sayaka Ito, Naoya Kato

Backgrounds/Objectives: Hepatitis B virus (HBV) is a major risk factor for hepatocellular carcinoma (HCC), and HBV X protein (HBx) has been suggested to be associated with hepatocarcinogenesis. On the other hand, HBV integration is frequently observed in HCC and suggested to contribute to the development of HCC. However, the molecular mechanism of hepatocarcinogenesis by HBV integration remains unclear. In this study, we identified the fusion HBx, the HBx-human fusion protein derived from HBV integrant, in Hep3B cells and investigated its role in hepatocarcinogenesis.

Methods: 1) We identified fusion HBx translated from HBV integrant in Hep3B cells, and established stably HBx knocked-down (KD) cells by siRNA. 2) Using KD cells, we examined the effect of fusion HBx on cell growth and invasion ability in vitro, and tumorigenicity in vivo. 3) We compared anchorage-independent growth ability in soft agar and transactivation ability of wild HBx and fusion HBx. 4) Using microarray, we examined the expression profile of mRNAs and miRNAs in KD cells, and performed gene set enrichment analysis (GSEA) to investigate the signature of fusion HBx.

Results: 1) The fusion HBx consisted of 1-140 amino acids of HBx followed by 61 amino acids from human genome, and its expression was confirmed to disappear in KD cells by immunofluorescence. 2) In KD cells, cell proliferation and invasion ability was significantly reduced compared to the parental cells. Moreover, KD cells could not develop any visible tumor in nude mice while parental cells could. 3) The fusion HBx had anchorage-independent growth ability in soft agar although the fusion HBx completely abrogated its transactivation ability. 4) There were 306 up-regulated and 116 down-regulated genes in KD cells compared to the parental cells. On the contrary, there was no miRNA which was differentially expressed in KD cells. In GSEA, the up-regulated genes in KD cells were significantly enriched in an endoplasmic reticulum (ER) stress response. In further analysis, it was revealed that the fusion HBx dysregulated ER stress response via the modification of ATF3, ATF4, and ATF6 transcription. Interestingly, the effects of the fusion HBx on ER stress signaling pathway was similar to those of C-terminal truncated HBx but significantly different from those of wild HBx.

Conclusions: The fusion HBx from HBV integrant might alter ER stress response and may contribute to hepatocarcinogenesis, and could be an attractive target for the treatment of HBV-induced HCC.

2. Small molecules for MICA modulation

Kaku Goto, Jun Arai, Yasushi Tanoue, Sayaka Ito, Anthony Stephanou, Ryosuke Muroyama, Yasuo Matsubara, Ryo Nakagawa, Sayuri Morimoto, Yoshimi Kaise, Toshio Fujisawa, Lay Lim, Naoya Kato

An immunoactivating ligand MHC class I polypeptide-related sequence A (MICA) was identified to be a genetic susceptibility factor for hepatitis C virus (HCV)-induced hepatocellular carcinoma (HCC) in our genome-wide association study (Kumar et al., Nat Genet 2011). Lower MICA expression level was associated with the elevated risk of HCC in patients, indicating protective effects of MICA against HCC. Towards this end, we aimed to find drugs for restoration of MICA expression, and our new reporter system identified the anti-cancer agent vorinostat (VOR) as the top hit in a screen for an FDA-approved drug library. VOR treatment upregulated the expression of MICA in HCC cells, boosting natural killer (NK) cell-mediated cytotoxicity in coculture and inhibiting tumor growth via NK cells in vivo (Goto et al., Sci Rep 2016). The mode of MICA expression induction and further strategies for pharmacological modulation of MICA are currently investigated. All findings would subserve novel chemoimmunotherapies for management of HCC (Goto et al., J Gastroenterol 2015; Goto et al., Nippon Rinsho 2015; Goto et al., J Hepatol 2017).

3. The AMPK-related kinase SNARK in viral hepatocellular carcinoma

Kaku Goto[1], Raymond T. Chung[2], Naoya Kato[1]: [1]Division of Advanced Genome Medicine, IMSUT; [2]GI Unit, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

Our genome-wide RNAi screen for host cellular cofactors for hepatitis C virus (HCV) replication (Tai et al., Cell Host Microbe 2009) discovered sucrose, non-fermenting 1/AMP-activated protein kinase-related kinase (SNARK). We demonstrated that SNARK supported HCV replication in cell culture and SNARK expression was upregulated by HCV in cell culture and patients. The effects were abrogated by SNARK kinase inhibitor, presenting SNARK as an effective target of therapies against the virus and pathogenesis (Goto et al., J Hepatol 2013). Subsequently we explored an FDA-approved drug library for SNARK kinase inhibitors in vitro, and found the anti-alcoholism drug disulfiram (DSF). Indeed DSF suppressed HCV replication, SNARK-promoted signaling, and hepatocellular carcinoma (HCC) (Goto et al., Oncotarget 2016). Further investigation into molecular properties of the kinase is underway, expectedly clarifying details of hepatitis virus pathogenesis and HCC development for novel biological insights and pharmacotherapeutic options.

4. Ras inhibitors suppress the inflammatory cytokine production in T cells of primary biliary cholangitis

Ryo Nakagawa, Ryosuke Muroyama, Yoshimi Kaise, Kaku Goto, Ito Sayaka, Jun Arai, Lay Ahyong Lim, Yasuo Matsubara, Sayuri Morimoto[1], Naoya Kato

Background/Aim: Primary biliary cholangitis (PBC) is an autoimmune liver disease of unknown pathogenesis. Since CD4+ T cells play a pivotal role in the immunological disorder of PBC, we pursued therapeutic targets by profiling the gene expression in CD4+ T cells of PBC comprehensively using microarray. Then, N-Ras was suggested to regulate the inflammatory cytokine production in CD4+ T cells of PBC. Therefore, we assessed the possibility of N-Ras inhibition as a novel PBC therapy.

Methods: Clinically and pathologically diagnosed 14 PBC patients and 10 healthy controls, who agreed to provide samples with written informed consent, were enrolled in this study. First, N-Ras, IL-2, and IFN-γ expression in CD4+ T cells of PBC patients and healthy controls was measured by qRT-PCR. Then, the correlation between N-Ras and 2 inflammatory cytokines in PBC patients was analyzed by Pearson's correlation coefficient. The cytokine productive function of N-Ras was analyzed by overexpression assay and T cell receptor (TCR) stimulation assay using cultured cells. Moreover, cytokine suppressive effect of 10 Ras inhibitors, ursodeoxycholic acid (UDCA), and obeticholic acid (OCA) were measured by IL-2 ELISA using cultured cell supernatant.

Results: The expression of N-Ras, IL-2, and IFN-γ was significantly upregulated in CD4+ T cells of PBC compared to those of healthy controls ($p < 0.05$). N-Ras and 2 inflammatory cytokines were positively correlated ($p < 0.05$). Then, IL-2 and IFN-γ expression was upregulated in N-Ras overexpressing cells compared to mock control by qRT-PCR ($p < 0.05$). IL-2 production was upregulated in N-Ras overexpressing cells compared to mock control ($p < 0.05$) by ELISA. Moreover, all Ras inhibitors suppressed IL-2 production of cultured cells, however UDCA and OCA didn't suppress IL-2 production of cultured cells ($p < 0.05$).

Conclusion: N-Ras regulated inflammatory cytokine production in CD4+ T cells of PBC. Moreover, Ras inhibitors suppressed inflammatory cytokine production of PBC although drugs of PBC therapy did not. Therefore, Ras inhibitors may become a novel therapeutic strategy for PBC by suppressing inflammatory cytokines production.

5. Novel zinc finger protein in gastrointestinal tract

Yasuo Matsubara[1,2], Kazuaki Takahashi[2], Masahiro Arai[2], Jun Miwa[2], Shunji Mishiro[2], Ryosuke Muroyama[1], Kaku Goto[1], Ryo Nakagawa[1], Jun Arai[1], Sayaka Ito[1], Sayuri Morimoto[1], Yoshimi Kaise[1], Naoya Kato[1]: [1]Division of Advanced Genome Medicine, IMSUT; [2]Department of Medical Science, Toshiba General Hospital

The gastrointestinal tract has definite anatomical and functional boundaries between its contiguous segments. Because some human cancers arise in a background of tissue metaplasia, e.g. Barrett's esophagus and intestinal metaplasia of the stomach, it is important to clarify the molecular and cellular basis of region formation and preservation. Some genetic markers that delimit gastrointestinal boundaries have been reported, but it is still unknown how such boundaries are established and maintained.

We identified novel zinc finger protein in the gastric biopsy specimen by mass spectrometry. Its mRNA sequence and other mRNAs with similar sequences were determined by RACE. The expression vector was constructed and the transfection was performed. Apparent phenotype change was not shown in transfected cultured cells of upper gastrointestinal tract. Knock-down studies using siRNA revealed growth inhibition effect. For further functional analysis, immunoprecipitation will be conducted.

6. Regorafenib is a promising medicine for HCC increasing membrane-bound MICA through ADAM 9 suppression

Jun Arai[1,2], Kaku Goto[1], Sayaka Ito[1], Yoshimi Kaise[1], Sayuri Morimoto[1], Ryo Nakagawa[1], Ryosuke Muroyama[1], Yasuo Matubara[1], Hitoshi Yoshida[2], Naoya Kato[1]: [1]Division of Advanced Genome Medicine, IMSUT; [2]Division of Gastroenterology, Department of Medicine, Showa University School of Medicine

Background and Aims: Regorafenib (REG) was shown to be effective for hepatocellular carcinoma (HCC) patients who got resistance to previous sorafenib (SOR) treatment (Bruix et al. Lancet 2016). In our previous genome-wide association study, MHC class I-related chain A (MICA) was associated with HCC development in chronic hepatitis C (Kumar et al. Nat Genet 2011). Moreover, MICA shedding is thought to be one of the mechanisms of HCC to escape immune surveillance by NK cells. As SOR was shown to increase membrane-bound MICA (mMICA) in HCC cells through decreasing MICA shedding by ADAM9 suppression, we investigated the effect of REG on MICA shedding in HCC cells.

Methods: HepG2 and PLC/PRF/5 cells were used. Soluble MICA (sMICA) and mMICA were measured by ELISA and flow cytometry, respectively. mRNA and protein levels of individual genes were analyzed by qRT-PCR and western blotting, respectively.

Results: REG significantly suppressed ADAM9 mRNA expression at much lower concentrations than those in plasma, surpassing the potency of SOR. ADAM9 knockdown reduced sMICA only to 70%, whereas REG reduced sMICA to 50% at even lower concentrations. Furthermore, REG increased the mMICA level more than SOR.

Conclusion: REG inhibited MICA shedding more than SOR through ADAM9 suppression. This could explain higher potency of REG than SOR, confirming ADAM9 as an attractive target for anti-HCC therapies.

7. IL-13Rα2 as a novel prognostic biomarker for human pancreatic cancer

Toshio Fujisawa[1,2], Yasushi Tanoue[1,3], Kaku Goto[1], Sayaka Ito[1], Ryosuke Muroyama[1], Ryo Nakagawa[1], Yasuo Matsubara[1], Naoya Kato[1]: [1]Division of Advanced Genome Medicine, IMSUT; [2]Department of Gastroenterology, NTT Medical Center Tokyo, Tokyo; [3]Department of Gastroenterology and Hepatology, JCHO Tokyo Takanawa Hospital, Tokyo

Interleukin-13 Receptor α2 (IL-13Rα2) is known as a cancer testis antigen and a potential target for cancer immunotherapy. IL-13Rα2 is overexpressed in about 70% pancreatic cancer samples and involved in cancer invasion and metastasis. We investigated a possible correlation between IL-13Rα2 expression in pancreatic cancer and patient prognosis.

Totally, 236 samples of surgically resected pancreatic cancer tissue (adenocarcinoma) were obtained from two different institutions. Samples were immunohistochemically stained for IL-13Rα2 expression and its intensity was evaluated by total 4 investigators. The expression of IL-13Rα2 was correlated with patient characteristics including prognosis and clinicopathological parameters.

As a result, 63% - 68% of pancreatic cancer samples overexpressed IL-13Rα2. Kaplan-Meier survival analysis revealed that patients with IL-13Rα2 positive pancreatic cancer had significantly lower

survival compared to patients with IL-13Rα2 negative cancer ($P<0.0057$ and 0.0002). On multivariate analysis, only IL-13Rα2 expression and UICC-stages were identified as common prognostic factor at both institutions. Interestingly, tumors that had significant nerve invasion frequently correlated with high IL-13Rα2 expression. IL-13Rα2 could be a prognostic biomarker of surgically resected pancreatic cancer patients. Because IL-13Rα2 expression is related with invasion to nerve, immunotherapy targeting IL-13Rα2 might not only prolong patient survival but also suppress symptoms by nerve invasion including cancer pain.

Samples used in this study were all Japanese. Therefore, we performed additional analysis using TCGA data from US national cancer institute. TCGA data revealed that the level of IL-13Rα2 mRNA expression was low, and not correlated with survival rate. The possible explanation of this contradictory results may be due to differences in tumor samples from totally different geographic population. The other more likely reason may be due to technology differences used between two data sets e.g., IHC and RNA-seq

These results in this study were summarized into the manuscript and being submitted to the journal now.

8. Development of anti-metastasis treatment targeting IL-13Rα2 for pancreatic cancer

Toshio Fujisawa[1,2], Kaku Goto[1], Ryosuke Muroyama[1], Naoya Kato[1]; [1]Division of Advanced Genome Medicine, IMSUT; [2]Department of Gastroenterology, NTT Medical Center Tokyo, Tokyo

Pancreatic cancer is an aggressive disease with only limited therapeutic options available. Interleukin-13 receptor α2 chain (IL-13Rα2), which is a high-affinity receptor for IL-13, is overexpressed in a variety of human solid cancers including pancreatic cancer. We have previously reported that histone acetylation was a key mechanism to control IL-13Rα2 expression in pancreatic cancer cells, and histone deacetylation (HDAC) inhibitors upregulated IL-13Rα2 expression and enhanced the efficacy of immunotoxin targeting IL-13Rα2. On the other hand, IL-13Rα2 helps invasion and metastasis of pancreatic cancer through activation of extracellular signal-regulated kinase 1/2 and activator protein-1 (AP-1) nuclear factors. We, thereupon, hypothesized that histone acetyltransferase (HAT) inhibitors, which oppositely works from HDAC inhibitors, and AP-1 inhibitors decrease IL-13Rα2 expression and metastasis of pancreatic cancer. Last year, we examined IL-13Rα2 expression in pancreatic cancer cell lines and picked up two cell lines, HS766T and MIA-PaCa2, because of their aggressive metastasis. And we took notice of three HAT inhibitors for inhibiting IL-13Rα2 expression. This year, we examined the efficacy of these drugs for inhibiting IL-13Rα2 expression in the pancreatic cancer cells. Epigallocatechin gallate showed the strongest effect for IL-13Rα2 inhibition and it was promising to prevent pancreatic cancer metastasis. Continuously, the possibility of the drugs for inhibition of cancer invasion and metastasis was examined. All three drugs showed a certain measure of inhibiting effects for cancer invasion in vitro. As well as IL-13Rα2 inhibition, Epigallocatechin gallate most strongly prevent pancreatic cancer invasion and metastasis. The pilot animal study revealed that epigallocatechin gallate inhibited cancer invasion and metastasis. However, the treatment decreased animal appetite and caused body weight loss. Now we are planning another animal study for confirming the data of the pilot study.

9. Regulation of membrane-bound MICA for hepatocellular carcinoma treatment

Yasushi Tanoue[1,2], Kaku Goto[1], Jun Arai[1], Toshio Fujisawa[1,3], Anthony Stephanou[1], Sayaka Ito[1], Ryosuke Muroyama[1], Yasuo Matsubara[1], Ryo Nakagawa[1], Sayuri Morimoto[1], Yoshimi Kaise[1], Lay Lim[1], Naoya Kato[1]: [1]Division of Advanced Genome Medicine, IMSUT; [2]Department of Gastroenterology and Hepatology, JCHO Tokyo Takanawa Hospital, Tokyo; [3]Department of Gastroenterology, NTT Medical Center Tokyo, Tokyo

Identification of an anti-tumor ligand MHC class I polypeptide-related sequence A (MICA) as a genetic susceptibility factor for HCV-induced hepatocellular carcinoma (HCC) in our genome-wide association study (Kumar et al., Nat Genet 2011) indicated the protective potential of MICA against HCC. Indeed innate immunity to HCC was potentiated by upregulated expression of MICA on cell surfaces (Goto et al., Sci Rep 2016), which was proved to be harnessed for immunotherapy. Multiple sheddases, hitherto, have been reported to cleave membrane-bound MICA (mMICA) abrogating its function, and recognized as pharmacological targets. Here we sought for new MICA sheddase inhibitors, and a screen for an FDA-approved drug library was conducted using our newly established in vitro system for measuring the activity of a proteinase. Several drugs were isolated and validated to inhibit the enzyme; currently their effects on mMICA and immune activity as well as mechanisms are examined. The data would be important for novel HCC immunotherapies.

10. Chemical compounds that bind to HBx inhibit HBV DNA replication

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Background and aims: Multifunctional protein HBx is associated with HBV replication and carcinogenesis. Chemical compounds that suppress the function of HBx by binding to it could elucidate the mechanism of HBx-dependent HBV replication and carcinogenesis. Their modes of action being completely different from that of nucleoside analogues or IFN treatment, they would provide a novel means to treat HBV-related diseases.

Methods: GST-tagged HBx was expressed and purified in E.coli. The library of 1018 FDA-approved drugs was screened for the binding to HBx by surface plasmon resonance analysis. The compounds' effects on carcinogenesis were measured by cell proliferation assay using Hep3B cells that demonstrate HBx-dependent proliferation and Huh7 cells that do not contain integrated HBV DNA. The compounds' effects on HBV replication were measured by the amount of HBV DNA in the culture medium using real-time PCR. In this experiment, HepG2.2.15.7 cells having stable HBV expression were used.

Results: Of the 1018 FDA-approved drugs in the library, 22 were found to bind to HBx, and 6 of them could strongly bind to HBx compared to others. These 6 compounds were further tested and cell proliferation assay revealed that there was no difference in the rate of cell proliferation between Hep3B and Huh7 cell lines. On the other hand, 2 compounds showed more than 50% inhibition of the amount of HBV DNA. One of them showed more than 90% inhibition, almost as good as 95% inhibition of nucleoside analogue entecavir.

Conclusion: Some FDA-approved drugs that bind to HBx were found to inhibit HBV DNA replication. These compounds, working in a different manner than entecavir, could be an attractive option for the treatment of HBV-related diseases.

11. Dysfunctional DENND1B induces T cell activation in PBC.

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Background: Genome wide association studies have revealed 23 susceptibility genes in primary biliary cholangitis (PBC). However, the pathological function of PBC susceptibility genes was still unclarified. Therefore, we comprehensively profiled the expression of PBC susceptibility genes in CD4+ T cells and analyzed those functions using microarray with gene set enrichment analysis (GSEA).

Methods: In this study, we used the microarray data of CD4+ T cells of PBC patients (n=7) and controls (n=7). To elucidate dysfunctional susceptibility genes in CD4+ T cells of PBC, microarray data were analyzed using GSEA and enrichment map analysis. Elucidated PBC susceptibility gene was analyzed in vitro.

Results: Among 23 PBC susceptibility genes, the expression of 13 genes was enriched in CD4+ T cells of PBC by GSEA (rank metric score$>$0.48). DENND1B was the most enriched susceptibility gene among them and was upregulated in PBC compared to control ($P>0.05$). Moreover, GSEA revealed 43 pathways and 17 gene ontology (GO) terms correlating with DENND1B expression. Among DENND1B correlating pathways and GO terms, 6 pathways and 12 GO terms were associated with T cell activation. In DENND1B overexpressing cells, IL-2 production by anti-CD3 antibody was decreased compared to that in parental cells ($P<0.05$).

Conclusions: Microarray study showed DENND1B was associated with T cell activation in PBC. However, DENND1B overexpression suppressed IL-2 production. This may suggest that DENND1B negatively regulates T cell activation, and the dysfunction of DENDD1B or the dysregulation by DENDD 1B is possible to underlie the pathogenesis of PBC.

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The main project of our division is to promote clinical development of novel gene therapy for cancer and chronic intractable diseases. We are currently engaged in clinical development of immuno-gene therapy with chimeric antigen receptor (CAR)-modified T cells for relapsed and refractory hematological malignancies.

1. Immuno-gene therapy with CD19-directed CAR-modified T cells (CD19-CAR-T cells) for adult patients with relapsed and refractory B-precursor acute lymphoblastic leukemia (B-ALL)

Sumimasa Nagai, and Keiya Ozawa

It has been reported that CD19-CAR-T gene therapy is highly effective for relapsed and refractory B cell malignancies, especially B-ALL. In order to develop this novel promising gene therapy in Japan, we are currently preparing Japanese multicenter clinical trial of CD19-CAR-T cell therapy for adult patients with relapsed and refractory B-ALL. This trial will start in 2017. CD19-CAR-T gene therapy for malignant B-cell lymphoma was conducted in two patients at Jichi Medical University Hospital as Phase I/II clinical research.

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