Advanced Clinical Research Center

Division of Molecular Therapy
分子療法分野

Professor	Arinobu Tojo, M.D., D.M.Sc.	教　授	医学博士	東　條　有　伸
Associate Profesor	Satoshi Takahashi, M.D., D.M.Sc.	准教授	医学博士	高　橋　　聡
Assistant Professor	Jun Ooi, M.D.	助　教		大　井　　淳
Assistant Professor	Yasushi Soda. M.D., D.M.Sc.	助　教	医学博士	曽　田　　泰

The main theme of our research is toward the development of novel therapeutic options against intractable hematological disorders including leukemia and lymphoma. For this purpose, we are making every effort to master the mechanisms of normal and neoplastic hematopoiesis on the basis of molecular and cellular biology.

(1) Preclinical study of therapeutic gene transfer mediated by various viral vectors:
We have two main research projects in this field. One is a murine therapeutic model of tumor vaccine secreting GM-CSF (GVAX) in combination with non-myeloablative allogeneic HSCT. The other is a human experimental model of ribozyme technology for inactivation of leukemogenic fusion mRNA such as BCR-ABL.

(2) Preclinical study of targeted drug delivery using various cell-targeting strategies and novel molecular target agents:
We are developing various cell-targeting strategies using cytokines, adhesion molecules as well as monoclonal antibodies. PEG-liposome has been applied for this purpose. In addition, we have made two types of cytokine derivatives by genetic engineering for preclinical study. We are also studying anti-leukemic effects of a novel signal transduction inhibitor and anti-GvHD effects of a novel cytokine synthesis inhibitor for the future clinical trial.

(3) Analysis of tumor stem cells and search for molecular targets for their elimination:
Cure of malignant tumors requires eradication of tumor stem cells. As a representative model for tumor stem cells, we are studying the identification and characterization of leukemia stem cells using cell tracking strategies and flow cytometry.

(4) Analysis of normal and neoplastic hematopoiesis based on their interaction with microenvironments:
Not only normal but also neoplastic hematopoiesis can be supported by the specific interaction between stem/progenitor cells and bone marrow microenvironments. To simulate this cell to cell contact in vitro, we are using a co-culture system in which stem/progenitor cells are overlaid on the layer of hematopoiesis-supporting stroma cells. This co-culture system is applied for determination of drug sensitivities and gene transfer effects.

1. Cytogenetic remissions induced by interferon alpha and imatinib mesylate are immunologically distinct in chronic myeloid leukemia.

Nakayama S, Ohno N, Ooi J, Takahashi S, Uchimaru K, Tojo A.

We compared immunologic parameters of chronic myeloid leukemia (CML) patients in cytogenetic remission receiving imatinib mesylate (STI) treatment, CML patients receiving interferon alpha (IFN-alpha), and healthy volunteers. Each group comprised 14 subjects. Median treatment dosages and durations were 6 x 10(6) IU/week and 174 months, respectively, for IFN-alpha and 400 mg/day and 54 months for STI. The numbers of T-cells were significantly lower in the 2 patient groups ($P = .0006$), whereas the 3 groups were comparable with respect to the numbers of natural killer cells. Not only the absolute numbers of monocytes and B-cells but also serum immunoglobulin G (IgG) and IgA titers were significantly lower in the STI group than in the IFN-alpha group ($P < .0001$). For T-cell subsets, the ratio of CD4 T-cells to CD8 T-cells was significantly lower in the IFN-alpha group than in the STI group, but the proportion of CD26(high)CD4+ T-cells among CD4+ cells was significantly higher. Collectively, the 2 therapeutic agents induce a distinct immunologic status in CML patients whose hematopoiesis has returned to normal levels.

2. Identification and comparative analysis of Pax5 C-terminal isoforms expressed in human cord blood-derived B cell progenitors.

Sekine R, Tojo A.

We identified three Pax5 isoforms due to alternative splicing of the C-terminal exons of its gene in cord blood (CB)-derived B cell progenitors cultivated on the murine bone marrow stromal (HESS-5) cells. Apart from wild type (wt), one isoform skips exon 9 without subsequent frameshift (del9), while the other has a frameshift insert between exons 8 and 9, resulting in novel C-terminal sequences (ins8′). Quantitative reverse transcription-polymerase chain reaction analysis revealed that wt mRNA could be detected in CB CD34(+) cells, but that del9 and ins8′ isoforms only appeared after 1 or 3 weeks of co-culture, respectively. Expression of each isoform mRNA was markedly upregulated during B cell differentiation in vitro, and wild type continued to be the most abundant isoform. In a luciferase reporter assay using a synthetic CD19 enhancer, del9 isoform revealed slightly lower activity and ins8′ isoform showed much lower activity, compared with Pax5-wt. Furthermore, retroviral expression of each Pax5 isoform in CB CD34(+) cells induced aberrant CD19 expression in a fraction of immature myeloid cells after 1 week of culture, although del9 and ins8′ isoforms showed much less potent activity than Pax5-wt. These results suggest that Pax5-wt is quantitatively and qualitatively dominant over other C-terminal isoforms during human B cell differentiation.

3. Efficient retroviral transduction of human B lymphoid and myeloid progenitors: marked inhibition of their growth by the *Pax5* transgene

Sekine R, Tojo A

We applied a co-culture system to the genetic manipulation of human B lymphoid and myeloid progenitor cells, using a murine bone marrow stromal cell support and investigated the effects of forced Pax5 expression in both cell types. Cytokine-stimulated cord blood $CD34^+$ cells could be transduced at 85% efficiency and 95% cell viability by a single 24 h infection with RD114-pseudotyped retroviral vectors, produced by a packaging cell line Plat-F and bicistronic vector plasmids pMXs-Ig, pMYs-Ig, or pMCs-Ig, encoding EGFP. Infected $CD34^+$ cells were seeded onto HESS-5 cells in the presence of stem cell factor and granulocyte colony-stimulating factor, allowing extensive production of B progenitors and granulocytic cells. We examined cell number and CD34, CD33, CD19, and CD20 lambda and kappa expression by flow cytometry. Ectopic expression of Pax5 in $CD34^+$ cells resulted in small myeloid progenitors coexpressing CD33 and CD19 and inhibited myeloid differentiation. After 6 weeks, the number of Pax5-transduced CD19+ cells was 40-fold lower than that of control cells. However, expression of CD20 and κ/λ chain on Pax5-transduced CD19+ cells suggests that Pax5 transgene may not interfere with their differentiation. This report is the first description of the effects of forced Pax5 expression in human hematopoietic progenitors.

4. RNAi-mediated silencing of $p190^{Bcr\text{-}Abl}$ inactivates Stat5 and cooperates with imatinib mesylate and 17-allylamino-17-demetoxygeldanamycin in selective killing of $p190^{Bcr\text{-}Abl}$- expressing leukemia cells.

Futami M, Tojo A

The 190kD (p190) and 210kD (p210) Bcr-Abl

proteins are responsible for the development and progression of Philadelphia chromosome (Ph)-positive leukemia. We applied RNA interference (RNAi) technology to specific killing of $p190^+$ cells. After screening a series of small hairpin RNA (shRNA) targeting p190, we determined the optimal sequences for gene silencing in the BCR, junctional and ABL region of p190, respectively. Then, $p190^+$, $p210^+$ and both negative cell lines were infected with lentiviral vectors encoding these shRNAs, resulting in efficient killing of $p190^+$ cells, while $p210^+$ cells were also sensitive to the two other than junction-specific shRNA and both negative cells were resistant to all. In p190-transformed Ba/F3 cells, RNAi-mediated silencing of p190 specifically inhibited tyrosine phospohorylation of stat 5 prior to their death, but did not affect phosphorylation of Jak2, Akt, or MEK1/2. In contrast, down-regulation of p190 by their treatment with 17-allylamino-17- demetoxygeldanamycin (17-AAG) was associated with reduced protein levels of Jak2, Akt, and MEK1/2. shRNA targeting p190 collaborated additively with imatinib and 17-AAG in growth inhibition of Ba/F3-p190wt and BaF/3-p190Y253H cells. Collectively, RNAi-mediated silencing of p190 is a promising option both for delineating signal transduction and for therapeutic application in p 190-expressing leukemia.

5. Bioimaging analysis of stem cell signal activity in cancer cells using lentiviral reporter vector system

Kobayashi S, Izawa K, Tojo A

Accumulating findings suggest a hierarchical organization of developmental potential, so called stem cell system, in normal as well as malignant tissues. Cancer stem cells have been identified mainly on the basis of cell surface marker and/or side population phenotype, but not of functional parameters they present. We introduce a novel strategy to detect stem cell signals including TERT, Wnt, Notch and NF-kB, which are important for development and/or maintenance of stem cell function. Cancer cells were transduced with lentiviral reporter vectors harboring yellow fluorescent protein (YFP) analogue (Venus) or firefly luciferase (Luc) driven by responsive elements to individual stem cell signals, and then subjected to FACS or CCD camera for visualizing as fluorescence or bioluminescence intensity, respectively. This reporter assay enabled us to evaluate each stem cell signal activity, particularly in a single living cell. High transduction efficiency of VSV-G pseudotyped lentiviral vectors made it possible to analyze primary samples such as leukemia cells. Such a bioimaging analysis combined with the standard procedures will contribute to elucidate the nature of cancer stem cells and to identify target molecules for therapy.

6. Microenvironmental upregulation of NF-kB activity suggests a novel hepatic niche for Philadelphia chromosome-positive acute lymphoblastic leukemia.

Tsai HJ, Kobayashi S, Izawa K, Tojo A

We lentivirally transduced IMS-PhL1 cells with NF-kB/luciferase (kB/Luc) reporter construct and established a bioluminescence imaging model of Ph-ALL for *in vitro* and *in vivo* analysis. Unstimulated PhL1-kB/Luc cells revealed a weak but significant Luc activity over the background, verifying constitutive activation of NF-kB. Among a panel of cytokines, only TNFa potently up-regulated Luc activity in PhL 1-kB/Luc cells about 10-fold over the basal level, but did not affect their growth and survival. DHMEQ, a specific inhibitor of nuclear translocation of p65, eradicated constitutive and TNFa-inducible NF-kB activity of PhL1 cells and induced their substantial apoptosis dose-dependently. A series of Ph-ALL cell lines were similarly sensitive to treatment with DHMEQ, suggesting the critical role of constitutive NF-kB activity in survival of Ph-ALL cells. When PhL1-kB/Luc cells were seeded onto a layer of murine HESS-5 stroma cells, Luc activity was not changed. Intriguingly, TNFa stimulation of PhL1-kB/Luc cells in the presence of HESS-5 cells caused synergistic enhancement of Luc activity up to 20 fold over the basal level. This up-regulation was canceled by blocking cell to cell contact with a transwell membrane, suggesting that the direct cell contact may be essential for such a synergistic up-regulation of NF-kB activity. In HESS-5 cells, NF-kB activity was markedly augmented in response to TNFalpha, but this up-regulation was not sensitive to DHMEQ. Furthermore, the inhibitory effects of DHMEQ on Luc activity as well as viability of TNFalpha-treated PhL1-kB/Luc cells were significantly alleviated in the presence of HESS-5 cells. Taken together, TNFalpha-triggered HESS-5 cells are likely to up-regulate NF-kB activity of PhL1 cells through DHMEQ-insensitive alternate pathway, which has been confirmed in a gel-shift assay. Finally, PhL1-kB/Luc cells were transplanted into NOD-SCID mice and subjected to periodic monitoring with a CCD camera. We successfully detected constitutive and TNFalpha-inducible bioluminescent signals during the expansion of engrafted leukemia cells, and unex-

pectedly, the very strongest signal was captured in the liver, although homogeneous leukemic infiltration was observed in other tissues including bone marrow and spleen, implying that hepatic microenvironment may offer proper stimuli to NF-kB activity and may constitute leukemic niche in this cell context. The present bioimaging model helps us to dissect NF-kB signals among complex cellular components.

7. Bioluminescence tracking of engraftment and propagation of cord blood stem/progenitor cells in immunno-deficient mice

Izawa K, Tojo A

We retrovirally transduced cord blood $CD34^+$ cells with firefly luciferase (Luc) gene for bioluminescence tracking of their engraftment and propagation in non-obese severe combined immuno-deficient (NOD-SCID) mice. Mice were conditioned with anti-asialoGM1 antibody and 3.5Gy total body irradiation, followed by intravenous injection of 6×10^3 -2×10^4 $CD34^+$ cells with or without 10-fold cell doses of either $CD4^+$ or $CD8^+$ T cells. Engraftment of $CD34^+$ cells was confirmed in all the recipient mice by CCD camera, and propagation of their progenies could be traced for as long as 21 weeks. Bioluminescent signal was predominantly detected in spine, skull, pelvis as well as femur. In addition, continuous monitoring of the whole body signal intensity revealed the biphasic hemopoietic reconstitution, namely early (around 6 weeks post -transplant) and late (thereafter) phase. Simultaneous infusion of either $CD4^+$ or $CD8^+$ T cells did not significantly accelerate engraftment of $CD34^+$ cells. This noninvasive system will contribute to verify the efficacy of ex vivo expansion of hemopoietic stem cells by various methods.

Publications

1 Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kato S, Kasahara S, Iseki T, Yamaguchi T, Tojo A, Asano S. Impact of cytomegalovirus serostatus on outcome of unrelated cord blood transplantation for adults: a single-institute experience in Japan. Eur J Haematol. 2007 Dec 19; [Epub ahead of print]

2 Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kobayashi T, Takasugi K, Iseki T, Tojo A, Asano S. Preemptive therapy with ganciclovir 5 mg/kg once daily for cytomegalovirus infection after unrelated cord blood transplantation. Bone Marrow Transplant. 2007 Nov 5; [Epub ahead of print]

3 Nagamura-Inoue T, Kodo H, Takahashi T, Mugishima H, Tojo A, Asano S. Four cases of donor cell-derived AML following unrelated cord blood transplantation for adult patients: experiences of the Tokyo Cord Blood Bank. Cytotherapy. 2007 Oct 4;1-2 [Epub ahead of print]

4 Nakayama S, Nagamura-Inoue T, Yokoyama K, Ohno N, Ooi J, Takahashi S, Uchimaru K, Iseki T, Tojo A. Cytogenetic remissions induced by interferon alpha and imatinib mesylate are immunologically distinct in chronic myeloid leukemia. Int J Hematol. 86: 208-11, 2007

5 Inoue Y, Izawa K, Yoshikawa K, Yamada H, Tojo A, Ohtomo K. In vivo fluorescence imaging of the reticuloendothelial system using quantum dots in combination with bioluminescent tumour monitoring. Eur J Nucl Med Mol Imaging. 34: 2048-56, 2007

6 Sekine R, Kitamura T, Tsuji T, Tojo A. Identification and comparative analysis of Pax5 C-terminal isoforms expressed in human cord blood-derived B cell progenitors. Immunol Lett. 111: 21-5, 2007

7 Inoue Y, Izawa K, Tojo A, Nomura Y, Sekine R, Oyaizu N, Ohtomo K. Monitoring of disease progression by bioluminescence imaging and magnetic resonance imaging in an animal model of hematologic malignancy. Exp Hematol. 35: 407-15, 2007

8 Inoue Y, Yoshikawa K, Nomura Y, Izawa K, Shimada M, Tojo A, Ohtomo K. Gadobenate dimeglumine as a contrast agent for MRI of the mouse liver. NMR Biomed. 20: 726-32, 2007

9 Tomonari A, Takahashi S, Ooi J, Tsukada N, Konuma T, Kobayashi T, Sato A, Iseki T, Yamaguchi T, Tojo A, Asano S. Impact of ABO incompatibility on engraftment and transfusion requirement after unrelated cord blood transplantation: a single institute experience in Japan. Bone Marrow Transplant. 40: 523-8, 2007

10 Tomonari A, Tsukada N, Takahashi S, Ooi J, Konuma T, Kobayashi T, Fukuno K, Takasugi K, Fujii T, Endo T, Iwamoto A, Oyaizu N, Tojo A, Asanoa S. Early-onset pulmonary complication showing organizing pneumonia pattern following cord blood transplantation in adults. Int J Hematol. 85: 364-6, 2007

11 Takahashi S, Ooi J, Tomonari A, Konuma T,

Tsukada N, Oiwa-Monna M, Fukuno K, Uchiyama M, Takasugi K, Iseki T, Tojo A, Yamaguchi T, Asano S. Comparative single-institute analysis of cord blood transplantation from unrelated donors with bone marrow or peripheral blood stem cell transplantation from related donors in adult patients with hematological malignancies after myeloablative conditioning regimen. Blood. 109: 1322-30, 2007

Advanced Clinical Research Center

Division of Cellular Therapy
細胞療法分野

Professor	Toshio Kitamura, M.D., D.M.Sc.	教　授	医学博士	北　村　俊　雄
Associate Professor	Kohichiro Tsuji, M.D., D.M.Sc.	准教授	医学博士	辻　　浩一郎
Assistant Professor	Toshiyuki Kawashima, M.D., D.M.Sc.	助　教	医学博士	川　島　敏　行
Assistant Professor	Jiro Kitaura, M.D.	助　教	医学博士	北　浦　次　郎

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, myelodysplastic syndromes, myeloproliferative disorders.

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

Toshiyuki Kawashima, Yukinori Minoshima, Ying Chun Bao, Tomonori Hatori, Yasushi Nomura, Takaya Satoh[1], Yoshito Kaziro[2], Hideaki Nakajima[3], Tetsuya Nosaka, David Williams[4] and Toshio Kitamura: [1]Kobe University, [2]Biochemistry and Cell Biology Unit, HMRO, Kyoto University Graduate School of Medicine, [3]Project of mesenchymal stem cells, The 21st century center of excellence program, Institute of Medical Science, The University of Tokyo, [4]Cincinnati Children's Hospital Medical Center, USA

In the 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 growth suppression and macrophage differentiation. Interestingly, MgcRacGAP localized to the nucleus in interphase, accumulated to the mitotic spindle in metaphase, and was condensed in the midbody during cytokinesis. These findings indicate that MgcRacGAP dynamically moves during cell cycle progression and plays critical roles in cytokinesis. Moreover, the experiment using a GAP-inactive mutant showed that 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. Recently, we have found that MgcRacGAP directly binds transcription factors STAT3 and STAT5, and enhances transcriptional activation of STAT proteins probably as a Rac GAP. In summary, our results suggest that MgcRacGAP plays distinct roles depending on the cell cycle thereby co-ordinating cell division and cell differentiation/proliferation.

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

Toshiyuki Kawashima, Akiho Tsuchiya, Yukinori Minoshima, Ken Murata and Toshio Kitamura:

Internal tandem duplications of the juxtamembrane region of the *Flt-3* (ITD-Flt3) are found in about 30% of the human acute myeloid leukemia patients. We previously identified small molecule compound GTP14565, a specific inhibitor of ITD-Flt3. GTP14564 preferentially inhibited the growth of the Ba/F3 cells transformed by the mutant *Flt-3*, but not Ba/F3 cells driven by the Flt-3 ligand/wild type Flt-3. Based on the in vitro results, we found that ITD-Flt3-induced cell growth was dependent on STAT5 activation while wild-type Flt3-induced cell growth was dependent on ERK and MAPK activation, suggesting the difference in signaling between phathological and physiological conditions. However, GTP14564 is unstable and insoluble, and cannot be used for preclinical trials.

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 already established an efficient screening protocol for identification of STAT3 inhibitors, and identified several compounds that inhibit STAT3 activation. One of the compounds RJSI-2 that inhibited STAT3 activation also inhibited activation of STAT1, STAT5, JAK1 and JAK2. Interestingly, RJSI-2 is not a kinase inhibitor. We are now in the process of analyzing molecular basis of RJSI-2 inhibition of these signaling molecules, and evaluating its effects in a tumor-burden model.

3. Molecular mechanisms of hematopoietic stem cell-supportive activities of ISF a subunit of proton pump-associated ATPases.

Hideaki Nakajima[3], Fumi Shibata, Yumi Fukuchi, Yuko Goto-Koshino, Miyuki Ito, Atsushi Urano, Tatsutoshi Nakahata[2], Hiroyuki Aburatani[5], and Toshio Kitamura: [5]Research Center for Advanced Science and Technology, The University of Tokyo

In the search for stromal-derived growth factors, we have identified a novel secreted short form of immune suppressor factor (ISF) using a combination of a genetic approach and retrovirus-mediated functional screening. This protein was isolated based on its ability to support proliferation of a mutant clone S21, which was established from Ba/F3 cells that are usually interleukin-3-dependent but became dependent on a stroma cell line ST2 after chemical mutagenesis. ISF is a membrane protein harboring six transmembrane domains, and turned out to be a subunit of vacuolar H (+)-ATPase subunit. When overexpressed in bone marrow stroma cells, ISF conferred the cells with an ability to support the growth of S21 cells as well as hematopoietcn stem cells (HSCs). To elucidate the molecular mechanisms, we analyzed the expression profiles using DNA chips, and found that ISF overexpression resulted in the up-regulation of MMP3, and down-regulation of TIMP3 and SFRP-1. We also demonstrated that down-regulation of TIMP3 and SFRP-1 could lead to maintainance of HSCs.

4. Integrin αIIbβ3 induces the adhesion and activation of mast cells through interaction with fibrinogen.

Toshihiko Oki, Jiro Kitaura, Koji Eto[6], Yang Lu, Yoshinori Yamanishi, Hideaki Nakajima[3], Hidetoshi Kumagai, and Toshio Kitamura: [6]Laboratory of Stem Cell Therapy, Institute of Medical Science, The University of Tokyo

Integrin αIIb, a well-known marker of megakaryocyte-platelet lineage, has been recently recognized on hemopoietic progenitors. We demonstrate that integrin αIIbβ3 is highly expressed on mouse and human mast cells and that mast cells, with exposure to various stimuli, adhere to extracellular matrix proteins such as fibrinogen and von Willebrand factor in an integrin αIIβ3-dependent manner. In addition, the binding of mast cells to fibrinogen enhanced proliferation, cytokine production and migration and induced the uptake of soluble fibrinogen, implicating integrin αIIbβ3 in a variety of mast cell functions. Our goal is to delineate the biological significance of integrin αIIbβ3 on mast cells by in vivo allergy and inflammation models using integrin αIIb knockout mice.

5. Identification and characterization of a new pair of immunoglobulin-like receptors, leukocyte mono-Ig-like receptors (LMIRs).

Yoshinori Yamanishi, Jiro Kitaura, Kumi Izawa, Toshihiko Oki, Yutaka Enomoto, Ayako Kaitani, Masahiro Sugiuchi, Takayuki Matsuoka, Fumi Shibata, Kaori Tamitsu, Si-Zhou Feng, Hideaki Nakajima[3], Hidetoshi Kumagai, and Toshio Kitamura

We originally identified and characterized two mouse cDNAs in a mouse bone marrow-derived

mast cell cDNA library. They encode 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, 5, 6 are activation type receptors like LMIR2.). LMIRs are also called CLMs or MAIRs. LMIR4, 5 are expressed in myeloid cells (LMIR4 is highly expressed in granulocytes, but is missing in humans), whereas LMIR3 is expressed in myeloid cells and B cells. Association of LMIR4 with FcRγ and LMIR5 with DAP12 is indispensable for LMIR4- and LMIR5-mediated functions of bone marrow-derived mast cells, respectively. Interestingly, the triggering of LMIR4 and TLR4 synergistically caused robust cytokine production in accordance with enhanced activation of ERK.

Investigation to elucidate the biological roles of LMIRs on immune cells is now underway; we attempt to identify the ligands and analyzing LMIR-deficient mice.

6. Molecular basis of acute leukemia, myelodysplastic syndromes (MDS), MDS overt leukemia, and myeloproliferative disorder (MPD).

Naoko Watanabe, Yukiko Komeno, Naoko Kato, Toshihiko Oki, Koichiro Yuji, Yuka Harada[7], Hironori Harada[7], Toshiya Inaba[8], Hideaki Nakajima[3], Tetsuya Nosaka, Jiro Kitaura, and Toshio Kitamura: [7]Department of Hematology/Oncology and [8]Department of Molecular Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University

To elucidate the molecular mechanisms of leukemia, MDS, and MPD, we established mouse model using bone marrow transplant (BMT); we transduced mouse bone marrow cells with genes of leukemogenic mutations such as MLL-fusions or AML-1 using retroviruses. In the result, we are now able to reproduce acute leukemia, MPD and MDS-like symptoms in mice, and are now in the process of characterizing these mouse models. We also establish bone marrow-derived immature cell lines transduced with MLL fusions and AML-1 with mutations. While the differentiation of these cell lines are blocked probably through the dominant negative effects of MLL-fusions and the mutated AML-1, they still remain dependent on cytokines including SCF, IL-3, and Flt-3 ligand. In the mouse BMT model, we are beginning to understand that leukemogenesis (acute leukemia as well as MDS overt leukemia) require multiple mutations; mutations that block differentiation, and mutations that block apoptosis or induce factor-independent proliferation. Based on the mouse BMT model, we assume that there are the second hit mutations in addition to mutations such as MLL-fusions and AML-1 in patients' leukemic cells. To identify such mutations, we use retrovirus-mediated expression cloning method. To this end, we make cDNA libraries of patients' leukemic cells, and will isolate cDNAs that give rise to the autonomous growth of the cytokine-dependent cell lines established as stated above. In this way, we isolated ITD-Flt3 (constitutively activated Flt3 mutant found in 30% of patients with acute myeloid leukemia) and some proteins that activate Ras pathways.

7. Identification of TSC-22 as a potential tumor suppressor.

Yang Lu, Jiro Kitaura, Toshihiko Oki, Yukiko Komeno, Katsutoshi Ozaki, Mari Kiyono, Hidetoshi Kumagai, Hideaki Nakajima[3], Tetsuya Nosaka, Hiroyuki Aburatani[5], and Toshio Kitamura

Two types of FMS-like tyrosine kinase-3 (Flt3) mutations are frequently found in acute myeloid leukemia: Flt3-ITD harboring internal tandem duplication in the juxtamembrane domain associated with poor prognosis and Flt3-TKD harboring a point mutation in the kinase domain. Comparison of gene expression profiles between Flt3-ITD and Flt3-TKD-transduced Ba/F3 cells revealed that constitutive activation of Flt3 by Flt3-TKD, but not Flt3-ITD, upregulated the expression of transforming growth factor-β (TGF-β)-stimulated clone-22 (TSC-22). Forced expression of TSC-22 suppressed the growth and accelerated the differentiation of several leukemic cell lines into monocytes, in particular, in combination with differentiation-inducing reagents. These results suggest that TSC-22 is a possible target of leukemia therapy.

Publications

Izawa, K., Kitaura, J., Yamanishi, Y., Matsuoka, T., Oki, T., Shibata, F., Kumagai, H., Nakajima, H., Maeda-Yamamoto, M., Hauchins, J. P., Tybulewicz, V.L.J., Takai, T. and Kitamura, T. Functional analysis of an activating receptor LMIR4 as a counterpart of an inhibitory receptor LMIR3. J. Biol. Chem. 25: 17997-18008, 2007.

Nakayama, M., Underhill, D.M., Peterson, T.W., Li, B., Kitamura, T., Takai, T. and Aderem, A. Paired Ig-like receptors bind to bacteria and shape TLR-mediated cytokine production. J. Immunol. 178: 4250-4259, 2007.

Sekine, R., Kitamura, T., Tsuji, T. and Tojo, A. Identification and comparative analysis of Pax 5 C-terminal isoforms expressed in human cord blood-derived B cell progenitors. Immunol. Letters 111: 21-25, 2007.

Miyanishi, M., Tada, K., Koike, M., Uchiyama, Y., Kitamura, T. and Nagata, S. Identification of TIM4 as a phosphatidylserine receptor for engulfment of apoptotic cells. Nature 450: 435-440, 2007.

Lu, Y., Kitaura, J., Oki, T., Komeno, Y., Ozaki, K., Kiyono, M., Kumagai, H., Nakajima, H., Aburatani, H. and Kitamura, T. Identification of TSC-22 as a potential tumor suppressor that is upregulated by Flt3-D835V but not Flt 3-ITD. Leukemia 21: 2246-2257, 2007.

Shinoda, G., Umeda, K., Heike, T., Arai, M., Niwa, A., Ma, F., Suemori, H., Luo, H.Y., Chui, D.H.K., Torii, R., Shibuya, M., Nakatsuji, T. and Nakahata, T. 4-integrin^{+} endothelium derived from primate embryonic stem cells generates both primitive and definitive hematopoietic cells. Blood 109: 2406-2415, 2007.

Sugiyama, D., Ogawa, M., Nakao, K., Ma, F., Osumi, N., Nishikawa, S., Nishikawa, S., Katsuki, M., Asano, S., Arai, K., Nakahata, T. and Tsuji, K. B cell can be obtained from precirculatory yolk sac, but with low frequency. Dev Biol 301: 53-61, 2007.

Ma, F., Wang, D., Hanada, S., Ebihara, Y., Kawasaki, H., Zaike, Y., Heike, T., Nakahata, T. and Tsuji K. Novel method for efficient production of multipotential hematopoietic progenitors from human embryonic stem cells. Int J Hematol 88: 371-379, 2007.

Kitamura, T., Oki, T., Watanabe-Okochi, N., Komeno, Y., Kato, N., Yuji, K., Ono, R., Nakajima, H., Tojo, A., Nosaka, T. and Kitaura, J. Identification of leukemia-related gene alterations: Molecular pathogenesis of leukemia, myeloproliferative disorders (MPD), and myelodysplastic syndromes (MDS). Blood Cells, Molecules and Diseases in press.

Sugano, Y., Takeuchi, M., Hirata, A., Atsushita, H.M., Kitamura, T., Tanaka, M. and Miyajima, A. Junctional adhesion molecule-A, JAM-A, is a novel cell surface marker for long-term repopulating hemotopoietic stem cells. Blood 111: 1167-1172, 2008.

Yamanishi, Y., Kitaura, J., Izawa, K., Matsuoka, T., Oki, T., Lu, Y., Shibata, F., Yamazaki, S., Kumagai. H., Nakajima, H,, Maeda-Yamamoto, M., Tybulewicz, V.L.J., Takai, T. and Kitamura, T. Analysis of mouse LMIR5/CLM7 as an activating receptor: differential regulation of LMIR5/CLM7 between mouse and human. Blood 111: 688-698, 2008.

Watanabe-Okochi, N, Kitaura, J., Ono, R., Harada, H., Harada, Y., Nakajima, H., Nosaka, T., Inaba, T. and Kitamura, T. AML1 mutations induced MDS and MDS/AML in a mouse BMT model. Blood 111: 4297-4308, 2008.

Morikawa, Y., Komori, T., Hisaoka, T., Ueno, H., Kitamura, T. and Senba, E. Expression of mKirrein the developing sensory pathways: its close apposition to nephrin-expressing cells. Neuroscience 150: 880-886, 2007.

Kawashima, T. and Kitamura, T. Rac and Nuclear Translocation of STAT Transcription Factors. Methods in Enzymology 431: 171-180, 2008.

Ma, F., Kambe, N., Wang, D., Shinoda, G., Fujino, H., Umeda, K., Fujisawa, A., Ma, L., Suemori, H., Nakatsuji, N., Miyachi, Y., Torii, R., Tsuji, K., Heike, T. and Nakahata T. Direct development of functionally mature tryptase/chymase double positive tissue-type mast cells from primate ES cells. Stem Cells, in press.

Ebihara, Y., Manabe, A., Tsuruta, T., Ishikawa, K,. Hasegawa, D., Ogami, K., Wada, Y., Kanda, T. and Tsuji, K. The effect of donor leukocyte infusion (DLI) on refractory pure red blood cell aplasia after allogeneic stem cell transplantation in a patient with myelodysplastic syndrome developing from Kostmann syndrome. Int J Hematol, in press.

Mizutani, T., Tsuji, K., Ebihara, Y., Ohba, Y., Taniguchi, T. and Honda, K. Homeostatic erythropoiesis by the transcription factor IRF2 through attenuation of type I interferon signaling. Exp Hematol, in press.

Konuma, T., Ooi, J., Takahashi, S., Tomonari, A., Tsukada, N., Kobayashi, T., Sato, A., Kato, S., Kasahara, S., Ebihara, Y., Nagamura-Inoue, T., Tsuji, K., Tojo, A. and Asano, S. Cardiovascular toxicity of cryopreserved cord blood cell infusion. Bone Marrrow Transplant, in press.

Advanced Clinical Research Center

Division of Infectious Diseases
感染症分野

Professor	Aikichi Iwamoto, M.D., D.M.Sc.	教 授	医学博士	岩 本 愛 吉
Lecturer	Takeshi Fujii, M.D., D.M.Sc.	講 師	医学博士	藤 井 毅
Assistant Professor	Ai Kawana-Tachikawa, D.M.Sc.	助 教	医学博士	立川(川名) 愛
Assistant Professor	Tomohiko Koibuchi, M.D., D.M.Sc.	助 教	医学博士	鯉 渕 智 彦

The long-term goal of our division is medical sciences on infectious diseases in two directions, from clinic to bench and from bench to clinic. Our current main subjects are immune-pathogenesis and drug-resistance in HIV-1-1 infection. We wish to clarify how cellular immune responses can control HIV-1-1 infection in some patients but not in others. We also wish to develop new methods to detect drug-resistance in HIV-1. 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. Characterization of HIV-1-specific CD8＋ T cells recognizing a variant epitope

Ai Kawana-Tachikawa, Eriko Miyazaki, Mariko Tomizawa, Tomohiko Koibuchi, Takeshi Fujii, Takashi Odawara[1], Tetsuya Nakamura[1], Aikichi Iwamoto: [1]Department of Infectious Diseases and Applied Immunology

It has been shown that antigen-specific CD8＋ T cells play a crucial role in controlling HIV-1. HIV-1 easily mutates and changes the amino acid sequences in the epitope for HIV-1-specific CD8＋ T cell recognition,. However, precise mechanisms for the escape and immune-evasion have poorly been understood. In this study, we wished to perform both quantitative and qualitative analyses of HIV-1-specific CD8＋ T cells recognizing the wild type or mutated epitopes.

In the previous study we found a common variant of HLA-A*2402- (A24-) restricted CTL epitope Nef138-10 (RYLPTFGWCF). Nef138-10 is an immunodominant epitope but the same amino acid substitution (phenylalanine (F) for tyrosine (Y) at 2nd position) occurred in 80% of A24＋ patients (Nef138-10(2F)). We made two tetramers labeled with different fluorochrome; one presented the wild type Nef138-10, while the other presentedNef138-10(2F) in the context of A24. PBMCs from HIV-1-infected patients were stimulated with Nef138-10 peptide and stained by these tetramers. The expression of Programmed Death-1 (PD-1) was evaluated on these cells as well. We found there were CTLs with different specificity for Nef138-10 and Nef 138-10(2F). When Nef138-10-specific CD8＋T cells were stained, Tet(wt)-single positive and Tet(wt)(2F)-dual positive populations were identified. PD-1 expression on Tet(wt)(2F)-dual positive cells was much higher than Tet(wt)-single positive T cells, implying that Tet(wt)(2F)-dual positive cells could be dysfunctional.

We also analyzed TCR repertoire of the tetramer-positive cells. TCR repertoire of Tet(wt) -single positive cells was diverse in both Vβ and Jβ regions. Interestingly, however, dual positive cells used very restricted TCR-Vβand Jβ genes. Our results suggest that there is a qualitative difference between CTLs which recognize original and mutant epitopes.

2. HIV-1 escape from antigen-specific CTL responses and its effect on the disease progression

Michiko Koga, Ai Kawana-Tachikawa, Tomohiko Koibuchi, Takeshi Fujii, Takashi Odawara[1], Aikichi Iwamoto

Japanese population have high incidence of HLA-*2402 (A24). Our previous study revealed that HIV-1 harboring a stereotypic mutation in a CTL epitope (Nef138-10(2F)) is prevailing among Japanese patients recently infected by sexual intercourse. We wished to analyze the impact of this mutation in the disease progression of Japanese people living with HIV/AIDS (PLWHA). Literatures from abroad suggest that HLA class I types are associated with the disease progression. Since the genetic diversity is less in Japanese than Caucasians as exemplified by the high allelic frequency of HLA-A24 (36.5 %), we wished to examine the influence of HLA -A24 in Japanese PLWHA.

We compared viral loads and CD4 cell counts between HLA-A24 positive and negative Japanese patients infected recently, but no statistically significant difference was revealed. HIV-1 having the wild type Nef138-10 or the mutant Nef138-10(2F) showed similar replication capacity *in vitro*. These results suggested that CTL escape mutation at Nef138-10 is neutral in disease progression as reported in Caucasians.

3. Generation of monoclonal antibodies against an immunodominant CTL epitope of HIV-1

Junichi Nunoya, Ai Kawana-Tachikawa, Aikichi Iwamoto

The cellular immune response plays an important role in HIV-1 infection. HIV-1-specific CTL responses have been characterized using several methods. However, HIV-1 antigen presentation has hardly been analyzed because the tools for the analysis have not been established. Monoclonal antibody can be a powerful tool to address the issue. We tried to isolate monoclonal antibodies against an immunodominant Nef 138-10 epitope presented in the context of HLA-A24*2402.

We isolated single-chain Fvs (scFvs) that directly bind to MHC class I/peptide complexes (HLA-A24/Nef138-10) from non-immunized human scFv phage display libraries. Two scFvs (clones 3 and 27) bound directly to HLA-A24/Nef138-10 complexes but did not bind any other HLA-A24 molecules loaded with unrelated peptides. Then, we generated fully humanized monoclonal antibodies using baculovirus expression system. The fully humanized IgG 1 molecules generated by baculovirus expression system did not lose their binding specificity to the antigen. Using surface plasmon resonance, we analyzed the kinetics of antigen-antibody interaction. Dissociation constants of clones 27 and 3 proved to be 20μM and 23μM, respectively.

Although we isolated monoclonal antibodies that specifically bind to particular MHC/peptide complexes, these antibodies had a relatively low affinity against the antigen. We are trying to increase the affinity of these antibodies for the analysis of HIV-1 antigen presentation and for potential therapeutic use.

4. Genetic manipulation of dendritic cells by Sendai virus vectors for immunotherapy

Tomohiko Koibuchi, Noriaki Hosoya, Ai Kawana-Tachikawa, Takashi Odawara[1], Tetsuya Nakamura[1], Aikichi Iwamoto

Dendritic cells (DCs) are efficient antigen presenting cells that are critical for induction of T-cell responses. At present the most useful method for genetic manipulation of DCs is to use viral vectors. Adenovirus (AdV) vector had been reported to be efficient at transduction of DCs. Sendai virus (SeV) is also expected to be an efficient vector for transduction of DCs. SeV has several unique features, such as cytoplasmic localized replication cycle and brief contact time for cellular uptake. In this study, we analyzed the capacity of SeV as a vector to transduce GFP or HIV-1 genes into human DCs. We showed that SeV vector transduced GFP gene efficiently into monocyte-derived immature DCs. DCs infected with the SeV vector expressed high amount of GFP 24 h after infection at an MOI of 2. A fusion protein (F)-defective SeV (dF-SeV) vector, which is replication-deficient, also showed the same level of transduction of GFP gene. The expression levels of HIV-1 structural genes, such as *env* and *gag*, by SeV vectors were higher than those by AdV vectors. Our results proved the high ability of gene expression by SeV vectors in human DCs.

Both SeV and dF-SeV vectors killed nearly 30 % of target DCs at the lowest MOI examined (MOI of 0.5). Several studies reported that SeV could induce apoptosis in infected host cells. This cytopathic effect may enhance specific T-cell responses through cross-presentation by DCs. In fact DCs transduced by SeV vector elicited higher IFN-γ Elispot responses of stimulated HIV-1-1 specific T-cells than DCs transduced by AdV vector. Presentation of virus-infected apoptotic cells by nearby DCs may

have activated T-cells efficiently.

Our study demonstrated that SeV vector induced maturation of DCs in terms of their phenotype and stimulated HIV-1 specific T-cell responses efficiently. Although further studies are required to apply SeV vector in a clinical setting, SeV vector may be a promising candidate for genetic manipulation of DCs to be utilized for immunotherapy.

Publications

1. Hosoya N, Miura T, Kawana-Tachikawa A, Koibuchi T, Shioda T, Odawara T, Nakamura T, Kitamura Y, Kano M, Kato A, Hasegawa M, Nagai Y, and Iwamoto A. Comparison between Sendai virus and Adenovirus vectors to transducer HIV-1-1 genes into human dendritic cells. J Med Virol. (in press)
2. Fujii T, Nakamura T, Iwamoto A. Pneumocystis pneumonia in patients with HIV-1 infection: clinical manifestations, laboratory findings, and radiological features. J Infect Chemother. 13: 1-7, 2007.
3. Maeda T, Fujii T, Oyaizu N, Endo T, Odawara T, Iwamoto A, Nakamura T. Pneumocystis jiroveci pneumonia in an AIDS patient: Unusual manifestation as multiple nodules with expansive multiloculated cavities. Eur J Radiol extra, 61, 49-52, 2007.
4. Maeda T, Yamada H, Akao H, Iga M, Endo T, Koibuchi T, Nakamura T, Odawara T, Iwamoto A, Fujii T. Unusual radiological findings of *Fasciola hepatica* infection with huge cystic and multilocular lesions. Intern Med. (in press)
5. Wichukchinda N, Kitamura Y, Rojanawiwat A, Nakayama EE, Song H, Pathipvanich P, Auwanit W, Sawanpanyalert P, Iwamoto A, Shioda T, Ariyoshi K. The polymorphisms in DC-SIGNR affect susceptibility to HIV-1 type 1 infection. AIDS Res Hum Retroviruses. 23: 686-92, 2007
6. Liu H, Nakayama EE, Theodorou I, Nagai Y, Likanonsakul S, Wasi C, Debre P, Iwamoto A, Shioda T. Polymorphisms in CCR5 chemokine receptor gene in Japan. Int J Immunogenet. 34: 325-35, 2007.
7. Hoshino, S., Sun, B., Konishi, M., Shimura, M., Segawa, T. Hagiwara, Y., Koyanagi, Y., Iwamoto, A., Mimaya, J., Terunuma, H., Kano, S., and Ishizaka, Y. Vpr in plasma of HIV-1-1-positive patients is correlated with the HIV-1-1 RNA titers. AIDS Research and Human Retroviruses. 23: 391-7, 2007.
8. Song H, Nakayama EE, Likanonsakul S, Wasi C, Iwamoto A, Shioda T. A three-base-deletion polymorphism in the upstream non-coding region of human interleukin 7 (IL-7) gene could enhance levels of IL-7 expression. Int J Immunogenet. 34: 107-13, 2007.
9. Nakayama EE, Carpentier W, Costagliola D, Shioda T, Iwamoto A, Debre P, Yoshimura K, Autran B, Matsushita S, Theodorou I. Wild type and H43Y variant of human TRIM5alpha show similar anti-human immunodeficiency virus type 1 activity both in vivo and in vitro. Immunogenetics. 59: 511-5, 2007.
10. Tomonari A, Tsukada N, Takahashi S, Ooi J, Konuma T, Kobayashi T, Fukuno K, Takasugi K, Fujii T, Endo T, Iwamoto A, Oyaizu N, Tojo A, Asanoa S. Early-onset pulmonary complication showing organizing pneumonia pattern following cord blood transplantation in adults. Int J Hematol. 85: 364-6, 2007.
11. Yoshioka S, Mukae H, Ishii H, Kakugawa T, Ishimoto H, Sakamoto N, Fujii T, Urata Y, Kondo T, Kubota H, Nagata K, Kohno S. Alpha-defensin enhances expression of HSP 47 and collagen-1 in human lung fibroblasts. Life Sci. 80: 1839-45, 2007.

Advanced Clinical Research Center

Division of Bioengineering
臓器細胞工学分野

Professor	Hideaki Tahara, M.D., D.M.Sc.		教　授	医学博士	田　原　秀　晃
Project Associate Professor	Kenji Nakano, M.D., D.M.Sc.		特任准教授	医学博士	中　野　賢　二
Assistant Professor	Masahisa Jinushi, M.D., D.M.Sc.		助　教	医学博士	地　主　将　久
Assistant Professor	Marimo Sato, Ph.D.		助　教	学術博士	佐　藤　まりも

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 of Department of Surgery in our research hospital. 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.

I. Development of innovative cancer therapy using immunologic approaches.

a. Systemic administration of IL-23 induces potent anti-tumor immunity primarily Mediated through Th1-type response in association with the endogenously expressed IL-12

Teruo Kaiga, Marimo Sato, Hide Kaneda, Hideaki Tahara

Interleukin-23 (IL-23), a cytokine which is composed of the p40 subunit shared with IL-12 and the IL-23-specific p19 subunit, has been shown to preferentially act on Th1 effecter/memory CD4+ T-cells and induce their proliferation and IFN-γ production. The IL-23 is also reported to act on Th17-CD4+ T-cells which are involved in inducing tissue injury. In this study, we examined the anti-tumor effects associated with systemic administration of IL-23 and their mechanisms in mouse tumor system. Systemic administration of high-dose IL-23 was achieved using *in vivo* electroporation of IL-23 plasmid DNA into the pre-tibial muscles of C57BL/6 mice. The IL-23-treatment was associated with significant suppression of the growth of pre-existing MCA205 fibrosarcoma and prolongation of the survival of treated mice without significant toxicity, when compared with those of the mice treated with EGFP. Although the therapeutic outcomes were similar to those with the IL-12-treatment, the IL-23-treatment induced characteristic immune responses distinctive to those of IL-12-treatment. The IL-23 administration even at the therapeutic levels did not induce detectable IFN-γ concentration in the serum. *In vivo* depletion of CD4+ T-cells, CD8+ T-cells or NK cells significantly inhibited the anti-tumor effects of IL-23. Furthermore, the CD4+ T-cells in the lymph nodes in the IL-23-treated mice showed significant IFN-γ- and IL-17-response upon anti-CD3 mAb stimulation *in vitro*. These results and the ones in the IFN-γ- or IL-12-gene knock-out mice suggest that potent anti-tumor effects of IL-23 treatment could be achieved when the Th1 type response is fully promoted in the presence of endogenously expressed IL-12.

b. The role of molecules involved in apoptotic cell phagocytosis in compromising antitumor immunity and promoting tumorigenesis

Masahisa Jinushi, Hideaki Tahara

Recent studies have unfolded that the engulfment and processing of apoptotic cells by professional phagocytes mediate an important role in inducing immunosuppression and attenuating antitumor immunity. We demonstrated that apoptotic cell-recognized opsonins milk-fat globule-EGF8 (MFG-E8) as well as T cell immunoglobulin mucin protein (TIM)-1/4 critically contribute to form immunosuppressive environments through the peripheral expansion of Foxp 3+regulatory T cells. We further identified MFG -E8 as a major regulator of antitumor immunity in murine and human cancer. MFG-E8 is produced at high levels by tumor-associated macrophages, dendritic cells, and myeloid suppressor cells, and mediates tumor growth and metastasis through the acquisition of apoptosis resistance, epithelial-mesenchymal transition, and the promotion of tumor angiogenesis.

Notwithstanding the pivotal role of MFG-E8 in accelerating malignant phenotype, it remains unclear whether MFG-E8, and other family of opsonins, such as Tim-1/4, and their downstream signaling components, have functional properties on rendering tumors with resistance to various anti-cancer regimens, such as chemotherapy and targeted therapy. We have been in process of elucidating the molecular and immunological machinery of MFG-E8-mediated modification of the therapeutic efficacy of anti-cancer modalities, by which may explore new strategy to restrain tumorigenesis in clinical settings.

c. Identification of human leukocyte antigen-A24-restricted epitope peptides derived from gene products upregulated in lung and esophageal cancers as novel targets for immunotherapy.

Takako Suda, Takuya Tsunoda, Hideaki Tahara

For the development of cancer vaccine therapies, we have searched for possible epitope peptides that can elicit cytotoxic T lymphocytes (CTL) to the TTK protein kinase (TTK), lymphocyte antigen 6 complex locus K (LY6K) and insulin-like growth factor (IGF)-II mRNA binding protein 3 (IMP-3), which were previously identified to be transactivated in the majority of lung and esophageal cancers. We screened 31, 17 and 17 candidate human leukocyte antigen (HLA)-A*2402-binding peptides to parts of TTK, LY6K and IMP-3, respectively. As a result, we successfully established strong CTL clones stimulated by TTK-567 (SYRNEIAYL), LY6K-177 (RYCNLEGPPI) and IMP-3-508 (KTVNELQNL) that have specific cytotoxic activities against the HLA-A24-positive target cells pulsed with the candidate peptides. Subsequent analysis of the CTL clones also revealed their cytotoxic activities against lung and esophageal tumor cells that endogenously express TTK, LY6K or IMP-3. A cold target inhibition assay further confirmed that the CTL cell clones specifically recognized the MHC class I-peptide complex. Our results strongly imply that TTK, LY6K and IMP-3 are novel tumor-associated antigens recognized by CTL, and TTK-567 (SYRNEIAYL), LY6K-177 (RYCNLEGPPI) and IMP-3-508 (KTVNELQNL) are HLA-A24-restricted epitope peptides that can induce potent and specific immune responses against lung and esophageal cancer cells expressing TTK, LY6K and IMP-3.

II. Development of innovative cancer therapy using gene therapy strategies.

a. Development of targeted HSV vector

Kenji Nakano, Hideaki Tahara

We have previously demonstrated HSV targeted entry using a bi-specific bridging molecule (adapter) against glycoprotein-D and epidermal growth factor (EGF) receptor [Mol Ther 2005; 11: 617-26]. This study was to address whether type of ligands, antibody versus natural ligand, to cellular receptor affects on the efficiency of adapter-mediated HSV infection. Adapter proteins, comprising of V-domain of nectin-1 fused to single chain of antibody (scFv) or EGF for EGF receptor, were generated in prokaryotic expression system. Adapter-mediated HSV infection was determined by the expression of reporter genes (β-galactosidase and GFP) 16 hours after incubation of replication-deficient HSV virus (QOZHG) with adapter proteins into the cells. Binding activity and post-binding kinetics of both adapters to EGF receptor were examined for several time points by immunostaining for c-myc tag of adapters in CHO-EGFR and J-EGFR cells. The tracking of virus particles was visualized by transmission electron microscopy. The pathway of HSV entry via membrane fusion or endocytosis was assessed by inhibition assays using lysosomotropic agents. The kinetics and activity of EGF receptor following the inoculation of HSV and adapters were compared between the two adapters by Western blot analysis. Entry efficiency was significantly higher for

scFv-adapter than EGF-adapter (β-galactosidase activity: 2.3 ± 0.2 versus 0.24 ± 0.02; $P < 0.01$), although both adapters similarly adsorbed to the membrane of EGFR-expressing cells. After adsorption, signal of EGF-adapter was shifted to cytoplasmic locus at 15 min and decreased with spotted distributions at 30 min when incubated the cells at 37°C. In contrast, scFv-adapter was detectable predominantly in the cytoplasmic area at 30 min with homogenous distributions, suggesting that natural ligand induces rapid degradation, whereas scFv is resistant. Electron microscopy showed that viral particles were detected in endosome-like vesicles in both cells treated with scFv- and EGF-adapters, and viral degradation in lysosomes was evident at 30 min for EGF-adapter incubation. The attachment of virus to cell membranes was significantly tighter when incubated with scFv-adapters than EGF-adapters, which might be reflected in higher fusion activity. Lysosomotropic agents did not affect the HSV entry significantly. Signaling of EGF receptor, evaluated by phosphorylated EGFR and FAK immunoblots, was activated in cells treated with EGF-adapter, but not with scFv-adapter. In conclusion, binding to cellular target receptor by antibody derivatives, but not natural ligand EGF, induces tight attachment and protects rapid degradation, resulting in efficient HSV infection. (under preparation for manuscript) Based on the above results, we are developing an oncolytic HSV integrated with adapter to amplify the infection and oncolysis to EGFR-expressing cancers.

b. Development of RNAi gene therapy

Kenji Nakano, Hideaki Tahara

A series of evidence has demonstrated that Y-box binding protein-1 (YB-1) plays an important role for multidrug and irradiation resistance in cancer. In the present study, we examined whether nuclear localization of YB-1 is associated with expression of epidermal growth factor receptors (EGFRs), hormone receptors, and other molecules affecting breast cancer prognosis. Expression of nuclear YB-1, clinicopathologic findings, and molecular markers (EGFR, HER2, estrogen receptor-α (ERα), ERβ, progesterone receptor (PgR), chemokine (C-X-C motif) receptor 4 (CXCR4), phosphorylated Akt (p-Akt), and major vault protein/lung resistance protein (MVP/LRP)) were immunohistochemically analyzed. The association of the expression of nuclear YB-1 and the molecular markers was examined in breast cancer cell lines using microarrays, quantitative real-time polymerase chain reaction, and Western blot analyses. Knockdown of YB-1 with small-interfering RNA significantly reduced EGFR, HER2, and ERα expression in ERα-positive, but not ERα-negative, breast cancer cell lines. Expression of nuclear YB-1 was positively correlated with HER2 ($P = 0.0153$), and negatively correlated with ERα ($P = 0.0122$) and CXCR4 ($P = 0.0166$), in human breast cancer clinical specimens, but was not correlated with EGFR expression. Nuclear YB-1 expression was an independent prognostic factor for overall ($P = 0.0139$) and progression-free ($P = 0.0280$) survival. In conclusion, nuclear YB-1 expression might be essential for the acquisition of malignant characteristics via HER2-Akt-dependent pathways in breast cancer patients. The nuclear localization of YB-1 could be an important therapeutic target against not only multidrug resistance but also tumor growth dependent upon HER2, ERα, and CXCR4. We are currently developing an RNAi vector/carrier system for YB-1 to overcome the robustness of cancer against radiation and chemotherapy.

Publications

Kaiga T, Sato M, Kaneda H, Iwakura Y, Takayama T, and Tahara H. Systemic Administration of IL-23 Induces Potent Anti-tumor Immunity Primarily Mediated through Th1-type Response in Association with the Endogenously Expressed IL-12. J Immunol 178: 7571-80, 2007

Jinushi M, Hodi FS, & Dranoff G. Improving antitumor efficacy of GM-CSF secreting tumor cell vaccines. Immunol Rev., in press.

Jinushi M, Vanneman M, Munshi NC, Tai Y-T, Prabhala RH, Ritz J, Neuberg D, Anderson KC, Carrasco DR & Dranoff G. MHC class I chain-related protein A antibodies and shedding are involved in theprogression of multiple myeloma. Proc. Natl. Acad. Sci. U.S.A., in press.

Kobayashi N, Dorfman DM, Pena-Cruz V, Jinushi M, Chernova I, Umetsu SE, Nagumo H, Zhu B, Dranoff G, Kaplan GG, Casasnovas JM, Umetsu DT, DeKruyff RH, & Freeman GJ. T cell Immunoglobulin Mucin Protein (TIM)-4 binds phosphatidylserine and mediates uptake of apoptotic cells. Immunity, 27, 927-940, 2007

Jinushi M and Dranoff G. Triggering tumor immunology through angiogenesis targeting. Clin. Cancer Res. 13, 3762-3764, 2007

Jinushi M, Nakazaki Y, Dougan M, Carrasco DR, Mihm M, & Dranoff G. MFG-E8 mediated uptake of apoptotic cells links the pro- and anti-inflammatory activities of GM-CSF. J. Clin. Invest. 117, 1902-1913, 2007

Tatsumi T, Takehara T, Yamaguchi S, Sasakawa A, Miyagi T, Jinushi M, Sakamori R, Kohga A, Uemura A, Ohkawa K, Storkus WJ & Hayashi N. Injection of IL-12-transduced dendritic cells into mouse liver tumor lesions activates both innate and acquired immunity. Gene Ther. 14, 863-871, 2007

Jinushi M, Takehara T, Tatsumi T, Yamaguchi S, Sakamori R, Hiramatsu N, Kanto T, Ohkawa K, & Hayashi, N. Natural killer cell and hepatic cell interaction via NKG2A leads to dendritic cell-mediated induction of CD4+CD25+T cells with PD-1 dependent regulatory activities. Immunology 120, 73-82. 2007

SudaT, Tsunoda T, Daigo Y, Nakamura Y, Tahara H Identification of HLA-A24-restricted epitope-peptides derived from gene products up-regulated in lung and esophageal cancers as novel targets for immunotherapy. Cancer Science 98: 1803-1808, 2007

Takahashi H, Ishizaki H, Tahara H, Tamaki Y and Yanagi Y. Vaccination with epitope peptide derived from human VEGF Receptor 2 prevent choroidal neovascularization in an Animal Model. Investigative Ophthalmology & Visual Science (In press)

Fujii T, Kawahara A, Basaki Y, Hattori S, Nakashima K, Nakano K, Shirouzu K, Kohno K, Yanagawa T, Yamana H, Nishio K, Ono M, Kuwano M, and Kage M.Expression of erbB2/Her2, estrogen receptor alpha, and chemokine (C-X-C Motif) receptor 4 depends upon nuclear localization of Y-box binding protein-1 in human breast cancers. Cancer Res (in press)

Advanced Clinical Research Center

Division of Clinical Immunology
免疫病態分野

Professor	Chikao Morimoto, M.D., D.M.Sc.	教　授	医学博士	森　本　幾　夫
Associate Professor	Hirotoshi Tanaka, M.D., D.M.Sc.	准教授	医学博士	田　中　廣　壽
Lecturer	Satoshi Iwata, M.D., D.M.Sc.	特任講師	医学博士	岩　田　哲　史
Project Assistant Professor	Hiroto Yamazaki, M.D., D.M.Sc.	特任助教	医学博士	山　崎　裕　人
Project Assistant Professor	Tadanori Yamochi, Ph.D.	特任助教	医学博士	矢　持　忠　徳

Our long term goal is to define the molecular and structural basis for the mechanisms of the immune abnormalities observed in various immune-mediated disorders such as autoimmue disease as well as to cure patients suffering from the above immune-mediated disorders. To accomplish this goal, we have focused on defining the structure and function of cell surface and intracellular molecules expressed in human T cells and other cells and on understanding how the immune regulatory system works in normal and disease conditions. Moreover, we will establish the translational research to cure such diseases. Our study will provide new insights into understanding the precise molecular mechanisms that underlie immune abnormalities found in various autoimmune diseases as well as other immune-mediated disorders and will lead to the development of new rational therapy for the manipulation of the abnormalities found in such diseases.

I. β1 integrins and Cas-L/NEDD9

Satoshi Iwata, Yutaka Hashizume, Koji Yo, Shunsuke Kondo, Sayaka Nomura, Yukiko Nakamura, Akiko Souta-Kuribara, Osamu Hosono, Hiroshi Kawasaki, Hirotoshi Tanaka, and Chikao Morimoto.

β1 integrins play crucial roles in a variety of cell processes such as adhesion, migration, proliferation, and differentiation of lymphocytes. Previously we showed that co-immobilized anti-β1 integrin mAbs or its ligand with a submitogenic dose of anti-CD3 mAb induced a marked increase of IL-2 secretion and proliferative response of T cells, indicating that β1 integrins are costimulatory molecules of T cells. Pp105 was first described in our laboratory as a protein predominantly tyrosine phosphorylated by the ligation of β1 integrins in H9 T cells. By cDNA cloning, we demonstrated that pp105 was a homologue of p130Cas (Crk-associated substrate)/BCAR1 (Breast Cancer Antiestrogen Resistance 1), and designated as Cas-L (Cas lymphocyte type). It has been shown that Cas-L, HEF1 (human enhancer of filamentation), and NEDD9 (neural precursor cell expressed, developmentally down-regulated 9) are identical gene products. We found that transfection of Cas-L cDNA into Jurkat T cells restored β1 integrin-mediated costimulation and cell migration, indicating that Cas-L plays a key role in the β1 integrin-mediated T cell functions.

We demonstrated that the levels of protein expression and tyrosine phosphorylation of Cas-L was markedly elevated in HTLV-I (human T lymphotropic virus type I) Tax transgenic mice, which develop polyarthritis similar to rheumatoid arthritis in human. Furthermore, we found the possible involvement of Cas-L in the patho-

genesis of ATL (adult T cell leukemia) through Tax-mediated overexpression and hyperphosphorylation of Cas-L, which resulted in markedly enhanced motility of lymphocytes. By yeast two-hybrid screening, we revealed that the association of Tax and Cas-L may specifically modulate Tax-mediated transactivation of NF-κB.

Our present projects aim at investigating the molecules associating with Cas-L, and biological significance of those interactions in vitro and in vivo. We believe that our approach will shed a light on the clinical relevance of Cas-L-mediated signaling pathways in inflammatory diseases and malignancies.

a. Cas-L and TGF-β (transforming growth factor-β) pathway

By yeast two-hybrid screening, we demonstrated that Cas-L potentiated TGF-β signaling pathway by interacting with Smad6 and Smad7. Immunoprecipitation experiments revealed that single domain deletion of full length Cas-L completely abolished its docking function with Smad6 and Smad7, suggesting that the natural structure of Cas-L is necessary for its association with Smad6 and Smad7. On the other hand, both N-terminal and C-terminal deletion mutants of Smad6 and Smad7 still retained their docking ability to Cas-L, suggesting that Smad6 and Smad7 possess several binding motifs to Cas-L. Moreover, Cas-L interaction with MH2 domain, but not with MH1 domain of Smad6 or Smad7, ameliorated TGF-β-induced signaling pathway. Finally, depletion of Cas-L by siRNA oligo attenuated TGF-β induces growth inhibition of Huh-7 cells, with a concomitant reduction in phosphorylation of Smad2 and Smad3. These results strongly suggest that Cas-L is a potential regulator of TGF-β signaling pathway.

In order to clarify the effect of Cas-L on the TGF-β signaling in vivo, mouse model of glomerulosclerosis induced by graft versus host disease(GVHD) is a suitable system. Splenocytes from DBA/2 mouse transfused into (DBA/2xC 57B6)F1 mouse induce GVHD in two weeks in the recipient mice causing severe glomerulosclerosis that is a common final pathology of diabetic nephropathy, chronic glomerulonephritis, chronic interstitial nephritis, and obstructive nephropathy. There have been a series of trials that intended to inhibit the progress of mouse glomerulosclerosis by targeting the TGF-βreceptors by monoclonal antibodies. These in vivo experimental trials were only partially successful, because the TGF-β receptor system per se is not the sole activator of R-Smad. Advanced Glycation Endproduct and Angiotensin-II are also potent activators of R-Smad. Thus blockade of TGF-β receptors is not sufficient for arresting the glomerulosclerosis.

We recognize the importance of inhibiting common intracellular signaling molecules. To pursue this issue, we developed Cas-L knockout mice in C57B6 background. By selecting out with PCR, we can selectively develop Cas-L null phenotype of (DBA/2xC57B6)F1 mouse. We plan to induce GVHD both in wild type F1 and mutant F1 to find out the protective effect of Cas-L depletion on the development of glomerulosclerosis. The manipulation of Cas-L could be a way of suppressing the development of chronic kidney disease.

b. Tyrosine phosphorylated Cas-L associates with adaptor protein Nck in lipid raft

We showed that Cas-L associated with adaptor protein Ncks (Nck1 and Nck2) upon β1 integrin- and TCR-mediated stimulation. Co-precipitation experiments revealed that the association of Ncks and Cas-L was dependent of tyrosine phosphorylation of Cas-L, and that Ncks bound to the substrate domain of Cas-L. Co-localization study showed that phosphorylated Cas-L merged with Ncks in the cytoplasm. In addition, it was shown that endogenous tyrosine-phosphorylated Cas-L associated with Ncks in H9 cells upon the stimulation with fibronectin or anti-CD3 mAb. Furthermore, we demonstrated that Cas-L localizes in the lipid raft, where tyrosine-phosphorylated Cas-L interacts with Nck in H9 cells upon stimulation with anti-CD3 mAb. The depletion of Cas-L by siRNA reduced IL-2 production and migration of H9 cells. Finally, we demonstrated that anti-CD3 mAb and SDF-1-induced translocation of Nck into lipid raft was abrogated in splenic T cells from Cas-L -/- mice. Based on the recent study showing that Nck is an important component for the immunological synapse conformation in the lipid raft following the TCR-mediated signaling, our data thus suggest that Cas-Land Ncks may play pivotal role at the immunological synapse in the β1 integrin- and TCR-mediated signaling and cell migration.

c. SHP-2 regulates beta-1 integrin signaling by dephosphorylation of Cas-L

We showed that SHP-2, a non-receptor tyrosine phosphatase containing SH2 domains, associated with Cas-L by co-precipitation study. By immunofluorescence staining, transfected Cas-L and SHP-2 co-localized at protruding edges in 293T cells. Co-precipitation study employing wild type Cas-L and wild type and mutant

SHP-2 (C459S, D425A, and DA/CS) in the presence or absence of Fyn revealed that the association of Cas-L and SHP-2 was enhanced in the presence of the constitutive active form of Fyn, and that the SHP-2 mutants C459S and DA/CS strongly associated with Cas-L. Furthermore, coprecipitation study using deletion mutants of Cas-L and SHP-2 C459S showed that substrate domain of Cas-L was necessary for the association. These result suggested that SHP-2 associates with substrate domain of Cas-L in a tyrosine phosphorylation-dependent manner. In vitro phosphatase assay using GST/SHP-2 fusion proteins showed that GST/PTP domain of SHP-2 dephosphorylated Cas-L in vitro, suggesting that Cas-L is one of the substrates for SHP-2 tyrosine phosphatase. Furthermore, exogenous SHP-2 inhibited migration of A549 lung cancer cell line on the FN-coated Transwell insert in a phosphatase activity-dependent manner.

These results suggest the possibility that SHP-2 negatively regulates the tyrosine phosphorylation of Cas-L, and β1 integrin-mediated cell migration.

d. Cas-L and EGF (epidermal growth factor) pathway

Growth factor-mediated signaling is mediated by receptor protein tyrosine kinases. We evaluated the role of EGF-receptor in tyrosine phosphorylation of Cas-L, since previous study showed that p130Cas/BCAR1 was tyrosine-phosphorylated both by integrins and growth factor receptors. Subsequently we found that EGFR stimulation promote tyrosine phosphorylation of Cas-L in human non small cell lung cancer cell lines (PC-9 and A549), which was abrogated by inactivation of EGFR using Gefitinib. Introduction of siRNA for Cas-L reduced the migratory activity of PC-9 and A549 cells. The extent of reduction was significantly higher in the case with siRNA for Cas-L compared to that of p130Cas. These results indicate that the crosstalk between EGF and integrin signaling pathways might be occurred at the level of Cas-L/NEDD9, and that Cas-L/NEDD9 may be a therapeutic target for the treatment of malignancies such as lung cancer.

II. Structural basis for CD26 mediated T cell costimulation and function in normal and disease conditions.

Kei Ohnuma, Tadanori Yamochi, Wakae Fujimaki, Nozomu Takahashi, Hiroyuki Kayo, Satoshi Iwata, Osamu Hosono, Hiroshi Kawasaki, Hirotoshi Tanaka and Chikao Morimoto (in collaboration with Nam H Dang, Nevada Cancer Institute, USA).

CD26 is a 110-kDa cell surface glycoprotein that posseses dipeptidyl peptidase IV(DPPIV) (EC. 3.4.14.5) activity in its extracellular domain and a primary marker of activated T cells. In the resting state, CD26 is preferentially expressed on a subset of CD4 memory T cells where they account for the majority of IL-2 secretory capabilities and help for B cell Ig production and are the primary responders to recall antigen such as tetanus toxoid. CD26 is also capable of providing a potent costimulatory or "second" signal which can augment other activation pathways leading to proliferation, cytokine production and effector functions. The mechanism of costimulation remains unclear since the cytoplasmic domain consists of only 6 amino acid and lacks a phosphorylation site, leading to the conclusion that CD26 interacts with other cell surface molecules. We have already shown that CD26 may interact with CD45RO which modulates TcR/CD3 activity through its intracellular tyrosine phosphatase domain. Recently, we have detected another CD26 binding protein, the mannose-6-phosphate/insulin-like growth factor II receptor (M6P/IGFIIR) as being critical for this interaction for CD26 mediated T cell costimulation in addition to adenosine deaminase (ADA). More recently, we have shown that CD26 localizes into lipid rafts, and targeting of CD26 to rafts is necessary for signaling events through CD26. Importantly, aggregation of CD26 by anti-CD26 mAb crosslinking also causes coaggregation of CD45 into rafts. In addition, we have demonstrated that recombinant soluble CD26 (sCD26) has an enhancing effect on T cell proliferation in the presence of the recall antigen, tetanus toxoid. This enhancement resulted in an increase in the surface expression of the costimulatory molecule CD86 on monocytes following sCD26 binding to Caveolin-1 expressed on monocytes.

Currently we are focusing on the molecular and structural basis for CD26-mediated T cell activation signaling and are searching for its ligand directly involved in CD26-mediated T cell costimulation. Furthermore we are focusing on the translational research of utilization of anti-CD26 mAb as well as recombinant soluble CD26 for treatment of malignant tumors, immune-mediated disorders and immune deficiency diseases. Hopefully we will perform phase I clinical trial utilizing humanized CD26 antibody for the treatment of the above diseases, this year.

a. Caveolin-1 triggers T-cell activation via CD 26 in association with CARMA1

CD26 is a widely distributed 110-kDa cell surface glycoprotein, having an important role in T-cell costimulation. We previously demonstrated that CD26 binds to caveolin-1 in antigen-presenting cells (APC), and that following exogenous CD26 stimulation, Tollip and IRAK-1 disengage from caveolin-1 in APC. IRAK-1 is then subsequently phosphorylated to upregulate CD86 expression, resulting in subsequent T cell proliferation. However, it is unclear whether caveolin-1 is a costimulatory ligand for CD26 in T-cell. Using soluble caveolin-1-Fc fusion protein, we now showed that caveolin-1 is the costimulatory ligand for CD26, and that ligation of CD26 by caveolin-1 induces T-cell proliferation and NF-κB activation in a TCR/CD3-dependent manner. We also demonstrated that the cytoplasmic tail of CD26 interacts with CARMA1 in T-cells, resulting in signaling events that lead to NF-κB activation. Ligation of CD26 by caveolin-1 recruits a complex consisting of CD26, CARMA1, Bcl10, and IKKβ to lipid rafts. These our results demonstrated that caveolin-1 is the costimulatory ligand for CD26. Taken together, our findings hence provide novel insights into the regulation of T-cell costimulation via the CD26 molecule.

b. Humanized anti-CD26 monoclonal antibody as a treatment for malignant mesothelioma tumors.

CD26 is a 110-kDa cell surface antigen with a role in tumor development. In this report, we show that CD26 is highly expressed on the cell surface of malignant mesothelioma and that a newly developed humanized anti-CD26 monoclonal antibody (mAb) has an inhibitory effect on malignant mesothelioma cells in both in vitro and in vivo experiments. Using immunohistochemistry, 12 patients' surgical specimens consisting of seven malignant mesothelioma, three reactive mesothelial cells, and two adenomatoid tumors were evaluated for expression of CD26. The effects of CD26 on malignant mesothelioma cells were assessed in the presence of transfection of CD26-expressing plasmid, humanized anti-CD26 mAb, or small interfering RNA against CD26. The in vivo growth inhibitory effect of humanized anti-CD26 mAb was assessed in human malignant mesothelioma cell mouse xenograft models. In surgical specimens, CD26 is highly expressed in malignant mesothelioma but not in benign mesothelial tissues. Depletion of CD26 by small interfering RNA results in the loss of adhesive property, suggesting that CD26 is a binding protein to the extracellular matrix. Moreover, our in vitro data indicate that humanized anti-CD26 mAb induces cell lysis of malignant mesothelioma cells via antibody-dependent cell-mediated cytotoxicity in addition to its direct anti-tumor effect via p27 (kip1) accumulation. In vivo experiments with mouse xenograft models involving human malignant mesothelioma cells show that humanized anti-CD26 mAb treatment drastically inhibits tumor growth in tumor-bearing mice, resulting in enhanced survival. Our data strongly suggest that humanized anti-CD26 mAb treatment may have potential clinical use as a novel cancer therapeutic agent in CD26-positive malignant mesothelioma.

c. Comparative study of regulatory T cell function of human CD25+CD4+ T cells from thymocytes, cord blood and adult peripheral blood

CD25+CD4+ regulatory T cells suppress T cell activation in vitro and regulate multiple immune reactions in vivo. To define the regulatory function of human CD25+CD4+ T cells in various maturing stage, we investigated the functional differences of CD25+CD4+ T cells from thymocytes, cord blood (CB) and adult peripheral blood (APB) comprehensively. CB CD25+CD4+ T cells, being mostly CD45RA+, showed no suppressive activity and weak FOXP3 protein expression. In contrast, CD25+CD4+ T cells from thymocytes or APB displayed strong suppressive activity with strong expression of FOXP3 protein. Most of APB CD25+CD4+ T cells exhibited a CD45RA- phenotype with a minor population being CD45RA+. The suppressive activity of CD45RA+ type APB CD25+CD4+ T cells was between those of CD45RA- type APB CD25+CD4+ T cells and CB CD25+CD4+ T cells, and the FOXP3 protein expression of CD45RA+ type cells was weaker than that of CD45RA- type cells. Thymic CD25+CD4+ T cells contained both CD45RA+ and CD45RA- subsets, with both expressing the FOXP3 protein. Although CB CD25+CD4+ T cells showed no suppressive activity, striking suppressive activity was observed after expansion with increased FOXP3 expression and a shift from the CD45RA+ to CD45RA- phenotype. These functional differences in CD25+CD4+ T cells from Thy, CB and APB suggest the pathway to maturation of Treg in peripheral immune system.

III. Therapeutically targetting transcription factors

Hirotoshi Tanaka, Noritada Yoshikawa, Noriaki Shimizu, Chikao Morimoto.

We are interested in the mechanism of eu-

karyotic gene expression and development of novel therapy and/or drugs which target transcriptional machineries. For this purpose, our recent work is mainly focused on conditional regulation of transcription factors including the glucocorticoid receptor and hypoxia-inducible factor-1α.

a. Glucocorticoid receptor (GR) project

Glucocorticoid hormones are effective in controlling inflammation and immunity, but underlying mechanisms are largely unknown. It has been shown that both positive and negative regulation of gene expression are necessary for this process. The genes whose activity is negatively modulated in the anti-inflammatory process code for several cytokines, adhesion molecules. Most of them do not carry a classical binding site for regulation by the GR, but have instead regulatory sequences for transcription factors such as AP-1 or NF-κB. Considering various severe side effects of glucocorticoids, it may be pharmacologically important to dissociate these negative regulatory function of the GR from induction of genes for metabolic enzymes, expression of which have been shown to be positively regulated by the GR. We propose that a certain class of compounds (surprisingly, some of them are non-steridal chemicals) may dissociate transactivation and transrepression function of the GR and offer opportunities for the design of such compounds that could function more effectively as antiinflammatory drugs. In this line, we are developing novel therapeutic strategy.

(i) Redox Regulation of the GR

Redox regulation is currently considered as a mode of signal transduction for coordinated regulation of a variety of cellular processes. Transcriptional regulation of gene expression is also influenced by cellular redox state, most possibly through the oxido-reductive modification of transcription factors. The glucocorticoid receptor belongs to a nuclear receptor superfamily and acts as a ligand-dependent transcription factor. We demonstrate that the glucocorticoid receptor function is regulated via redox-dependent mechanisms at multiple levels. Moreover, it is suggested that redox regulation of the receptor function is one of dynamic cellular responses to environmental stimuli and plays an important role in orchestrated crosstalk between central and peripheral stress responses.

(ii) Development of Dissociating Ligand for the GR

The GR function could be differencially regulated by ligands. We have recently shown that not only synthetic glucocorticoids but also certain bile acids could differentially modulate GR function. Moreover, the effects of those compounds are indicated to be ascrived to the ligand binding domain of the receptor. In this line, we are going to isolate the dissociating ligand that preferencially promotes transrepression function of the GR. Recently we have demonstrated that certain ligands can modulate interdomain communication of the GR, which will eventually contribute to isolation of novel category of ligands.

On the other hand, receptor specificity is another important aspect of novel GR regulator. In this line, we have shown that cortivazol is extremely specific for GR and does not bind to MR. We are studying the molecular basis for this receptor specificity of the ligand using cortivazol as a model. Our recent microarray study demonstrated that GR and MR have differential role in homeostatic regulation in non-classical corticosteroid target tissues including the heart. Notably, collaboration with Professor Miyano's laboratory greatly contributed to development of this program.

(iii) Molecular biology of small nuclear RNA binding protein HEXIM1

Expression of HEXIM1 is induced by treatment of vascular smooth muscle cells with a differentiation inducer hexamethylane bisacetamide. It is shown that HEXIM1 binds 7 SK snRNA and inhibits P-TEFb-mediated transcriptional elongation process. On the other hand, we have found that HEXIM1 directly associates with the GR in the absence of 7SK and represses GR-mediated transcription. We are currently working on regulation of HEXIM1 expression, physiological role of HEXIM1 in GR action. Indeed, HEXIM1 has differential roles in gene regulation in a context and gene specific fashion. We have recently characterized that HEXIM1 may play an important role in tissue-specific regulation of glucocorticoid-mediated gene expression. Physiological significance of HEXIM1 is being studied using newly generated transgenic mice.

b. Hypoxia-inducible Factor (HIF)-1α project

HIF-1α is essential for not only angiogenesis but also development of certain organs. In this line, molecular biology of HIF-1α will provide us possible advantage to characterize and manupilate such processes.

Peripheral T cells encounter rapid decrease in oxygen tension as they are activated by antigen recognition and migrate into inflammatory sites or tumors. Activated T cells, therefore, are

thought to have such machineries that enable them to adapt to hypoxic conditions and execute immune regulation in situ. We have recently shown that survival of CD3-engaged human peripheral blood T cells is prolonged under hypoxic conditions and HIF-1 and its target gene product adrenomedullin play a critical role for the process. It is also shown that hypoxia alone is not sufficient but TCR-mediated signal is required for accumulation of HIF-1α in human peripheral T cells. In the present study, we showed that TCR-engagement does not influence hypoxia-dependent stabilization but stimulates protein synthesis of HIF-1α, most possibly via PI3K/mTOR system, and that expression of HIF-1α and its target gene is blocked by treatment with rapamycin. Since some of those gene products, e.g., glucose transporters and phosphoglycerate kinase-1, are considered to be essential for glycolysis and energy production under hypoxic conditions and adequate immune reaction in T cells, this TCR-mediated synthesis of HIF-1α may play a pivotal role in peripheral immune response. Taken together, our results may highlight a novel aspect of downstream signal from antigen recognition by TCR with giving insight of a unique pharmacological role of rapamycin. We are currently working with the mechanism of translational regulation of HIF-1α.

IV. Cancer Stem Cells

Hiroto Yamazaki, Hiroyuki Kayo, Hiroko Nishida, Ghani Farhana Ishrat, and Chikao Morimoto

a. Stem cell properties and the side population cell as a target for interferon-a in ATLL

The theory of cancer stem cells (CSC) suggests that chemoresistance and recurrence of tumors are often due to the similarity of stem cell properties between normal and cancer cells. Adult T-cell leukemia/lymphoma (ATLL) has poor prognosis, suggesting that ATLL cells possess common stem cell properties. We analyzed side population (SP), a characteristic phenotype of various stem cells, and cell surface antigens in ATLL cell lines. We found that 4 of 12 lines contained SP with expressions of some hematopoietic stem cell markers. On the other hand, treatment with interferon (IFN)-α is sometimes effective in ATLL, particularly in combination with other drugs. We examined the effect of IFNs on ATLL cells and found that IFN-α significantly reduced the SP proportion. Moreover, CD25-positive cells and phosphorylation of STAT1/5 and ERK were upregulated during this process. These data suggest that their stem cell properties render ATLL cells therapy-resistant, and IFN-α exerts its clinical effect through a reduction of the SP cell population.

b. Identification of cancer stem cells in human T-ALL

Although cancer stem cells (leukemia stem cells) have been recently identified in myeloid leukemia, published data on lymphoid malignancy have been sparse. T-acute lymphoblastic leukemia (T-ALL) is a lymphoid neoplasm characterized by the abnormal proliferation of T-cell precursors and is generally aggressive. To identify cells having stem cell properties in T-ALL, we performed extensive analysis of cell surface antigen markers in T-ALL cell lines by flow cytometry. We found that some of the tested cell lines consisted of heterogeneous populations of cells with various levels of surface marker expression. In particular, for CCRF-HSB-2, a small subpopulation of CD90 (Thy-1) and CD110 (thrombopoietin receptor, c-Mpl) double-positive cells were shown to possess stem cell characters, such as asymmetric cell division-like proliferation and tumorigenicity in immunodeficient mice. As both CD90 and CD110 are expressed in hematopoietic stem cells, CD90+/CD110+ cells were considered to be stem-like cells of T-ALL. We also examined peripheral blood samples of pediatric T-ALL patients and found that 3 of 8 cases contained abundant CD90+/CD110+ cells with significant correlation with CD34 expression. In addition, gene expression analysis revealed that the Src family proteins Lyn and Blk were down-regulated in CD90+/CD110+ cells of both the cell line as well as patient samples. These results suggest that CD90+/CD110 + cells are cancer stem cells and Src family proteins play an important role in regulating the stem cell potential of some cases of T-ALL.

Publications

Iwata S, Morimoto C. BCAR1 (p130Cas). Curr Biodata. In press (2008).

Iwata S, Nori M, Morimoto C. Effect of H1 receptor antagonists on cytokine production, various functions of human lymphocytes. Clin Exp Allergy Rev. In press (2008).

Ohnuma K, Takahashi N, Yamochi T, Hosono O, Dang NH, Morimoto C. Role of CD26/

dipeptidyl peptidase IV in human T cell activation and function. Front Biosci. 13: 2299-310 (2008).

Havre PA, Abe M, Urasaki Y, Ohnuma K, Morimoto C, Dang NH. The role of CD26/dipeptidyl peptidase IV in cancer. Front Biosci. 13: 1634-45 (2008).

Kayo H, Yamazaki H, Nishida H, Dang NH, Morimoto C. Stem cell properties and the side population cells as a target for interferon-alpha in adult T-cell leukemia/lymphoma. Biochem Biophys Res Commun. 2007 364: 808-14. (2007).

Kanai Y, Akatsu H, Iizuka H, Morimoto C. Could serum antibody to poly(ADP-ribose) and/or histone H1 be marker for senile dementia of Alzheimer type? Ann N Y Acad Sci. 1109: 338-44 (2007).

Sano M, Tokudome S, Shimizu N, Yoshikawa N, Ogawa C, Shirakawa K, Endo J, Katayama T, Yuasa S, Ieda M, Makino S, Hattori F, Tanaka H, Fukuda K (equal last authors). Intramolecular control of protein stability, subnuclear compartmentalization, and coactivator function of PGC-1alpha. J Biol Chem. 282: 25970-80 (2007).

Inamoto T, Yamada T, Ohnuma K, Kina S, Takahashi N, Yamochi T, Inamoto S, Katsuoka Y, Hosono O, Tanaka H, Dang NH, Morimoto C. Humanized anti-CD26 monoclonal antibody as a treatment for malignant mesothelioma tumors. Clin Cancer Res. 13: 4191-200 (2007).

Makino Y, Uenishi R, Okamoto K, Isoe T, Hosono O, Tanaka H, Kanopka A, Poellinger L, Haneda M, Morimoto C. Transcriptional up-regulation of inhibitory PAS domain protein gene expression by hypoxia-inducible factor 1 (HIF-1): a negative feedback regulatory circuit in HIF-1-mediated signaling in hypoxic cells. J Biol Chem. 282: 14073-82 (2007).

Thompson MA, Ohnuma K, Abe M, Morimoto C, Dang NH. CD26/dipeptidyl peptidase IV as a novel therapeutic target for cancer and immune disorders. Mini Rev Med Chem. 7: 253-73 (2007).

Ohnuma K, Uchiyama M, Yamochi T, Nishibashi K, Hosono O, Takahashi N, Kina S, Tanaka H, Lin X, Dang NH, Morimoto C. Caveolin-1 triggers T-cell activation via CD26 in association with CARMA1. J Biol Chem. 282: 10117-31 (2007).

Sano M, Izumi Y, Helenius K, Asakura M, Rossi DJ, Xie M, Taffet G, Hu L, Pautler RG, Wilson CR, Boudina S, Abel ED, Taegtmeyer H, Scaglia F, Graham BH, Kralli A, Shimizu N, Tanaka H, Makela TP, Schneider MD. Menage-a-trois 1 is critical for the transcriptional function of PPARgamma coactivator 1. Cell Metab. 5: 129-42 (2007).

Ohnuma-Ishikawa K, Morio T, Yamada T, Sugawara Y, Ono M, Nagasawa M, Yasuda A, Morimoto C, Ohnuma K, Dang NH, Hosoi H, Verdin E, Mizutani S. Knockdown of XAB2 enhances all-trans retinoic acid-induced cellular differentiation in all-trans retinoic acid-sensitive and -resistant cancer cells. Cancer Res. 67: 1019-29 (2007).

Inamoto, S., Iwata, S., Inamoto, T., Nomura, S., Sasaki, T., Urasaki, Y., Hosono, O., Kawasaki, H., Tanaka, H., Dang, NH., Morimoto, C. Crk-associated substrate lymphocyte type regulates transforming growth factor-beta signaling by inhibiting Smad6 and Smad7. Oncogene. 26: 893-904 (2007).

Takuya Fukazawa, Yutaka Maeda, Mary L. Durbin, Toru Nakai, Junji Matsuoka, Hirotoshi Tanaka, Yoshio Naomoto, and Noriaki Tanaka. Pulmonary adenocarcinoma-targeted gene therapy by a cancer- and tissue-specific promoter system. Mol. Cancer Ther. 6: 244-252 (2007).

Tanaka H, Yoshikawa N, Shimizu N, Morimoto C. Strategic targeting of the glucocorticoid receptor for anti-inflammation. Inflammation and Regeneration. 27: 486-493 (2007).

Advanced Clinical Research Center

Division of Medical Data Processing Network System
ゲノム医療情報ネットワーク分野

Professor Tetsuo Shimizu

教 授 清 水 哲 男

The purpose of Research Laboratory of Medical Data Processing Network System is to research and develop advanced system engineering methodology and computer technology suitable for the 21st century type research hospital. The system is called "Infrastructure for Translational Research of Genome Based Medicine", which is expected to strongly support bi-directional translation between genome based life-science and clinical medicine. Our main research objectives are as follows;
-Construction of knowledge database system for translational research between genomic science and clinical medicine called "e-pathfinder",
-Development of clinical protocol management system using computer technologies called "e-protocol",
-Research and development of agent based simulation methodology for epidemiological and biological analysis and application of "Logical Atomism" and "Hypercycle Theory" to systems medical sciences.

1. Integrated knowledge database system (E-pathfinder)

In post-genomic era, bridging genomic science and clinical medicine is the most important issue. To make bi-directional migration of knowledge in both fields efficient, it is necessary to integrate knowledge of genomic science and clinical science in a single architecture with Information Technology. To this end, we defined EBKA (Evidence Based Knowledge Architecture as the single architecture. EBKA has LEAHS (Logically Extended Anatomically Hierarchical Structure) as a knowledge backbone that enables to integrate knowledge from macro to micro. In LEAHS, knowledge is represented in knowledge unit that is a set of logical unit and its supportive evidence.

Focusing on hematology and immunology, we have tried to develop an integrated knowledge database system (E-pathfinder) prototype, which is able to be customized by medical users. This system is intended to bridges between clinical and biological knowledge under LEAHS and to represent the huge amount of knowledge using GUI (Graphical User Interface). For instance, it shows clinical information (epidemiology, pathogenesis, diagnosis, treatment and prognosis etc.), models of molecular mechanism of diseases, information of genes involved in diseases, treatment protocols, information of clinical examination and drugs, and so on, in an easy-to-understand way. Furthermore, we plan to incorporate E-pathfinder with other systems useful for translational research, including clinical protocol management system described as below.

In this year we have continued to develop the system for blood and immunological diseases. We believe that many clinicians and basic scientists might have great insights from this knowledge database system in the near future.

2. Clinical protocol management system for translational research

The realization of a clinical method that brings from molecular biological findings requires experiment "in human," that is, "experimental care." Because experimental care entails various risks in the process of the care, it should be done in the maximum of safety and efficiency, which is essential for the success of translational research. We are developing a protocol management system that supports experimental care. In this year we have designed the concept of the protocol management system and also have constructed a prototype of the system.

The system manages optimized operation of experimental care by means of the combination of (1) the protocol development support and (2) the implementation management of protocols. By the word "protocol," we mean a detailed procedure of experimental care, which should ensure the maximum of patient's safety. The first subsystem, protocol development support system, helps to construct protocols. A protocol for an experimental care is constructed by integrating new methods into an existing or standard care procedure. The integrated procedure should be well broken down so that it clarifies what each clinical staff should do in every situation of the care. We call such a concrete level of procedures, "working activity plan." The working activity plan should be developed considering various conditions, for example, personal health conditions and resources of a health care provider. The protocol development support system gives tools for the integration and the break-down task.

At the same time, the compliance with the constructed protocol is also important to accomplish the safe and efficient care. The second subsystem, protocol implementation management system, assists each clinical staff smoothly to comply with the protocol. Based on the working activity plan, the subsystem gives appropriate instructions to members of the staff at the proper timing. When a member completes a task, the result will be recorded on the electric medical record database. Making use of the working activity plan and the database, the subsystem manages and analyzes the experimental data, as well as the support for clinical decisions. These features enable the prevention of wrong care, and the early detection of patient's abnormal conditions. In this year we have developed the system especially for chord blood cell plantation protocol.

3. *Research and development of agent based simulation methodology for epidemiological and biological analysis and application of "Logical Atomism" and "Hyper-cycle Theory" to systems medical sciences*

Agent based modeling is becoming more important for evidence based policy making. This modeling method is expected to provide traceability of the evidence for economical, social and organizational planning. We apply the method of agent based simulation for the analysis of epidemiological and biological issues.

We have developed a new framework of agent based modeling named SOARS (Spot Oriented Agent Role Simulator). In this development, we first aimed at the modeling of the SARS (Severe Acute Respiratory Syndrome) infection in hospital where many kinds of human agents interact with each other. However, it was difficult to represent the complex social behavior of human agents properly by the present modeling platforms. So we developed SOARS as a multipurpose agent based modeling platform implemented in Java computational language.

In hospital, there are various persons who play each role such as "doctor", "nurse" and "patient". They act complex social behavior as their own rules and interact with each other. We regard the persons as autonomous agents with various interactions like cooperation, opposition, or unaware infection. We treat them as a kind of Complex Systems, and abstract the model of agents' rule actions and represent them as computational script language.

Also, in biological view, a human body consists of various organs, and the organs consist of numerous cells. The ordinary biological analyses handle the cells by quantitative methods mainly using differential equation, but the analyses of some diseases caused by functional abnormality of cells need representation of qualitative changes and may properly described by the method of agent based simulation. We are also developing hybrid simulation platform of continuous and discrete agent based system using SOARS. In this year we have developed prototype simulation system especially for blood cell interaction taking Hyper-cycle theory, which was developed by Manfred Eigen, into consideration.

"Logical Atomism" is the basic language system proposed by Bertrand Russell for the foundation of all physical sciences. This theory combined with "Hyper-cycle Theory" will provides

us a powerful tool to describe ontological system consisting of many types and classes of lives, because one logical atom is corresponding to one hyper-cycle, one computer-program, or one recursive function. We are planning and preparing its application to genome based medical sciences through constructing "e-pathfinder" system.

Publications

清水哲男：「一握の砂」から生・老・病・死の宇宙へ―システム医科学序論―, preprint, 2007.

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

Our division was launched in April 2007. For the application of human genome data to clinics, we have been working on the following three projects; 1) the identification of novel diagnostic and therapeutic strategies of human cancer, 2) the prediction of sensitivity of anticancer drugs, and 3) genetic diagnosis and care of patients with hereditary cancer (HNPCC). 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

Fabio Pittella Silva, Masaki Kunizaki, Kiyoshi Yamaguchi, Yoichi Furukawa, Yusuke Nakamura[1]: [1]Laboratory of Molecular Medicine, Human Genome Center

To identify novel target molecules for diagnosis and treatment of human cancers arising in various gastrointestinal organs, we have been studying their global gene expression profiles using genome-wide cDNA microarray. Profiles of colorectal cancer (CRC), gastric cancer, hepatocarcinoma (HCC), and intrahepatic cholangiocarcinoma (ICC) have been obtained. Among the gene expression profiles of 25 ICCs, we selected RAD51 associating protein-1 gene (*RAD51AP1*) as a candidate for clinical application, because its expression was frequently elevated in our microarray data. Quantitative PCR confirmed that RAD51AP1 expression was elevated in the great majority of ICCs examined. Immunohistochemical analysis with anti-RAD51AP1 antibody further corroborated its accumulation in 14 of 23 ICC tissues (61%). Notably, suppression of RAD 51AP1 by short-interfering RNA resulted in growth suppression of cholangiocarcinoma cells. Since RAD51AP1 interacts with RAD51, a molecule involved in DNA repair, we investigated whether RAD51AP1 is implicated in DNA strand-breaks using γ-irradiation. As a result, γ-irradiation augmented RAD51AP1 protein expression, and brought a focus formation in the nuclei where accumulated RAD51AP1 co-localized with phospho-histone 2AX (γ-H2AX) or RAD51. These data suggest that RAD51AP1 may play a role in cell proliferation as well as DNA repair. Our findings may contribute to the better understanding of cholangiocarcinogenesis, and open a new avenue to the development of novel therapeutic and/or diagnostic approach to this type of tumor.

2. Functional analysis of SMYD3, a molecular target for the treatment of CRC, HCC, and breast cancer

Fabio Pittella Silva, Masaki Kunizaki, Kiyoshi Yamaguchi, Yoichi Furukawa, Yusuke Nakamura[1]: [1]Laboratory of Molecular Medicine, Human Genome Center, IMSUT

We earlier showed that SMYD3, a histone H3-Lysine 4-specific methyltransferase, is frequently up-regulated in human colorectal, liver, and breast cancer compared to their matched non-cancerous cells, and that its activity is associated

with the growth of these tumors. In the present study, we found that human cancer cells express both the full-length and a cleaved form of SMYD3 protein. Amino acid sequence analysis uncovered that the cleaved form lacks the 34 amino acids in the N-terminal region of the full-length protein. Interestingly the cleaved protein and mutant protein containing substitutions at glycines 15 and 17, two highly conserved amino acids in the N-terminal region, revealed a higher histone methyltransferase (HMTase) activity compared to the full-length protein. Furthermore, the N-terminal region is responsible for the association with heat shock protein 90 alpha (HSP90α). These data indicate that the N-terminal region plays an important role for the regulation of its methyltransferase activity and suggest that a structural change of the protein through the cleavage of the region or interaction with HSP90α may be involved in the modulation of euzyme activity. These findings may help for a better understanding of the mechanisms that modulate the histone methyltransferase activity, and contribute to the development of novel anticancer drugs targeting SMYD 3.

In addition, we found that vascular endothelial growth factor receptor-1 (VEGFR1) was also methylated by SMYD3. We further identified the methylated residue at VEGFR1 lysine 831 that is located in the kinase domain and is conserved among VEGFR1 orthologues. The lysine is followed by serine, which is conserved among some of the methylation targets of histone methyltransferases. Furthermore, methylation of VEGFR1 enhanced its kinase activity in cells. These data should be helpful for the profound understanding of the biological role of SMYD3 and regulatory mechanisms of VEGFR1. Additionally our finding may facilitate the development of strategies that may inhibit the progression of cancer cells.

3. Prediction of sensitivity of Gefitinib to lung cancer, and that of Imatinib to CML.

Yoichi Furukawa, Yusuke Nakamura[1,2], Toyomasa Katagiri[1], Yataro Daigo[2], Noriharu Sato[3], Naoyuki Takahashi[3], Tsuyoshi Fujii[4]: [1]Laboratory of Genome Technology, Human Genome Center, IMSUT, [2]Laboratory of Molecular Medicine, Human Genome Center, IMSUT, [3]Department of Applied Genomics, Research Hospital, IMSUT, [4]Department of Infectious Diseases and Applied Immunology, Research Hospital, IMSUT

In the second project, we are working on a prospective study of the prediction of sensitivity to Gefitinib (Iressa) in patients with lung adenocarcinoma, in collaboration with Human Genome Center, and Department of Applied Genomics and Department of Infectious Diseases and Applied Immunology in Research Hospital, IMSUT. In our earlier study, we investigated expression profiles of lung adenocarcinomas that were treated with Gefitinib (Iressa), which identified 12 genes that can discriminate tumors with sensitivity to the drug from those without sensitivity. Two other groups reported that genetic alteration of *EGFR*, the target of Gefitinib, was associated with the efficacy. Since these studies and ours analyzed a limited number of clinical samples, sensitivity and reliability of the two prediction methods remain unresolved. Therefore, we started a prospective study to analyze both expression profile and *EGFR* mutation of tumor tissues prior to treatment with Gefitinib. An outpatient clinic for consultation of the applicants was opened in Research Hospital, IMSUT in September 2004, and department of respiratory medicine, Kawasaki Medical University joined to this project in 2005. In 2007, five patients were enrolled in this study after informed consent was obtained. Tumor specimens from the five were obtained by surgical operation, and all five samples were successfully analyzed.

In another study, we investigated expression profiles of 26 chromic myeloid leukemia (CML) with high-sensitivity and those with low-sensitivity to Imatinib (Glivec), and identified a total of 79 genes differently expressed between high- and low-sensitivity groups. We developed a prediction system of the sensitivity using expression of 15 genes among the 79. For the application of the findings into clinics, we launched a prospective study to evaluate the system, and analyzed expression profiles of additional CML samples in collaboration with Applied Medicine in Research Hospital, IMSUT. An outpatient clinic for consultation of applicants was opened in June, 2004, and one patient with CML visited the clinic in 2007. After written informed consent was obtained, blood samples were taken from the patient. FISH and expression profile analyses were carried out, and calculated prediction score of the sensitivity to Imatinib was reported to the patient in the clinic.

4. Genetic diagnosis of HNPCC

Yoichi Furukawa, Yusuke Nakamura[1]: [1]Laboratory of Molecular Medicine, Human Genome Center, IMSUT

Hereditary non-polyposis colorectal cancer

(HNPCC) is an autosomal dominant hereditary disease accompanied by tumors arising mainly in the colon and other associated organs, such as stomach, renal pelvis, and endometrium. The frequency of HNPCC in Caucasian patients with colorectal cancer is estimated between two and five percent. However the frequency in Japanese patients with colorectal cancer remains undetermined. Therefore, Japanese Study Group for Colorectal Cancer started a collaborative project of registration of Japanese HNPCC patients and genetic analysis of mutations in *MSH2*, *MLH1*, and *MSH6*, the responsible genes for HNPCC. All patients with colorectal cancer and those who are diagnosed as HNPCC by Amsterdam's II criteria in the collaborative hospitals have been registered, and the frequency of HNPCC in registered patients with colon cancer has been determined. Collaborating to this project, we have analyzed genetic alteration in a total of 128 patients using PCR-direct sequencing and Multiplex Ligation-dependent Probe Amplification. Among the 128 cases, 11 cases were analyzed in 2007. The data will provide valuable information for the understanding of the frequency, penetrance and phenotypes of Japanese HNPCC. The results will be also used for genetic diagnosis of affected family members of the probands.

Publications

1. Obama K, Satoh S, Hamamoto R, Sakai Y, Nakamura Y, Furukawa Y. Enhanced expression of RAD51AP1 is involved in the growth of intrahepatic choloangiocarcinoma cells. Clin. Cancer Res, 14 (5): 1333-1339, 2007.
2. Silva FP, Hamamoto R, Kunizaki M, Tsuge M, Nakamura Y, Furukawa Y. Enhanced methyltransferase activity of SMYD3 by the cleavage of its N-terminal region in human cancer cells. Oncogene, 2007 Nov (12, Epub).
3. Kidokoro T, Tanikawa C, Furukawa Y, Katagiri T, Nakamura Y, Matsuda K. CDC20, a potential cancer therapeutic target, is negatively regulated by p53. Oncogene, 27 (11): 1562-1571, 2008.
4. Kunizaki M, Hamamoto R, Silva FP, Yamaguchi K, Nagayasu T, Shibuya M, Nakamura Y, and Furukawa Y. The lysine 831 of VEGFR1 is a novel target of methylation by SMYD3. Cancer Res, 67 (22): 10759-10765, 2007.