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.

(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 echanism to develop ATL.

(5) Translational research on tissue engineering:
To accomplish this goal, we are focusing on the issues including a) identification and characterization of somatic stem cells, b) search for molecules to affect the growth and differentiation of stem cells, and c) basic and clinical studies on bone tissue engineering.

1. BRAF-V600E mutation on circulating cell-free DNA is a promising biomarker of high-risk adult Langerhans cell histiocytosis.

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Langerhans cell histiocytosis (LCH) is a rare disorder characterized by clonal proliferation of Langerhans cells and significant infiltration of immune cells. Oncogenic BRAF-V600E mutation could be detected in LCH lesions from the majority of patients. Recently, it was found that patinets with active, high-risk LCH carried BRAF-V600E in circulating CD11c+/CD14+ cell fractions. In patients with various kinds of cancers, circulating cell-free DNA (cfDNA) in peripheral blood contains cancer-derived genomic DNA and has been applied to non-invasive diagnostic procedure, so called liquid biopsy. In this study, we evaluated BRAF mutation on cfDNA as a potential biomarker of LCH using allele-specific quantitative polymerase chain reaction (ASQ-PCR). We cloned normal and mutant BRAF alleles that include exon 15 and neighboring sequences into pCR2.1 to make the standard curve. cfDNA was prepared from plasma of adult LCH patients and was subjected to genotyping BRAF alleles by ASQ-PCR, which was specifically designed for detection of BRAF-V600E with a 3'-phosphate-modified oligonucleotide blocker according to Thierry AR, et al. Mutant BRAF load was estimated from the standard curve in each assay and was expressed as the percentage of mutant alleles to total number of alleles. Plasma cfDNA was prepared from 8 adult patients with LCH as well as normal subjects including cancer-free patients. The mean quantity of recovered cfDNA in LCH vs normal was 316.5pg/ml (median, 290.4) vs 92.0pg/ml (median, 91.8). Three high-risk patients with active multiple lesions were positive for BRAF-V600E. In these patients, the mean ratio of mutant BRAF alleles to total was 3.25% (median, 2.59%). Next, we compared the sensitivity of ASQ-PCR of BRAF-V600E between cfDNA and cellular DNA in the same blood sample, and the results suggested that LCH-derived genomes are significantly enriched in cfDNA compared with cellular DNA, and that cfDNA is more adequate for liquid biopsy in LCH with BRAF-V600E. Then, in a BRAF-V600E-positive patient, we followed the mutant BRAF load during the course of initial chemotherapy. The ratio of mutant to total alleles was estimated as 1.00% prior to chemotherapy and not detectable after one course of chemotherapy consisting of vinblastine, prednisolone, methotrexate and 6-mercaptopurine. The validity of this ASQ-PCR data was confirmed by a series of routine imaging analysis performed at the same time. Taken together, ASQ-PCR of BRAF-V600E on cfDNA may contribute to planning of risk-based treatment as well as monitoring of treatment efficacy in LCH, especially in an active, high-risk group. A number of BRAF-targeted inhibitors have been approved or under clinical trial for various cancers with BRAF mutant, and one of those, vemurafenib is also active against LCH with BRAF-V600E (Haroche J, et al. Blood. 2013). Hereafter, the utility of BRAF-V600E in cfDNA should be validated in a large cohort of LCH patients.

2. Impact of Ph+ stem cell burden on clinical findings and molecular responses to first-line nilotinib in newly diagnosed chronic myeloid leukemia: the results from the interim analysis of N-road, a multi-center phase II study.

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We are conducting a phase II study (N-road) for newly diagnosed CML-CP pts, in which nilotinib 300mg BID is given for 24 M and is to be escalated to 400mg BID if no optimal response at any check points. The primary endpoint is CMR rate by 24 M, and secondary endpoints include $MR^{3.0}$/$MR^{4.0}$ by 12 M. In this setting, the impact of initial Ph+ stem cell burden on clinical findings and therapeutic responses has been investigated in a sub-study. By July 2014, 48 pts were enrolled and BM $CD34^+$ cell fractions could be evaluated by FACS-FISH analysis at diagnosis in 43 pts, among those 35 pts passed 3 M, 34 pts 6 M and 15 pts 12 M, respectively. MR3.0 rate was 8/35 at 3M, 23/34 at 6M and 10/15 at 12M. When 43 pts were classified into two groups (higher: H, lower: L) according to the mean $CD34^+$ cell counts at diagnosis (5995/μL of BM aspirates), there were significant differences ($p<0.05$) in BCR-

ABL transcripts indicated as IS (77.66 vs 64.03%, p = 0.030) and WBC count (81.3 vs 22.3 /mL, p = 0.012), but no differences in molecular responses at 3, 6 and 12 M between the two groups. There was a positive correlation between $CD34^+$ cell count and WBC count. The median percentage of Ph+ cells, as measured by FISH, in $CD34^+CD38^-$ fraction at diagnosis was 97.1% compared to 98.6% in $CD34^+$ $CD38^+$ fraction. The proportion of Ph+ cells in $CD34^+CD38^-$ fraction correlated with PLT count but inversely with RBC count, Hb and Ht, respectively. Between the two groups divided by the median percentage, there were significant differences in RBC count (406 vs $460 \times 10^4/\mu L$, p = 0.046), Hb (11.7 vs 14.5 g/dL, p = 0.009) and Hct (38.8 vs 44.7 %, p = 0.040). There were no significant differences in molecular responses at any check points. On the other hand, when divided by the mean percentage (81.1%), there was only a significant difference in PLT count (48.1 vs 27.2 /μL, p = 0.028). Absolute Ph + cell counts in $CD34^+$ $CD38^-$ fraction were estimated in each patient by combining 3 parameters of $CD34^+$ cell counts/μL of BM aspirates, proportion of $CD38^-$ fraction and percentage of Ph+ cells. Ph+ $CD34^+CD38^-$ cell counts siginificantly correlated with WBC count and inversely with RBC count, Hb and percentage of lymphocytes. Between the 2 groups divided by the median cell counts (256/μL), there were significant differences in RBC (406.5 vs $472.0 \times 10^4/\mu L$, p = 0.005), Hb (11.8 vs 14.3 g/dL, p = 0.004), Hct (39.0 vs 44.6%, p = 0.024). Although we could not find significant difference in molecular responses at any check points, patients with lower number of Ph+ $CD34^+CD38^-$ cells tend to achieve $MR^{3.0}$ faster than those with higher number of cells (p = 0.059). When divided by the mean cell counts (578/μL), there was a significant difference in WBC count (94.45 vs 22.44 /mL, p = 0.016). In conclusion, increased Ph+ stem cell burden apparently affects the level of leukocytosis and anemia at diagnosis, but not $MR^{3.0}$/$MR^{4.0}$ rate by 12 M on nilotinib, although it is likely to extend time to achieve $MR^{3.0}$.

3. Thymidine kinase-deleted, let7a-regulated vaccinia virus for a novel oncolytic therapy against multiple myeloma

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Vaccinia virus is a novel tool for a cancer therapy that infects and lyses cancer cells. We found that multiple myeloma shows an exceptionally high susceptibility to vaccinia virus. Because tumor specific infection is the key for a successful oncolytic therapy, we modified thymidine kinase (TK) gene and B5R gene in the vaccinia genome to make it more tumor-specific. Efficacy and safety was determined by the in vitro and mouse in vivo infection models. To confirm that regulation of B5R by let-7a improves myeloma-specific infection, we infected a myeloma cell line RPMI8226 as well as normal skin fibroblasts with the recombinant B5Rgfp-let7a vaccinia virus. *In vitro* infection of a myeloma cell line (RPMI8226) showed significant infectivity even with a very low titer (MOI = 0.1), while human skin fibroblasts were not infected at all. Without the let7 a-regulation, the vaccinia virus infected both myeloma cells and normal cells at the same titer, suggesting that let-7a clearly works. As an *in vivo* infection model, 1×10^7 RPM8226-Rluc cells were injected into immunodeficient mice (CB.17-SCID) subcutaneously. Four weeks later, 1×10^6 pfu of virus (parent; TK-deleted; TK, let7a double regulated) was administered via an intravenous injection, and tumor volume and the amount of virus were determined weekly by the *in vivo* imaging system with renilla and firefly luciferases. Without any regulation, vaccinia virus infected not only myeloma cells but also normal tissues, and mice developed pock lesions in the ear, nose, mouth, foot, and tail, resulting in death within 21 to 24 days. TK-deletion significantly alleviated viral toxicity but still caused death 42days after infection. In contrast, infection with TK and let-7a double regulated vaccinia virus was clearly limited in myeloma, all mice remained alive and the size of myeloma shrunk continuously. These data suggest that the TK-let7a-double regulated vaccinia virus infects and kill myeloma cells specifically, and will be a good candidate for the future clinical application.

4. CADM1 expression and stepwise downregulation of CD7 are closely associated with clonal expansion of HTLV-I-infected cells in adult T-cell leukemia/lymphoma.

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Cell adhesion molecule 1 (CADM1), initially identified as a tumor suppressor gene, has recently been reported to be ectopically expressed in primary adult T-cell leukemia-lymphoma (ATL) cells. We incorporated CADM1 into flow-cytometric

analysis to reveal oncogenic mechanisms in human T-cell lymphotrophic virus type I (HTLV-I) infection by purifying cells from the intermediate stages of ATL development. We isolated CADM1/CD7-expressing peripheral blood mononuclear cells of asymptomatic carriers and ATLs using multicolor flow cytometry. FACS subpopulations were subjected to clonal expansion and gene expression analysis. HTLV-I-infected cells were efficiently enriched in CADM1+ subpopulations (D, $CADM1^+CD7^{dim}$ and N, $CADM1^+CD7^-$). Clonally expanding cells were detected exclusively in these subpopulations in asymptomatic carriers with high proviral load, suggesting that the appearance of D and N could be a surrogate marker of progression from asymptomatic carrier to early ATL. Further disease progression was accompanied by an increase in N with a reciprocal decrease in D, indicating clonal evolution from D to N. The gene expression profiles of D and N in asymptomatic carriers showed similarities to those of indolent ATLs, suggesting that these subpopulations represent premalignant cells. This is further supported by the molecular hallmarks of ATL, that is, drastic downregulation of miR-31 and upregulation of abnormal Helios transcripts. The CADM1 versus CD7 plot accurately reflects disease progression in HTLV-I infection, and CADM1+ cells with downregulated CD7 in asymptomatic carriers have common properties with those in indolent ATLs.

5. Advanced HTLV-1 carriers and early-stage indolent ATLs are indistinguishable based on the CADM1 vs. CD7 plot in flow cytometry

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In $CD4^+$ cells from peripheral blood, $CADM1^-CD7^+$ (P), $CADM1^+CD7^{dim}$ (D) and $CADM1^+CD7^-$ (N) subpopulations are observed. The D and N subpopulations increase as asymptomatic HTLV-1 carriers (ACs) progress to indolent adult T-cell leukemia-lymphoma (ATL), and the N subpopulation then expands in aggressive ATL. In this study we examined whether the analysis can estimate risk of developing ATL in advanced ACs. Peripheral blood samples from ACs (N = 41) and indolent ATL patients (N = 19) were analyzed by flow cytometry using the CADM1 vs. CD7 plot for $CD4^+$ cells and inverse long PCR (clonality analysis) of FACS-sorted subpopulations. Almost all ACs with a high HTLV-1 proviral load (>4 copies/100 cells) had a D + N frequency of >10%. ACs with 25%<D + N≤50% contained expanded clones similar to smoldering-type ATLs. In many patients in the 25%<D + N≤50% group, the proportion of abnormal lymphocytes was distributed around the 5% line which divides ACs and smoldering-type ATL in Shimoyama's classification. In conclusion, the CADM1 vs. CD7 plot is useful for selection of putative high-risk ACs. Some ACs and smoldering ATLs are indistinguishable from each other and should be considered as the same subgroup of HTLV-1 infections.

6. Enforced HoxB4 sustains $CD45^-$c-kit^+ pre-hematopoietic stem cells (HSCs) derived from murine induced-pluripotent stem cells, from which long-term and short-term repopulating HSCs emerge.

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We established iPSCs (GGH-iPSCs) from BMCs of a B6/Ly5.2 GATA2-GFP knock-in mouse (PNAS 103:2202, 2006) and transduced them with a 4-hydroxytamoxyfen (4-HT)-inducible *HoxB4* construct. GGH-iPSCs were induced toward hematopoietic differentiatin under enforced HoxB4, so that almost all the resulting cells expressed c-kit but not CD45 and approximately half of them were highly positive for GFP (GATA2) after 2 months of culture. We also found that these $CD45^-$ cell populations expressed HSC marker genes such as *SCL* and *LMO2*, and that they produced $CD45^+$ blood cells after HoxB4 was switched off. These results suggest that $CD45^-$ pre-HSCs, which can develop LT-HSCs, may be included in HoxB4-sustained iPSC-derived cell populations. Next, according to the GFP (GATA2) expression level, we divided $CD45^-$c-kit^+ cells on HoxB4 into two fractions; $CD45^-$c-kit^+GATA2^{high} and $CD45^-$c-$kit^+GATA2^{low/-}$ cells, and cultured FACS-sorted cells over an OP9 monolayer with cytokines and without 4-HT to switch off HoxB4. Then, $CD45^+$ blood cells emerged from both cell fractions over time, and $CD45^-$c-$kit^+GATA2^{low/-}$ cells produced much more $CD45^+$ cells than $CD45^-$c-kit^+GATA2^{high} cells one month later. Moreover, to test a long-term repopulating activity in mice, each cell fraction was cultured without 4-HT for 4 days after sorting, and then subjected to transplanttion into sublethally-irradiated B6/Ly5.1 recipient mice. Two weeks after transplantation, peripheral blood Ly5.2 donor cells were much more frequent in mice transplanted with $CD45^-$c-$kit^+GATA2^{low/-}$ cells, compared to those with $CD45^-$c-kit^+GATA2^{high} cells, whereas the $CD45^-$c-$kit^+GATA2^{low/-}$ fraction-derived donor cells rapidly decreased and disappeared around 2 months later. In contrast, $CD45^-$c-kit^+GATA2^{high} donor cells could repopulate in recipient mice over 20 weeks after transplantation. These results suggest that $CD45^-$c-$kit^+GATA2^{low/-}$ and $CD45^-$c-kit^+GATA2^{high} cell frac-

tions include short-term (ST) and long-term (LT) -repopulating HSCs, respectively. Taken together, in a hematopoietic differentiation model of murine GGH-iPSCs, long-term repopulating hematopoietic stem cells (LT-HSCs) emerge from $CD45^{-}$c-kit^{+} $GATA2^{high}$ pre-HSC fraction which can be sustained *in vitro* by enforced HoxB4. In this experimental setting, enforced HoxB4 is likely to hinder differentiation from $CD45^{-}$ pre-HSCs to $CD45^{+}$ HSCs.

7. FRS2beta, a feedback inhibitor for EGF receptor/ErbB family, may control malignancy of breast cancer, in association with overexpression of a polycomb protein, Ezh2

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It is well known that the more advanced and malignant cancer, the less chance that treatment will be curative. But the molecular mechanisms underlying such malignant change in cancer tissues are still unclear. In this research, we focused on FRS2beta adaptor protein, a feedback inhibitor for EGF receptor/ErbB family. We generated knockout mice of FRS2beta and crossed them with MMTV-ErbB2 mice in which overexpression of ErbB2. Tumor growth in the breast tissues of wild-type mice was much faster than those of the FRS2beta mutant mice. All the FRS2beta mutant mice survived longer than wild-type mice. Sphere forming activity of breast cancer cells of FRS2beta mutant mice was reduced. This result suggests that FRS2beta is important for maintenance of breast cancer stem cells. In order to comprehensively analyze changes in gene expression patterns under the influence of FRS2β, we conducted DNA microarray analysis by using spheres derived from breast cancer cells of wild-type mice and FRS2beta mutant mice. Gene set enrichment analysis (GSEA) revealed that a polycomb group protein Ezh2 pathway is among the top list of enriched pathways in wild-type breast cancer cells compared with those of mutant cancer cells. This result suggests that FRS2beta plays important roles for epigenetic regulation of breast cancer cells, leading to malignant changes of breast cancer.

8. HER2/3-PI3K-IGF2-ID1 circuit addiction as a fundamental mechanism for stabilization of stemness of breast cancer cells in their niche

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Many breast cancer patients suffer from relapse that is potentially due to cancer stem cells that are not eliminated by treatment. Not only cancer stem cells do not easily disappear, but also cancer cells appear to have plasticity by which they even acquire cancer stem cell properties. However, it is largely unknown how cancer stemness is maintained. Here we have shown the fundamental mechanisms to stabilize cancer stemness. Since the HER2/3-PI3K-NF-kappaB pathway is important for cancer stemness, we examined expression of downstream molecules in this pathway by comprehensive analysis of gene expression profiles over time after addition of the HER3 ligand heregulin (HRG). Insulin-like growth factor 2 (IGF2) was identified as a key downstream molecule, since anti-IGF2 antibody treatment blocked tumor sphere formation, a characteristic of stemness, even under conditions where other growth factors/cytokines were present. IGF2-PI3K signaling induced tumor sphere formation and enhanced the expression of genes favoring stemness, including the transcription regulator *ID1* and *IGF2* itself. Consistent with these data, ID1 and the IGF2 receptor IGF-1R were expressed at high levels in cancer stem cell population of the breast cancer patient-derived xenographt(s) (PDXs). Moreover, *ID1* knockdown suppressed IGF2-induced expression of *IGF2*. Thus HER2/3-PI3K-NF-κappaB signaling may trigger IGF2-PI3K-ID1-IGF2 positive feedback circuits and PI3K-mediated feed forward circuits by which cancer stem cells are addicted to stabilize stemness.

9. Clinical study on bone tissue engineering

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Atrophic maxilla or mandible are major obstacles for dental implant therapy. For example, severe periodontitis, which is a leading cause of tooth loss in the elderly, accompanied by significant bone absorption, makes dental implant therapy very difficult if not impossible to perform. Furthermore, alveolar bone regeneration is also required to improve the functional and esthetic aspects of treatment outcome. Although use of dental implants is already an established clinical procedure, there are a large number of patients without adequate bone

volume for placement of dental implants. For patients with severe atrophy of alveolar bone, autologous bone grafts from iliac bone, tibial bone, or mandible have been performed. However, these destructive procedures may not be feasible for all patients. Even when the amount of harvested bone is small, the procedure is inevitably accompanied by swelling and pain at the donor site. Although bioartificial bone substitutes have been frequently used, even with biological materials such as demineralized freeze-dried allografts or xenogeneic bone substitutes, the ability to induce bone regeneration is considered less efficient than native bone. Thus, the application is limited. We are carrying out a clinical study of alveolar bone tissue engineering for dental implant therapy using bone marrow stromal cells (BMSCs), with a goal of eventual commercialization. The study has been approved by the institutional committee and by the Minister of Health, Labour and Welfare of Japan and currently in progress.

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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 therapy targeting signal transduction pathways, (3) Characterization of a PIR (paired Ig receptors) family (LMIR/MAIR/CLM) and (4) 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.

Kohtaro Nishimura, Toshihiko Oki, Toshiyuki Kawashima, Yukinori Minoshima, Ying Chun Bao, Tomonori Hatori, Yasushi Nomura, Noriko Takahashi, Takaya Satoh[1], and Toshio Kitamura: [1]Osaka City University.

In search for key molecules that prevent murine M1 leukemic cells from undergoing IL-6-induced differentiation into macrophages, we 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 Geminin, suggesting that MgcRacGAP may play roles in G1 check point. In addition, we have recently identified that APCCDH1 targets MgcRacGAP for destruction by ubiquitination. In summary, our results indicate that MgcRacGAP plays distinct roles depending on the cell cycle thereby co-ordinating control of cell division and determination of cell fate, implicating multiple levels of regulation of MgcRacGAP including phosphorylation and ubiquitination in distinct biological roles in different cell cycles.

2. Molecular therapy targeting signal transduction pathways using small molecule compounds

Akiho Tsuchiya, Toshiyuki Kawashima, Yukinori Minoshima, 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 AndroScience in modification of RSJI-1 for optimization to develop anti-cancer drugs, and have developed JP1156 that kills the tumor cells with much lower IC50. In addition, JP1156 is effective in inhibiting growth of various tumor cell lines in mouse tumor-burden models, and we are now searching for a partner company to support phase studies of this compound. In addition to TAT3 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.

3. Identification and characterization of a novel family of paired Ig (immunoglobulin-like) receptors LMIRs.

Kumi Izawa, Akie Maehara, Masamichi Isobe, Toshihiro Matsukawa, Masahiro Sugiuchi, Ayako Kaitani, Mariko Takahashi, Yoshinori Yamanishi, Hideaki Nakajima[2], Toshio Kitamura, and Jiro Kitaura: [2]Keio University School of Medicine

We originally identified and characterized two mouse cDNAs from a mouse bone marrow-derived mast cell cDNA library. They encoded type I transmembrane proteins including a single variable immunoglobulin (Ig) motif in the extracellular domain with about 90% identity of amino acids. LMIR1 contains immunoreceptor tyrosine-based inhibition motif (ITIM) in the intracellular domain, while LMIR2 harbors a short cytoplasmic tail associating with immunoreceptor tyrosine-based activation motif (ITAM)-bearing molecules such as DAP12. In addition to LMIR1/2, related genes were identified by homology search in the close proximity on the same chromosome 11: LMIR3 is an inhibitory type receptor like LMIR1, and LMIR4-8 are activation type receptors like LMIR2. It is of note that LMIR3 has a unique property to associate with FcRγ and thereby functions as an activating receptor in concert with TLR4 stimulation. LMIRs are also called CLMs or MAIRs. Those receptors are mainly expressed in cells involved in innate immunity including mast cells, neutrophils, monocytes, and dendritic cells, indicating that these receptors play important roles in innate immunity.

LMIR5 is a DAP12-coupled activating receptor predominantly expressed in myeloid cells. We have identified T cell Ig mucin 1 (TIM1) as a possible ligand for LMIR5 by retrovirus-mediated expression cloning. TIM1 interacted only with LMIR5 among the LMIR family, whereas LMIR5 interacted with TIM4 as well as TIM1. Stimulation with TIM1 or TIM4 induced LMIR5-mediated activation of mast cells. Notably, LMIR5 deficiency suppressed TIM1-Fc-induced recruitment of neutrophils in the dorsal air pouch, and LMIR5 deficiency attenuated neutrophil accumulation in a model of ischemia/reperfusion injury in the kidneys in which TIM1 expression is up-regulated. In that model, LMIR5 deficiency resulted in ameliorated tubular necrosis and cast formation in the acute phase. Collectively, our results indicate that TIM1 is an endogenous ligand for LMIR5 and that the TIM1-LMIR5 interaction plays a physiological role in immune regulation by myeloid cells.

We have recently identified ceramides as ligands for LMIR3, and demonstrated that LMIR3 plays critical roles in inhibiting allergic response caused by mast cells using LMIR3 knockout mice. Our results suggest that ceramides present in the skin attenuate the activation of mast cells when they are activated by IgE and antigens. We have also identified sphingomyelin as another ligand for human LMIR3/CD300f, also inhibiting IgE-mediated mast cell activation. In addition to allergy, ceramide-LMIR3 binding suppresses experimental colitis by inhibiting ATP-mediated activation of colonic mast cells.

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

Daichi Inoue, Kojin C Kawabata, Reina Nagase, Sayuri Horikawa, Naoko Watanabe, Yukiko Komeno, Naoko Kato, Yutaka Enomoto, Toshihiko Oki, Hideaki Nakajima[2], Yuka Harada[3], Hironori Harada[3], Tetsuya Nosaka[4], Jiro Kitaura, and Toshio Kitamura: [3]Department of Hematology/Oncology, Juntendo University, and [4]Mie University School of Medicine.

To elucidate the molecular mechanisms of acute

leukemia, MDS, and MPD, we established mouse models using bone marrow transplant (BMT); we transduced mouse bone marrow cells with mutant genes derived from patients, including MLL-fusions, BCR-Abl, Runx1 and C/EBPa mutants, using retroviruses. Using this mouse BMT model, we have shown; 1) Combination of class I and class II mutations lead to development of acute leukemia; 2) A class II mutation (Runx1 mutations) induced an MDS-like disease, and overexpression of Evi1 or Bmi1 induced leukemic transformation of MDS as class I mutations; 3) Combination of BCR-Abl and Hes1 expression induced CML blast crisis (BC) like disease. Overexpression of Hes1 was observed in 8 of 20 patients with CML-BC but not in patients with CML-chronic phase. In addition, Hes1 is found to induce MMP9 expression, contributing progression of CML; 4) Two distinct mutants of C/EBPa (N-terminal and C-terminal mutants) collaborate with each other in inducing acute leukemia in mouse BMT models, probably working as class I and class II mutations, respectively.

Recent progress using high-speed sequencing has identified mutations in genes that are not categorized to class I and class II mutations. These include mutations of epigenetic factors, splicing factors, and molecules of the Cohesin complex. 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 H3K27 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 including p53, Bak1, Bmf, and Trp53inp1, we have identified Clec5a/MDL1. We have also found that Clec5a is required for differentiation of granulocytes, implicating its downregulation in the pathogenesis of MDS. ASXL1 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.

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Advanced Clinical Research Center

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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.

1. Impact of population-level viral adaptation on HIV-1 virulence.

Jiro Katoh, Ai Kawana-Tachikawa, Akihisa Shimizu, Dayong Zhu,Chungyong Han, Hitomi Nakamura[1], Michiko Koga, Tadashi Kikuchi, Eisuke Adachi[2], Tomohiko Koibuchi[2], Yusuke Sato[3], Atsushi Yamagata[3], Shuya Fukai[3], Yi Shi[4], George F. Gao[4], Zabrina L. Brumme[5,6], Aikichi Iwamoto: [1]Department of Infectious Diseases Control, International Research Center for Infectious Diseases, IMSUT, [2]Department of Infectious Diseases and Applied Immunology, IMSUT hospital, IMSUT, [3]Structural Biology Laboratory, Life Science Division, Synchrotron Radiation Research Organization and Institute of Molecular and Cellular Biosciences, The University of Tokyo, [4]CAS Key Laboratory for Pathogenic Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Science, Beijing, China, [5]Faculty of Health Sciences, Simon Fraser University, Burnaby, BC, Canada. [6]British Columbia Centre for Excellence in HIV/AIDS, Vancouver, BC, Canada.

Highly polymorphic human leukocyte antigen (HLA) class I (HLA-I) molecules present human immunodeficiency virus type 1 (HIV-1)-derived peptide epitopes on the surface of infected cells, targeting these for elimination by Cytotoxic T lymphocytes (CTLs) that recognize specific peptide-HLA complexes. Mutational escape from HLA-restricted CTL is a major force driving within-host HIV-1 evolution. By extension, HIV-1 sequences circulating in a given host population exhibit adaptations that reflect the HLA-I allele distributions of that population. Intuitively, higher frequencies of HIV-1 CTL escape mutations in circulation should result in their more frequent transmission−thus compromising host antiviral cellular immunity to the incoming viral strain. However the impact of ongoing viral adaptation to its host population on HIV-1 disease progression has yet to be conclusively demonstrated. Here we present evidence that acquisition of HIV-1 harboring a key escape mutation restricted by HLA-A*24:02, the most common HLA-A allele in Japan, accelerates disease progression in HLA-A*24:02-expressing individuals. Our finding supports population-level HIV-1 adaptation as a driver of enhanced viral virulence, and underscores ongoing viral adaptation as a major challenge for

HIV-1 vaccines, particularly those tailored to host populations with limited HLA diversity.

2. Anti-APOBEC3G activity of HIV-1 Vif protein is attenuated in elite controllers

Tadashi Kikuchi, Yukie Iwabu[1], Ai Kawana-Tachikawa, Michiko Koga, Noriaki Hosoya[2], Shigeru Nomura, Zabrina L. Brumme, Heiko Jessen[3], Florencia Pereyra[4], Alicja Trocha[4], Bruce D. Walker[4], Aikichi Iwamoto, Kenzo Tokunaga[1], Toshiyuki Miura: [1]National Institute of Infectious Diseases, [2]Department of Infectious Diseases Control, International Research Center for Infectious Diseases, IMSUT, [3]Jessen Praxis, Berilin, Germany, [4]MIT and Harvard, Ragon Institute of MGH, Charlestown, United States.

HIV-1 Elite Controllers (EC) are rare individuals who are able to control plasma viremia to undetectable levels without antiretroviral therapy. Understanding the pathogenesis and mechanisms underpinning this rare phenotype may provide important insights for HIV vaccine design. The EC phenotype is associated with beneficial host immunogenetic factors (such as HLA-B*57) as well as attenuated viral protein functions (e.g. Gag, Pol and Nef). In this study, we demonstrate that HIV-1 Vif sequences isolated from EC display relative impairments in their ability to counteract the host restriction factor APOBEC3G compared to Vif sequences from normal progressors and acutely-infected individuals. This result extends the growing body of evidence demonstrating attenuated HIV-1 protein function in EC, and in particular supports the relevance of viral factors in contributing to this rare HIV-1 phenotype.

3. Identification of frequently targeted viral epitopes in Japanese population for T cell therapy against opportunistic infections.

Ai Kawana-Tachikawa, Toshiaki Ono[1], Yuriko Fujita[2], Yukie Tanaka[2], Tomohiro Morio[1], Satoshi Takahashi[2]: [1]Department of Pediatrics and Developmental Biology, Tokyo Medical and Dental University Graduate School of Medical and Dental sciences, [2]Division of Molecular Therapy, IMSUT

Although substantial progress has been made in allogeneic hematopoietic stem cell transplantation (HSCT) in the last decade, severe viral diseases, such as CMV, EBV, and AdV infection, remain causes of morbidity and mortality in immunosuppressed patients after allogeneic HSCT. Immunotherapy to restore virus-specific immunity are considered to be an attractive strategy against viral diseases, and recent clinical trials showed the safety and effectiveness of adoptive transfer of virus specific T cells against viral diseases. We are developing multivirus-specific T cell culture system using overlapping peptides (OLPs) covering the sequence of viral proteins for clinical application. To assess how broad T cell responses are expanded in the culture and identify the frequently targeted regions of each viral protein in Japanese population, we established an efficient epitope mapping system for 6 viral proteins from CMV, EBV, and AdV with a peptide matrix-based approach. We found broad T cell responses were induced in our T cell culture system. We also found robust T cell responses in healthy Japanese subjects against OLPs in which no epitope has been reported.

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Our division has been conducting basic research projects related to the cancer and transplantation immunology. The reagents, modalities, and concepts developed in this division have been clinically applied as translational research projects by the clinicians involved in related clinical trials. We believe that bidirectional information exchange between the bench and the bed side would be one of the most important requirements for the successful development of novel and effective therapies.

Development of innovative cancer therapy using immunologic approaches

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

Hideaki Tahara

The progress of the basic immunology research has prompted the development of various types of cancer immunotherapy. Check-point blockades, which block the regulatory pathways of CTL activation with antagonistic antibodies to promote immunological responses, have been recently applied to the clinical trials.

Nivolumab (ONO-4538/BMS-936558/MDX-1106) is a fully human monoclonal IgG4 Antibody (HuMAb) to PD-1 which has high affinity for PD-1 (KD 2.6 nM/L) and block cross-linkage to both PD-L1 (BH-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, nivolumab has become the first government-approved drug in the world as a PD1 related drug.

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

Marimo Sato-Matsushita, Hideaki Tahara

The secreted protein, milk fat globule epidermal growth factor-8 (MFG-E8), stimulates disease progression through coordinated vβ3 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 finding 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).

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

Marimo Sato-Matsushita, 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.

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

Yoshihiro Hayakawa[###], Yoshitaka Kimura[#], Marimo Sato-Matsushita, Hideaki Tahara: [#]The University of Tokyo, [##]Institute natural Medicine, University of Toyama

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, CD11b$^+$ Ly-6G$^+$ neutrophils infiltrated into the inflammatory site primed by IL-17-producing NK1.1$^-$ CD27$^-$ 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.

V. 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[#], Hiroyuki Mizuguchi[##], Takafumi Nakamura[###], Hisako Katano[####], Hideaki Tahara: [#]Laboratory for Immune Signal, National Institute of Biomedical Innovation, Osaka, Japan [##]Laboratory of Biochemistry and Molecular Biology, Graduate School of Pharmaceutical Sciences, Osaka University, [###]Tottori University, [####]University 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) 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. Based on these results, we are now in the phase of preparing the phase I study for the patients with malignant pleura mesothelioma using this strategy.

Publications

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Advanced Clinical Research Center

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

Professor	Yoichi Furukawa M.D., Ph.D.	教　授	医学博士	古　川　洋　一
Associate Professor	Tsuneo Ikenoue M.D., Ph.D.	准教授	医学博士	池　上　恒　雄
Assistant Professor	Kiyoshi Yamaguchi Ph.D.	助　教	薬学博士	山　口　貴世志

We have been working on the following four projects, 1) development of novel therapeutic strategies of human cancer, 2) functional analysis of molecules associated with human cancer, 3) establishment and investigation of mouse models of human cancer, and 4) development of novel diagnostic strategies for hereditary tumors. 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[1,2]: [1]Laboratory of Sequence Analysis, [2]Laboratory of DNA Information Analysis, Human Genome Center, IMSUT

It has become increasingly recognized that aberrant epigenetic modifications play an important role in carcinogenesis. Two protein domain families, bromodomain and chromodomain proteins have been known as recognizing epigenetic imprints on histone tails. Notably, bromodomain proteins preferentially bind to acetylated lysine residues within histone proteins. Through this interaction, bromodomain proteins conduct the assembly of nuclear factor complexes to targeted sites on chromatin, resulting in transcriptional co-activation. We found that bromodomain containing 8 (BRD8) was frequently accumulated in colorectal cancer. Although BRD8 is a component in TRRAP/TIP60-histone acetyltransferase complex, its biological role and function are largely unknown. Therefore, we have investigated the mechanism of BRD8 accumulation and its function in cancer cells. Bromodomain-containing proteins can be attractive therapeutic target for the treatment of cancer.

It is well known that some of the epigenetic modification enzymes including histone methyltransferases, demethylases, and deacetylases are impaired in human carcinogenesis. We have identified SET and MYND domain containing 3 (SMYD3) as a novel therapeutic target for colorectal cancer. Accumulating evidence suggests that SMYD3 catalyzes methylation of histone and non-histone proteins, with implications for human carcinogenesis. Using genome-wide analysis of SMYD3 binding sites based on ChIP-Seq, we identified the candidate genes that were directly regulated by SMYD3. Further analysis should be helpful for the better understanding of human carcinogenesis involving SMYD 3 overexpression.

2. Functional analysis of Smyd3 *in vivo*

Kiyoshi Yamaguchi, Tsuneo Ikenoue, and Yoichi Furukawa

SMYD3 is a histone methyltransferase whose expression levels are enhanced in human colon, liver, and breast cancer. We have revealed that zebrafish Smyd3 plays a crucial role in morphogenesis of

heart and skeletal muscle. To clarify the physiological function of Smyd3 in mammal, we have established Smyd3 knockout mice. Now, the phenotypes of their heart, muscle, liver, as well as intestine are under investigation. We also analyze methylation status of histone tails in Smyd3 knockout mice. To clarify the roles of Smyd3 in colon tumorigenesis, further investigation will be performed by crossing the *Smyd3* conditional knockout mice with colon-specific *Apc* KO mice, which have been used as a model recapitulating human colon tumor.

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

Tsuneo Ikenoue and Yoichi Furukawa:

Genetically engineered mice are useful tools for studying human diseases, including cancer. In this project, we have successfully established mouse model of intrahepatic cholangiocarcinoma (ICC) by liver-specific Kras activation and Pten deletion. To investigate the origin of the tumor cells in this model, we have taken advantage of lineage tracing system using tamoxifen-inducible Cre mice and Rosa-GFP reporter mice. Furthermore, we have investigated the roles of Notch signaling pathway in the development of the ICC in this mouse model.

We have also generated a mouse strain carrying a conditional knockin allele of the *Fbxw7* gene, which is frequently mutated in human colon and liver cancer. Using this strain, we are now trying to establish novel mouse models of these tumors. Intensive investigation of these mice should provide better understanding of their carcinogenesis and facilitate the development of new therapies to these cancers.

4. Genetic diagnosis using next generation sequencer

Kiyoshi Yamaguchi, Tsuneo Ikenoue, Yoichi Furukawa, Seiya Imoto[1], Mitsuhiro Komura[1], Eigo Shimizu[1], Shinichi Kasuya[1], Rui Yamaguchi[2], Tetsuo Shibuya[2], and Satoru Miyano[1,2]: [1]Laboratory of DNA Information Analysis, [2]Laboratory of Sequence Analysis, Human Genome 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, we have been working on the following projects; 1) the determination of germline mutations in patients suspected for hereditary colon tumor, and 2) identification of somatic mutations in hematopoietic malignancies and solid tumors. These projects are aimed to return the data of personal genome and/or cancer genome to the patients in IMSUT Hospital, and apply the data for their diagnosis and treatment.

In the first project, we carried out whole genome sequencing of three patients who were diagnosed as familial polyposis of the colon (FAP). Since most of FAP cases are caused by a germline mutation in the *APC* gene, the three patients underwent genetic testing of *APC*. However, no pathogenic mutations were detected within the two-thirds region of the *APC* gene by conventional sequence analysis using the Sanger method. Therefore, we performed whole genome sequencing of the three patients. Consequently, we successfully identified three different types of pathogenic mutations in the patients. One of the three was a mosaic mutation of *APC* in a patient who suffered from mild type colonic polyposis without a family history. Another was a very rare mutation in the 3' terminal region of *APC* in an FAP patient with multiple desmoids. The third mutation was a structural variation lacking the promoter region of *APC.* The three mutations are difficult to identify by genetic testing using the conventional sequencing method. These data have corroborated the usefulness of NGS in clinical practice.

In the second project, we analyzed genetic alterations in Japanese biliary tract cancer and pseudomyxoma peritonei of the colon (PMP) using multiplex PCR-based targeted enrichment and NGS. We have identified different mutation profiles between low-grade and high-grade PMP. The data may give us important information for the development of personalized approaches to the cancer treatment.

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Advanced Clinical Research Center

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

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Assistant Professor	Motokazu Ito, M.D., Ph.D.	助　教	医学博士	伊　藤　元　一

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 a 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, cholangiocarcinoma, 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

Division of Advanced Medicine Promotion was established in 2011. Our mission is to assist the development 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 utilization. In this sense, our role is the translation from the results of basic science of our Institute to the conduct of clinical trials at the Research Hospital. At Research 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

Noriko Fujiwara, Minako Kouno, Makiko Karasawa, 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 2014, we supported 4 investigator-initiated investigational new drug application (IND) clinical trials and 2 non-IND clinical studies.

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

Noriko Fujiwara, Makiko Karasawa, 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 Ministry of Education, Culture, Sports, Science and Technology.

Makiko Karasawa, Hiroshi Yasui, Fumitaka Nagamura

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 program is expected to support TRs from basic science to seek obtaining intellectual property to early stage of clinical trial. In 2014, we supported 13 basic researches, 10 preclinical studies, and 5 clinical studies. The number of studies we assist has been increasing year by year. Organization reinforcement is the urgent problem.

Publications

Ohashi K, Nagamura-Inoue T, Nagamura F, Tojo A, Miyamura K, Mori T, Kurokawa M, Taniguchi S, Ishikawa J, Morishima Y, Atsuta Y, and Sakamaki H. Effect of graft sources on allogeneic hematopoietic stem cell transplantation outcome in adults with chronic myeloid leukemia in the era of tyrosine kinase inhibitors: a Japanese Society of Hematopoietic Cell Transplantation retrospective analysis. Int. J. Hematol., 100: 296-306 (2014)

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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. HBV induces an HBV-induced HCC associated gene MICA through transcriptional activation in SNPs dependent manner

Ryosuke Muroyama, Kaku Goto, Wenwen Li, Yasuo Matsubara, Ryo Nakagawa, Sayaka Ito, Naoya Kato

Backgrounds/Objectives: Hepatitis B virus (HBV) infection is one of the major factors for developing hepatocellular carcinoma (HCC). Interestingly, the clinical course after exposure to HBV considerably varies between individuals, and host factors such as single nucleotide polymorphisms (SNPs) might affect the clinical outcome of patients with HBV infection. Previously, we reported the SNP (rs2596542) located in the promoter region of MHC class I polypeptide-related chain A (MICA) was significantly associated with the risk of HBV-induced HCC and also with serum levels of soluble MICA. MICA is a ligand of the NKG2D receptor, and plays an important role in eliminating transformed cells as well as virus infected cells by activating mainly innate immune cells such as NK cells. In this study, we investigated the effects of SNPs and HBV replication/proteins on MICA expression from the point of view of microRNA-mediated regulation and transcriptional activity.

Methods: 1) The 3′-UTR (174 bp) and promoter (approximately 5.5 kb) sequences of MICA from HLE cells (G allele at rs2596542) and Huh7 cells (A allele) were subcloned into reporter vectors. We transfected them into Huh7 cells and compared the effect of SNPs on microRNA-mediated regulation and transcriptional activity of MICA by luciferase assay. 2) We cotransfected HBV replicating plasmid into Huh7 cells with reporter vectors, and investigated the effect of HBV replication on MICA expression by luciferase assay. 3) We constructed vectors expressing each HBV protein (LHBs, Precore, Core, HBx, and Pol), and examined the effects on transcriptional activity of MICA by luciferase assay.

Results: 1) There were 7 and 24 different alleles in the 3′-UTR and promoter sequence of MICA respectively. Although 3′-UTR SNPs didn't affect the regulation of MICA by microRNAs, the promoter sequence with G allele at rs2596542 exhibited 2-3 folds higher transcriptional activity than that with A allele. 2) Although HBV replication didn't affect microRNA-mediated regulation of MICA, transcriptional activity of MICA was enhanced by HBV replication. Interestingly, transcriptional activity in the promoter sequence with G allele at rs2596542 was more enhanced than that with A allele. 3) The expression of HBV proteins except Pol enhanced transcriptional activity of MICA.

Conclusions: SNPs in the promoter region of MICA and HBV replication/proteins affect MICA expression level through altering transcriptional activity. These results may partially explain the diversity of clinical course of patients with HBV infection.

2. Fusion HBx translated from HBV integrant is a responsible molecule for hepatocarcinogenesis and could be a universal treatment target

Ryosuke Muroyama, Kaku Goto, Wenwen Li, Yasuo Matsubara, Ryo Nakagawa, Sayaka Ito, Naoya Kato

Backgrounds/Objectives: Epidemiological studies have demonstrated that chronic infection with hepatitis B virus (HBV) is a major risk factor associated with hepatocellular carcinoma (HCC), and HBV X protein (HBx) has been suggested to play an important role in hepatocarcinogenesis. However, HBV asymptomatic carriers expressing a large amount of HBx rarely develop HCC. In this study, we identified fusion HBx (3′-trancated HBx + human peptides) from HBV integrant in human hepatoma cell line, and investigated its role in hepatocarcinogenesis.

Methods: 1) We could identified fusion HBx translated from HBV integrant in Hep3B cells, which consisted of 3′-trancated HBx following 61 amino acids translated from human sequences, 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 by MTT assay and matrigel invasion assay. 3) We injected KD cells subcutaneously into nude mice, and monitored the tumor growth. 4) We constructed the vectors expressing wild HBx and fusion HBx, and compared anchorage-independent growth ability and transactivation ability by soft agar assay and luciferase assay, respectively. 5) We examined the expression change of genes and miRNAs in KD cells using microarray.

Results: 1) Using real-time PCR and Northern blot analysis, we confirmed nearly 90% reduction of fusion mRNA expression in KD cells, and fusion HBx was disappeared in KD cells by immunofluorescence. 2) In KD cells, cell proliferation and invasion ability was reduced. 3) KD cells could not develop any visible tumor in nude mice when we injected KD cells subcutaneously into nude mice although Hep3B cells could. 4) Although fusion HBx had significantly decreased transactivation ability compared to wild HBx, only fusion HBx had anchorage-independent growth ability in soft agar whereas wild HBx did not. 5) In microarray analysis, 331 genes were significantly up-regulated and 124 genes were down-regulated in KD cells by more than two folds. On the other hand, there was no miRNA which has such an expression change. The profiling using Gene Ontology (GO) showed that 113 genes among differentially expressed in KD cells were associated with cell cycle, cell death and cell motility.

Conclusions: Not HBx but fusion HBx translated from HBV integrant played an important role in hepatocarcinogenesis. Fusion HBx could be a universal treatment target for HBV-related HCC.

3. Small molecules for MICA regulation

Kaku Goto[1], Masahisa Jinushi[2], Ryosuke Muroyama[1], Wenwen Li[1], Ryo Nakagawa[1], Sayaka Ito[1], Yasuo Matsubara[1], Naoya Kato[1]: [1]Division of Advanced Genome Medicine, IMSUT; [2]Research Center for Infection-Associated Cancer, Institute for Genetic Medicine, Hokkaido University

An anti-tumor ligand MHC class I polypeptide-related sequence A (MICA) was identified to be a genetic susceptibility factor for HCV-induced hepatocellular carcinoma (HCC) in our genome-wide association study (Kumar V *et al.,* Nat Genet 2011). Lower levels of MICA expression were associated with the enhanced risk of HCC development in patients therein, and preventive effects of MICA expression on hepatocarcinogenesis were indicated. We therefore sought to find drugs for regulation of MICA expression. Our stable cell clone harboring an active luciferase reporter encoding MICA promoter detected an anti-cancer agent as a signal inducer in a screen for an FDA-approved drug library. The compound in actuality upregulated the expression of MICA in hepatoma cells, and accordingly natural killer (NK) cell-mediated cytotoxicity was enhanced in coculture. The mode of MICA expression induction by the approved drug and further possibilities of pharmacological regulation of MICA are currently investigated. Findings here are expected to lead to development of anti-tumor immunotherapies in HCC (Goto K *et al.,* J Gastroenterol 2014).

4. AMPK-related kinase SNARK in viral hepatitis and HCC

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Our genome-wide RNAi screen for host cellular cofactors for hepatitis C virus (HCV) replication (Tai AW et al., Cell Host Microbe 2009) identified that sucrose, non-fermenting 1/AMP-activated protein kinase-related kinase (SNARK) positively regulated HCV replication. We therefore sought to clar-

ify the mechanisms of reciprocal regulation between SNARK and HCV. Knockdown and overexpression of SNARK revealed that SNARK supported HCV replication by its phosphorylation and phosphotransferase activity. Conversely, SNARK expression level was upregulated by HCV infection in patients and cell culture, interfering with intracellular signalings. These biological effects on both virus and host were cancelled by SNARK kinase inhibitor, raising SNARK as an effective target of therapies against the virus and pathogenesis (Goto K et al., J Hepatol 2013). Our investigation into substrates, interacting partners, and signalings targeted by the kinase is underway, clarifying involvement of SNARK in critical factors for hepatitis virus pathogenesis and HCC development. Also pharmacological regulation of the kinase activity and the targets above are being examined.

5. The Characteristic Changes in Hepatitis B virus X region for Hepatocellular Carcinoma: A Comprehensive Analysis Based on Global Data

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Mutations in hepatitis B virus (HBV) X region (HBx) play important roles in hepatocarcinogenesis while the results remain controversial. We sought to clarify potential hepatocellular carcinoma (HCC) characteristic mutations in HBx from HBV genotype C infected patients and the distribution of those mutations in different disease phases and genotypes. HBx sequences downloaded from an online global HBV database were screened and then classified into Non-HCC or HCC group by diagnosis information. Data of patient age, gender, country or area, and viral genotype were also extracted. Logistic regression was performed to evaluate the effects of mutations on HCC risk. We found that 1) Full length HBx sequences (HCC: 161; Non-HCC: 954) originated from 1115 human sera across 29 countries/areas were extracted from the downloaded 5956 HBx sequences. Genotype C occupied 40.6% of Non-HCC (387/954) and 89.4% of HCC (144/161). 2) Sixteen nucleotide positions showed significantly different distributions between genotype C HCC and Non-HCC groups. 3) Logistic regression showed that mutations A1383C (OR: 2.32, 95% CI: 1.34-4.01), R1479C/T (1.96, 1.05-3.64; 5.15, 2.53-10.48), C1485T (2.40, 1.41-4.08), C1631T (4.09, 1.41-11.85), C1653T (2.58, 1.59-4.19), G1719T (2.11, 1.19-3.73), and T1800C (23.59, 2.25-247.65) were independent risk factors for genotype C HBV-related HCC, presenting different trends among individual disease phases. 4) Several genotype C HCC risk mutations pre-existed, even as major types, in early disease phases with other genotypes. Mutations associated with HCC risk were mainly located in HBx transactivation domain, viral promoter, protein/miRNA binding sites, and the area for immune epitopes. Furthermore, the signatures of these mutations were unique to disease phases leading to HCC, suggesting molecular counteractions between the virus and host during hepatocarcinogenesis.

6. Novel drug screening system based on the essential role of HBx trans-activation activity in HBV replication

Wenwen Li, Kaku Goto, Yasuo Matsubara, Ryosuke Muroyama, Ryo Nakagawa, Sayaka Ito, Naoya Kato

Current efficacy of anti-hepatitis B virus (HBV) drugs is limited in eradicating nuclear covalently closed circular DNA (cccDNA), which is a vital factor helping the establishment of persistent HBV infection, necessitating the development of novel anti-HBV drugs for erasing cccDNA. One of the HBV proteins, HBx, has been proved to be essential for HBV replication probably through the interaction with cccDNA and the transactivation function. Therefore, we hypothesized that suppressing HBV cccDNA by inhibiting HBx transcriptional transactivity should be a promising therapeutic strategy against HBV. Hence, we constructed genotype C HBx-encoding plasmids and then examined the effects of HBx on major signaling pathways reported ever (i.e., NF-kappaB, AP-1, and SRE) by luciferase assay. NF-kB signal pathway was most stimulated in the presence of HBx. Subsequently, the NF-kB luciferase reporter was stably transfected in Huh7 cell line, and clones with the lowest background and high sensitivity to stimuli were isolated. A Tet-express system-controlled HBx-encoding plasmid was then transfected into the NF-kB-LUC Huh7 cell clone. A dual stable transfectant hepatoma cell line with two types of plasmid, a signaling pathway luciferase reporter and the HBx Tet-express system vector, was then established using multiple antibiotics selection. We used an FDA-approved drug library in order to discover potential inhibitors of HBx-stimulated NF-kB pathway and several candidates have been identified in the primary screening. Potential molecular mechanism of the interaction between viral onco-protein and host signaling pathways will be further analyzed.

7. Differentially expressed miRNA and target genes of $CD4^+$ T cells participates in the pathogenesis of primary biliary cirrhosis

Ryo Nakagawa[1,2], Ryosuke Muroyama[1], Wenwen

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Primary biliary cirrhosis (PBC) is a chronic inflammatory autoimmune liver disease. Although detailed mechanisms of the pathogenesis of PBC remain unknown, $CD4^+$ T cells are suggested to play an important role. Recently microRNA (miRNA) was reported to be involved in the pathogenesis of PBC.

Aim: We analyzed the expression profile of miRNA and their target genes in $CD4^+$ T cells of PBC patients to reveal their participation in pathogenesis of PBC. Clinically and pathologically diagnosed 7 PBC patients and 7 healthy controls, who agreed to provide samples with written informed consent, were enrolled in this study. Total RNA, including miRNA, was extracted from $CD4^+$ T cells purified from peripheral blood. The comprehensive analysis of miRNA was undergone using microarray and quantitative real-time PCR (qRT-PCR). We predicted the target genes of miRNA, which was expressed specifically in PBC, using bioinformatics. The dynamics of predicted target genes were analyzed by microarray and qRT-PCR. Then, luciferase assay and miRNA mimic assay were performed to examine the binding of the specific miRNA to 3′ untranslated region (3′ UTR) of target genes. Finally, we tested the potential role of specifically expressed miRNA against target genes by overexpressing miRNA in cultured cells. Microarray miRNA study showed 2 increased and 13 decreased miRNAs in PBC ($p<0.05$). Among them, 5 miRNAs were validated to be down-regulated in PBC ($p<0.05$) by qRT-PCR. A total of 4,855 target genes were predicted from 5 miRNAs by bioinformatics. In the mRNA microarray analysis, the expression of 2,565 genes was significantly different between PBC and control. Comparison of the target prediction and gene expression microarray study revealed 256 target genes were specifically expressed in PBC. Among 256 target genes, we analyzed 7 genes that were included in T cell receptor (TCR) signaling pathway to reveal the regulation of the target genes by miRNAs. The expression of 5 candidate target genes was validated by qRT-PCR. Luciferase assay and miRNA overexpression assay demonstrated that miR-425 regulate the expression of N-Ras including 5 candidate target genes by binding to their 3′ UTR. We have identified 5 decreased miRNA and 256 candidate target genes. Especially miR-425 regulate N-Ras expression in TCR signaling pathway. These miRNA may participate in the immunological pathogenesis of PBC through the regulation of the target genes in $CD4^+$T cells.

8. Novel zinc finger protein in gastrointestinal tract

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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 ZNF-114-like hypothetical 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 custom antibodies were made. Functional analysis will be executed using cultured cells.

9. Third-line rescue therapy with sitafloxacin after failure of two treatments (with clarithromycin and metronidazole) for *H.pylori* infection

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H. pylori eradication therapy with standard two treatments fails in 2～3% in cases. According to the prevalence of drug resistance, the rate of failure will increase, and an effective 3rd eradication regimen should be established. We evaluate an efficacy of a regimen with rabeprazole, amoxicillin and sitafloxacin, and in more than 90% of cases successful eradication was attained. Our study revealed that the sitafloxacin-containing regimen is well effective the 3rd rescue strategy for eradication of *H. pylori.*

10. Detection of HCV NS5A L31/Y93 mutations conferring treatment resistance

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Background and aims: Recently interferon-free regimen for HCV infection was attained by the combination treatment with NS5A inhibitor and protease inhibitor. However, amino acid mutations conferring resistance to NS5A inhibitor have been identified in the N-terminal region of NS5A protein. Here we focused on the resistance mutations at L31 and Y93 of NS5A reported in genotype 1b HCV infection, and examined their emergence in patients using our newly established detection system. Methods: Following the isolation of viral RNA from patient serum and its reverse transcription, the region covering the mutations was amplified by nested PCR. Subsequently L31/Y93 mutations were investigated by direct sequencing. Zero Blunt TOPO PCR Cloning Kit was used for the construction of plasmids encoding NS5A fragments with L 31F or Y93H. Results: Reverse transcribed viral RNA from serum of HCV-infected patient was used for the nested PCR with primers designed based on HCV genotype 1b genome sequences. Then we identified the primer set with the highest efficiency and specificity in amplification and subsequent direct sequencing. The established method was capable of sensing each mutation in the mixture of the plasmids encoding L31F or Y93H, with the individual mutation content of 25% or more and in a mixing ratio-dependent manner. So far L31F/M and Y 93H were observed in 5 (5%) and 12 (12%) out of 102 cases, respectively, irrelevant to patients' ages. Conclusions: We newly generated a detection system of NS5A inhibitor resistance mutations in NS5 A of HCV from patient serum. Our system is expected to be conducive for designing treatment strategy evading emergence of quasispecies highly resistant to NS5A inhibitors.

11. Induction of MICA expression by anti-allergic agent

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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 V et al., Nat Genet 2011) indicated the hepatocarcinogenesis-preventive potential of MICA expression. In our screen for an FDA-approved drug library using a luciferase reporter system, an anti-allergic agent was found to upregulate MICA promoter activity. The drug indeed induced the expression of MICA in hepatoma cells at non-cytotoxic concentrations. Currently its effects on MICA biogenesis and anti-HCC activities of NK cell are examined, with immunological prevention of carcinogenesis in view.

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

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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.

First, we examined IL-13Rα2 expression in ten pancreatic cancer cell lines and found 4 IL-13Rα2-positive cell lines and 6 IL-13Rα2-negative ones. Two cell lines, HS766T and MIA-PaCa2, were chosen for using in the further examinations because of their aggressive metastasis. In the next step, stable transfectants of IL-13Rα2-modulated were produced in the pancreatic cancer cell lines to confirm that the drugs affect cancer metastasis through IL-13Rα2. Drug candidates have already been narrow down to three HAT inhibitors. The candidates were included in the familiar items in our diet and enough safe for clinical use. The efficacy of them for suppressing IL-13Rα2 expression was examined and they decreased the IL-13Rα2 expression to less than 30% -50% on the drug concentration without cell toxicity.

Hereafter, the anti-metastatic effect of these drugs will be investigated for the pancreatic cancer cells both in vitro and in vivo.

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Division of Genetic Therapeutics
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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 cell 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.

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