Research Hospital

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先端診療部

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Department of Advanced Medical Science was established in September 1997. Our aim is to contribute to the performance and the development of advanced therapeutic approach to the diseases. We have been participating in the potentially important clinical trials and the several projects in line with our principles. Our research projects were 1) Analysis of adaptive transfer of allogeneic umbilical cord blood-derived cytotoxic lymphocytes, 2) Analysis of the role of Dnm3os, a non-coding RNA in skeletal development, 3) Analysis of the Gradient Expression of Genes in Human Colonic Mucosa, 4) Analysis on the mechanisms of cardiac outflow tract development, and 5) Analysis of microRNA expression during inflammatory response of human cord blood and peripheral blood mononuclear cells.

1. Adaptive transfer of allogeneic umbilical cord blood-derived cytotoxic lymphocytes

Nagayama H., Fujita S., et al.

We have intensively investigated the possibility of adaptive transfer of allogeneic umbilical cord blood-derived cytotoxic lymphocytes (CTLs) for the treatment of cancer. Cryopreserved umbilical cord blood was used for the source of CTLs. Various combinations of T cell growth factors and various methods for antigen stimulation were tested for massive expansion to use for adoptive transfer. Flowcytometric analysis using phenotypical markers, HLA-restricted tetramer analysis and cytoplasmic interferon-γ staining reveals the possibility of clinical application. (manuscript in preparation) Now we are claiming the patent of this method from the University of Tokyo (Japanese patent claiming No. 2009-188251.) and will be exposed later.

2. Analysis of the role of *Dnm3os*, a non-coding RNA in skeletal development

Nakaoka T. et al.

Dnm3os, a gene transcribed into a non-coding RNA (ncRNA), contains three micro RNAs; miR-199a, miR-199a* and miR-214, whose functions remain entirely unknown in mammals. We generated *Dnm3os* knock-out (KO) mouse in collaboration with Department of Physiological Chemistry and Metabolism, Division of Biochemistry and Molecular Biology, University of Tokyo. *Dnm3os* KO mice exhibited several skeletal abnormalities, including craniofacial hypoplasia, defects in dorsal neural arches and spinous processes of the vertebrae, and osteopenia. Importantly, the expression of miR-199a, miR-199a*, and miR-214 was significantly

down-regulated in *Dnm3os* KO embryos, supporting the assumption that *Dnm3os* serves as a precursor of these three miRNAs. Now, we are investigating the molecular mechanisms responsible for the skeletal abnormalities observed in *Dnm3os* KO mice.

3. Analysis of the Gradient Expression of Genes in Human Colonic Mucosa

Ohno H. et al.

Ulcerative colitis is characterized by continuous inflammation extending from rectum to oral colonic mucosa. Epidemiological data have provided incontrovertible evidence that both genetic and environmental factors are important in the disease susceptibility. We speculate that the gradient expression of genes in human colonic mucosa might be related to the disease development and progression. First, we selected the genes whose expression levels were reported to increase toward the distal colon. Next, we evaluated the expression levels of these genes throughout the GI tract and in other tissues by northern blot analysis. As a result of this analysis, some genes showed the expression gradient to increase toward the distal colon. Interestingly, one of the genes highly expressed in enteroendocrine cells of rectal mucosa. The data suggest that the gene may act as an endocrine hormone of GI tract. We are currently investigating the expression changes of the gene in human intestinal diseases.

4. Analysis on the mechanisms of cardiac outflow tract development

Nakaoka T. et al.

Malformations of the cardiovascular system in the human account for most of the premature deaths caused by congenital abnormalities and, most often, are linked to abnormalities in the formation of the cardiac outflow tract. The heart defect (hdf) mouse is a recessive lethal mutation that arose from a LacZ reporter containing a transgene insertional mutation. The most striking feature of the hdf homozygous embryo is the immature formation of the outflow tract. As a result of chromosome mapping and analysis of the DNA sequence flanking the transgene, *Cspg2* was identified as the gene disrupted by the insertional mutation in the *hdf* mouse line. The *Cspg2* gene, encodes the extracellular matrix protein, versican. We are analyzing the second heart field and the neural crest cells, which actively contribute to the formation of the cardiac outflow tract in hdf mouse embryos, in ordre to elucidate the role of versican during embryonic cardiac development.

5. Analysis of microRNA expression during inflammatory response of human cord blood and peripheral blood mononuclear cells

Takahashi N. et al.

Compared with allogeneic hematopoietic stem cell transplantation using other sources, cord blood transplantation (CBT) has clinical advantages in terms of incidence and severity of acute graft-versus-host disease (aGVHD) despite using allogeneic stem cells with more human leukocyte antigen mismatches. However, detailed molecular mechanism of aGVHD developed after CBT has not yet been elucidated. MicroRNAs (miRNAs), important regulators of many cellular processes including cell growth and differentiation, have been reported to show specific expression signatures in different blood cell lineages. To elucidate the roles of miRNAs in the pathogenesis of aGVHD after CBT, we examined expression of 69 miRNAs and 11 transcription regulator genes during inflammatory response of $CD4^+$, $CD8^+$, and $CD14^+$ cells derived from cord blood (CB) and adult peripheral blood (APB) by quantitative RT-PCR.

A total of 20 miRNAs showed differential expression between CB and APB-derived cells, three of which were down-regulated in all cell lineages of CB. Inflammatory stimulation altered miRNA expression almost exclusively in CB-derived cells. These differences in miRNA expression between CB and APB may contribute to the blunt response of CB-derived cells in inflammatory conditions.

Publications

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Research Hospital

Department of Medicine (Department of Hematology / Oncology)
内科（血液腫瘍内科）

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We have been challenging to cure intractable hematological disorders such as leukemia and lymphoma mainly with the aid of hematopoietic stem cell transplantation (HSCT). No less than 30 patients per year receive allogeneic HSCT in our facilities. In recent years, unrelated cord blood has been our major stem cell source for recipients who have no suitable family donors in HSCT. Since 1998 we have performed over 300 cases of cord blood transplantation (CBT) for adult patients, which appears a distinguished experience in the world. Recent advance in identification of signaling molecules activated in a tumor-specific manner or associated with tumor-specific genomic recombination have disclosed many candidate therapeutic targets in tumors. In the field of hematological malignancies, we have already experienced remarkable clinical efficacies of imatinib mesylate (ABL kinase inhibitor) for CML, rituximab (chimeric anti-CD20 monoclonal antibody) for B cell lymphoma as well as bortezomib (proteasome inhibitor) for multiple myeloma. We extensively apply these molecular targeted therapies for in- and out-patients. Furthermore, in recent years, our department has been a hub facility in the greater Tokyo area for treating patients with intractable adult T-cell leukemia/lymphoma.

1. Clinical study of nilotinib in patients with imatinib-resistant or -intolerant CML or relapsed/refractory Ph＋ ALL.

Tojo A, Uchimaru K, Yuji K, Ohno N

We are currently involved in clinical trial of newly developed agents. Nilotinib, one of these, is a second-generation BCR-ABL kinase inhibitor with improved potency and selectivity compared to imatinib. A Phase I/II dose-escalation study was designed to evaluate the efficacy, safety, and pharmacokinetics of nilotinib in Japanese patients with imatinib-resistant or -intolerant Philadelphia chromosome-positive (Ph＋) chronic myelogenous leukemia (CML) or relapsed/refractory Ph＋ acute lymphoblastic leukemia (ALL). A total of 34 patients were

evaluated in this analysis and had a median duration of drug exposure of 293 (range 13-615) days. All 6 CML-CP patients without complete hematologic response (CHR) at baseline rapidly achieved CHR. A major cytogenetic response was achieved in 94% of patients with CML-CP, including a complete cytogenetic response in 69 %. A major molecular response was achieved by 56%. These responses were also observed in patients with CML in advanced stages and Ph+ ALL. Non-hematologic adverse events were mostly mild to moderate. Grade 3 or 4 neutropenia and thrombocytopenia occurred in 50 and 28% of patients, respectively. Overall, the results of this study suggest that nilotinib induced significant responses in imatinib-resistant or -intolerant patients with CML-CP and CML in advanced stages and Ph+ ALL. The results of this study confirmed the efficacy and safety of nilotinib in Japanese patients.

2. Unrelated cord blood transplantation after myeloablative conditioning in adults with ALL.

Ooi J, Takahashi S, Tsukada N, Tojo A

We analyzed the disease-specific outcomes of adult ALL treated with cord blood transplantation (CBT) after myeloablative conditioning. Between October 2000 and November 2007, 27 adult patients with ALL were treated with unrelated CBT. All patients received four fractionated 12 Gy TBI and chemotherapy as myeloablative conditioning. The median age was 36 years, the median weight was 57 kg and the median number of nucleated cells was 2.47×10^7/kg. All patients received a single and HLA-mismatched cord blood unit. The cumulative incidence of neutrophil recovery at day 30 and platelet recovery at day 200 was 92.6 and 92.3%, respectively. With a median follow-up of 47 months, the probability of EFS at 5 years was 57.2%. The 5-year cumulative incidence of TRM and relapse was 3.7 and 27.4%, respectively. These results suggest that unrelated CBT after myeloablative conditioning could be safely and effectively used for adult patients with ALL.

3. Myeloablative unrelated cord blood transplantation for acute leukemia patients between 50 and 55 years of age: single institutional retrospective comparison with patients younger than 50 years of age.

Konuma T, Takahashi S, Ooi J, Tomonari A, Tsukada N, Uchimaru K, Tojo A.

Increasing recipient age is a well-known risk factor for graft-versus-host disease (GVHD) and treatment-related mortality (TRM) and has a negative impact on allogeneic hematopoietic stem cell transplantation. Since the incidence of severe GVHD after cord blood transplantation (CBT) is lower than that after transplants using bone marrow or mobilized peripheral blood grafts from adult cells, we should expect better outcomes from CBT in older patients. To evaluate the feasibility and efficacy of myeloablative unrelated CBT in patients aged between 50 and 55 years, we performed a retrospective comparison of 100 patients with acute leukemia who received cord blood grafts at our institution. Nineteen older patients (median age, 52; range, 50-55) and 81 younger patients (median, 36; range, 16-49) received a myeloablative conditioning regimen including 12 Gy of total body irradiation and chemotherapy. GVHD prophylaxis included cyclosporine with (n=96) or without (n =4) methotrexate. There were no significant differences in the incidences of grades II to IV acute GVHD, extensive-type chronic GVHD, TRM, and the probability of overall and disease-free survival between these groups. These results suggest that, in patients with acute leukemia, myeloablative CBT might be as safe and effective in patients aged between 50 and 55 years as in younger patients.

4. Second myeloablative stem cell transplantation (SCT) using cord blood for leukemia relapsed after initial allogeneic SCT.

Konuma T, Ooi J, Takahashi S, Tomonari A, Tsukada N, Uchimaru K, Tojo A.

There are many reports of second allogeneic stem cell transplantation (allo-SCT) using cord blood (CB) for graft failure after initial allo-SCT. However, the efficacy of second allo-SCT using CB for patients with leukemia relapsed after initial allo-SCT is unknown. We report the results of second allo-SCT using CB in seven adult patients with leukemia relapsed after initial allo-SCT. All patients received a myeloablative conditioning regimen including oral busulfan 16mg /kg, intravenously fludarabine 100mg/m^2 and cyclophosphamide 120mg/kg. All but one patient had myeloid reconstitution and four patients remain alive at between 4 and 40 months after second SCT. We conclude that second myeloablative allo-SCT using CB may be feasible in selected patients with the relatively younger age, less organ damage and longer time interval between first and second allo-SCT.

5. Migration effects increase asymptomatic carriers of HTLV-1 in greater Tokyo area.

Uchimaru K, Tojo A

We previously reported that immigrant from endemic area, offspring of mothers who moved from endemic area and individuals presumed to be involved in sexual transmission from spouse born in endemic area constitute approximately 70% of the HTLV-1 carriers in greater Tokyo area. This year we re-analyzed factors predisposing to HTLV-1 infection in residents of greater Tokyo area adding 48 HTLV-1 carriers who visited our outpatient clinic to the previous cohort. The HTLV-1 carriers moved from endemic area constitute 39.1% of 161 individuals analyzed in this study. Those who were born in greater Tokyo were 34.1% and markedly increased compared with those in the nation-wide study in 1988. In these carriers who were born in greater Tokyo, 42.6% has mothers from endemic area and 23.4% has spouse or father from endemic area. These data indicated that 66% of the HTLV-1 carriers in greater Tokyo related to migration from endemic area, which confirmed the results of our previous study.

6. Nation-wide cooperative cohort study of HTLV-1 carriers to reveal predisposing factors for ATL development (JSPFAD) and surveillance study of HTLV-1 carriers

Uchimaru K

JSPFAD is the nation-wide cohort study to reveal predisposing factors for ATL development established in 2002. We are acting as a member of JSPFAD and enrolled 55 new participants this year. From August 2002 to December 2009, 1905 participants of HTLV-1 asymptomatic carriers were enrolled in this study and 173 of them were enrolled at our department. During follow-up period, 14 participants progressed to overt ATL and their base-line HTLV-1 proviral load was higher than that of participants who didn't progress to ATL. None developed ATL whose proviral load was lower than 4%. Multivariate Cox analysis revealed that higher proviral load was an independent risk factor for progression of ATL from carrier status. Nation-wide surveillance of HTLV-1 carriers was started from 2008 after over 20 years period and we participated in the study. It was estimated from this study that there were 1.1 million HTLV-1 carriers in Japan and it was not to be expected that the number of carriers did not decrease compared with that in the previous study in 1988. Another important information from this study was that the distribution of HTLV-1 carriers in Japan had changed. As we predicted in our previous study described above, the proportion of HTLV-1 carriers residing in greater Tokyo would increase more and more in HTLV-1 carriers in Japan and national politics is needed. In this study, we are now making a manual for follow-up of HTLV-1 carriers.

Publications

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Research Hospital

Department of Infectious Diseases and Applied Immunology
感染免疫内科

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【兼任】
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（先端医療研究センター感染症分野）

Founded in 1981, Department of Infectious Diseases and Applied Immunology (DIDAI) started HIV clinic in 1986. In 2009, 64 new patients with HIV infection have visited or been admitted to our hospital and 447 patients in total are currently under our clinical management. The total number of in-patients with HIV-infection during 2009 was 29, and 5-7 beds in our ward have been constantly occupied by patients with not only HIV-infection but also other infectious diseases. Since the number of the staff members of DIDAI is too small to care both outpatients and in-patients, members of the Division of Infectious Diseases and the Department of Infectious Disease Control join the clinic. IMSUT hospital provides the most up-to-date medical treatment to HIV-infected patients in Japan. DIDAI is also a treatment center for international infectious diseases such as malaria and typhoid fever.

1. Treatment of and clinical research on HIV infection and related diseases.

a. Treatment of HIV infection in IMSUT hospital: Statistical characteristics of HIV infected patients in IMSUT hospital this year

Takeshi, Fujii, Tomohiko Koibuchi, Toshiyuki Miura[1], Hitomi Nakamura[1], Michiko Koga[1], Tadashi Kikuchi, Kentaro Imai, Takashi Odawara, and Aikichi Iwamoto[1]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center

64 new patients with HIV-1 infection visited our hospital this year (from January 1 to December 31, 2009), and 447 patients in total are under medical management in our outpatient clinic. The total number of HIV-infected in-patients during 2009 was 29. The number of total patients declined in 1997 because a part of patients as well as medical stuffs moved to newly established AIDS Clinical Center in International Medical Center of Japan. However, the number of patients started to increase again after 1998 in accordance with Japanese statistics of HIV-infected patients (Fig. 1). In contrast, the number of admission has decreased since 1997 and stable over the last decade (Fig. 2) because of the introduction of highly active anti-retroviral ther-

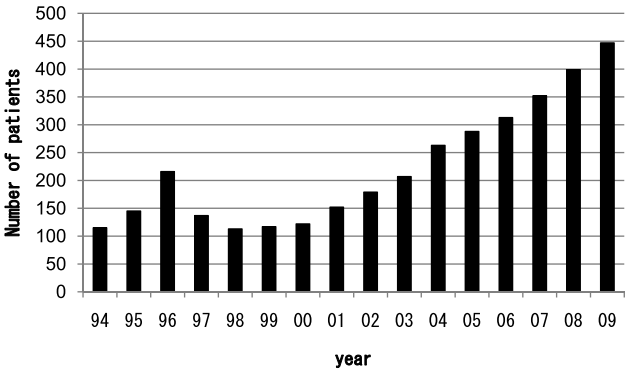

Figure 1. Number of HIV-infected outpatients in IMSUT Hospital

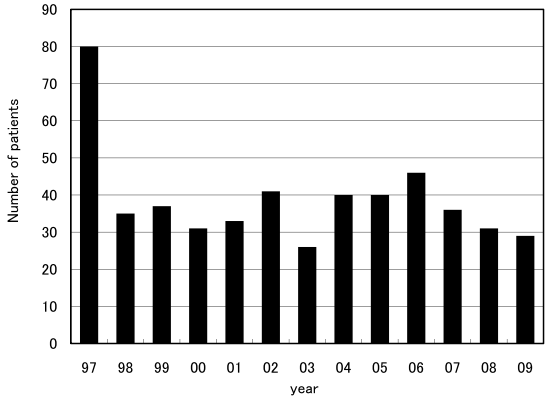

Figure 2. Number of HIV-infected inpatients in IMSUT Hospital

apy (HAART) which effectively suppresses the replication of HIV. Anti-retroviral therapy has been introduced to around 320 HIV-infected patients in our hospital, and most of their HIV viral loads have been well controlled. After one year of HAART, the viral loads become less than 400 copies/ml in more than 90% of patients, and their CD4 cell counts increase by approximately 200/μL in average. Consequently, the clinical management of HIV-infected patients changed from how to treat opportunistic infections into how to control patients with HAART.

2. Treatments and Clinical Research of Tropical Diseases

a. Treatment of Tropical Diseases in IMSUT hospital

Takeshi, Fujii, Tomohiko Koibuchi, Toshiyuki Miura1, Hitomi Nakamura[1], Michiko Koga[1], Tadashi Kikuchi, Kentaro Imai, Takashi Odawara, and Aikichi Iwamoto[1]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center

This year, more than hundred of overseas travellers visited our clinic. The reasons of their visit included prescription of malaria prophylaxis, hepatitis A/B vaccination, other general health consultation, or treatment of tropical diseases such as malaria, amoebic liver abscess, and post-exposure prophylaxis of rabies.

Supported by Department of Pharmacy in our hospital, we are managing the clinical use of orphan drugs for the treatment of tropical diseases in Japan. We take the consultation from the doctors facing tropical diseases all over Japan, and provide them with the appropriate medicines for the treatment of tropical diseases.

3. Comprehensive preoperative evaluation of patients with hemophiliac arthropathy

Tomohiko Koibuchi[1], Takeshi, Fujii, Takashi Odawara[1], and Aikichi Iwamoto[1]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center

In collaboration with the Department of Joint Surgery we evaluate medical status of patients with hemophiliac arthropathy who are going to be given surgery. The majority of hemophiliac patients who had received factor concentrates in the early 1980s were infected with hepatitis C virus (HCV). A considerable percentage of them were also infected with Human immunodeficiency virus (HIV). Among the hemophiliac patients who had orthopedic surgery in 2009, 89% were HCV-Ab-positive and 44% were HIV-positive. Appropriate preoperative evaluation of liver function and immunological status is essential to reduce the morbidity associated with the surgery and improve the clinical outcomes of these patients.

We have developed a comprehensive preoperative assessment system with a flow chart to evaluate the liver function and immunological status of hemophiliac patients. The flow chart employs several indices for evaluation, such as CD4 cell count, ICG retention test, prothrombin time, fibrosis markers (type IV collagen, hyaluronic acid), the finding of abdominal ultrasound and Child-Pugh score. Enhanced abdominal CT and/or upper gastrointestinal endoscopy are also required when we suspect the existence of hepatocellular carcinoma or esophageal varix. Based on the result of these indices, our multidisciplinary team assesses the risks and benefits of the surgery. The check-up system by the multi-disciplinary team using the flow chart has improved the care of the hemophiliac patients undergoing surgical operations.

4. Creating Practice Guidelines for Treatment of HIV-infected Patients in Japan

Tomohiko Koibuchi, Takeshi, Fujii, Toshiyuki Miura[1], Hitomi Nakamura[1], Michiko Koga[1], Tadashi Kikuchi, Kentaro Imai, Takashi Odawara, and Aikichi Iwamoto[1]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center

The Japanese guidelines for treatment of HIV-infected patients have been established since 1998 with support from Ministry of Health, Labor and Welfare. The representatives from our department have played critical roles in development of the current practice guidelines in Japan. It is vital to create practice guidelines that are specific for the unique genetic and social backgrounds of the HIV-infected population in Japan. In collaboration with other Japanese HIV-experts, the physicians from our department update the practice guidelines annually, as we deem necessary.

The guidelines are available at http://www.haart-support.jp/guideline.htm and used widely by Japanese clinicians. In Japan, where the number of HIV-experts are limited compared to other countries, the practice guidelines have substantially improved the standard of care for the HIV-infected patients in our country.

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Research Hospital

Department of Pediatric Hematology-Oncology
小児細胞移植科

Associate Professor Kohichiro Tsuji
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准教授 医学博士 辻 浩一郎
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Our major goal is to cure children suffering from a variety of life-threatening hematological disorders. Attempting to achieve it, we continue the commitment to treatment and follow-up care of such children, and to clinical and laboratory research that ultimately will help us devise better therapeutic approaches to the diseases. Currently efforts are directed toward treatment of acute leukemia in adolescence, establishment of novel therapies using hematopoietic or mesenchymal stem cells (HSC or MSC, respectively), and analysis of pathogenesis of hematopoietic disorders, especially pediatric myelodysplastic sundrome (MDS).

1. Hematopoietic stem cell transplantation for children with high-risk leukemia

Yasuhiro Ebihara, Kohichiro Tsuji

Although a standard regimen in hematopoetic stem cell transplatation (HSCT) has been available for children with acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), it has not been standardized for those with rare diseases including congenital bone marrow failure syndrome (CBMFS) and natural killer (NK) cell leukemia. A multi-institutional trial using regimens with a rationale should be proposed in a prospective manner. For CBMFS, we conducted *in vitro* and *in vivo* assays to assess the sensitivity of granulocyte colony-stimulating factor (G-CSF), and transplanted the patients whose leukemic cells had a high sensitivity to G-CSF using a regime including G-CSF. Thus, we could avoid intensive chemotherapy before HSCT for patients with a vulnerable normal bone marrow reserve. For patients with Fanconi anemia, in particular, we employed a regimen containing fludarabine to reduce the dose of alkylating agents and irradiation to avoid the toxicity, which was otherwise likely to occur in those patients. For patients with NK cell disease, we used a regimen combining alkylating agents (cyclophosphamide and thiotepa) and total body irradiation based on the results that NK leukemic cells strongly expressed multidrug-registant genes. Now we plan to extend our experience in nationwide collaborative studies.

2. Cooperative clinical trial for pediatric myelodysplastic syndrome

Kohichiro Tsuji, Yasuhiro Ebihara, Atsushi Manabe[1], Yuji Zaike[2]: [1]St. Luke's International Hospital, [2]Department of Laboratory Medicine, Research Hospital

Pediatric MDS is a rare disease, and only 50-100 children under the age of 16 suffer from the disease annually. The diagnosis and treatment have not been standardized and it should be determined in a nationwide manner. On behalf of the MDS committee of the Japanese Society of Pediatric Hematology, we began the pathologic central review in 1999 and reviewed all samples of patients suspected of having MDS. At present, over 300 patients have been enrolled, and

standard diagnostic criteria have been proposed for juvenile myelomonocytic leukemia (JMML), a subset of MDS. We also tested *in vitro* cell growth for patients with JMML using diagnostic samples. The results showed that spontaneous growth and hypersensitivity to granulocyte-macrophage colony-stimulating factor (GM-CSF) were observed in most children with JMML. We proposed a cooperative trial to establish the treatment for MDS (MDS99) and have enrolled over 100 patients from the whole country.

3. Novel approach to therapy in juvenile myelomonocytic leukemia

Yasuhiro Ebihara, Yoshitoshi Ohtsuka[3], Atsushi Manabe[1], Yuji Zaike[2], Kohichiro Tsuji: [3]Department of Pediatrics, Hyogo College of Medicine

JMML is a clonal myeloproliferative/myelodysplastic disorder of early childhood with poor prognosis. JMML cells are characterized by hypersensitivity to GM-CSF caused by continuously activated GM-CSF receptor-RAS signal transduction pathway through various molecular mechanisms, resulting in spontaneous colony formation *in vitro*. Bisphosphonate zoledronic acid (ZOL), a RAS-blocking compound, suppressed colony formation from bone marrow (BM) cells of JMML patients and normal volunteers without and with GM-CSF, respectively, in a dose-dependent manner in clonal culture. At 10 μM of ZOL, however, spontaneous colony formation decreased, but formation of granulocyte (G) colonies containing only granulocytes, but no macrophages was enhanced in culture of JMML BM cells, while granulocyte-macrophage (GM) colonies containing both granulocytes and macrophages retained and G colony formation was not affected in culture of normal BM cells with GM-CSF. In suspension culture, 10 μM of ZOL also inhibited spontaneous proliferation and differentiation along monocyte/macrophage lineage of JMML BM cells, but not development of normal BM cells by GM-CSF assessed in cytochemical and flow cytometric analyses. The inhibitory effect of ZOL on JMML cells was confirmed at a single-clone level, and observed even at 3 μM. The current result offers a novel approach to therapy in JMML.

4. Establishment of therapy for acute leukemia in adolescence and young adults

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In many area of medicine adolescents are regarded as a discrete group with specific therapeutic, psychological, educational, and resource needs. In acute leukemia age is a predictor of response. Thus, in ALL there is a clearly poorer treatment outcome after puberty, while in AML, which is more common in older adults, age is a continuous variable with poorer outcomes in each successive decade. Much is known about other prognostic factors and their relative incidence in each age stratum. Although there is some segregation of favorable factors with relative youth, age usually remains an independent factor with respect to prognosis. So far, adolescents are included in pediatric or adult-oriented treatment protocols, but it has been controversial which protocol is superior to acute leukemia in adolescence and young adults. We are now searching the most suitable therapy for them, especially cord blood HSCT, which recently has been shown by us to be safe and effective in acute leukemia in adolescence and young adults.

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

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Hemophilia is a congenital disease with a lack of coagulation factors. Arthropathy is a major cause of morbidity in the patients with hemophilia. Approximately one third of the patients need the mobility assistance. Although the pathogenesis of hemophilic arthropathy (HA) still have not been precisely clarified, the destruction of articular cartilage is the most prominent event in HA. Most surgical treatments for HA, such as synovectomy or total joint arthroplasty, in Japan is performed by Department of Joint Surgery in our hospital. So far, however, the efficacy of the treatment has been insufficient. Recently it has been shown that BM contains MSC, which can differentiate into various mesenchymal tissue cells, osteocytes, adipocytes and chondrocytes. Although the mechanism by which MSC are committed to differentiate into each mesenchymal tissue, the environment surrounding MSC plays an important role in the

commitment. We are then preparing for the clinical trial of the transplantation of autologous culture-expanded BM-derived MSC into the articular cartilage defect in the HA patients.

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We participate in cutting edge science of autoimmune, rheumatic and allergic diseases and novel treatments for patients with these disorders. In addition to conventional drug studies aimed to improve the efficacy and safety of current therapies, we are going to carry out experimental protocols of particular interest for patients not responding to conventional therapy and to perform the translational research.

I. Study on CD26 molecule in normal immune response and in patients with immune-mediated diseases

Osamu Hosono, Kei Ohnuma, Noritada Yoshikawa, Hiroshi Kawasaki, Hirotoshi Tanaka, Chikao Morimoto. (Department of Rheumatology and Allergy), Emi kumagai, Akiko Souta-Kuribara, (Division of Clinical Immunology)

CD26 is a T cell costimulatory molecule as well as an activation antigen with dipeptidyl peptidase IV (DPPIV) enzyme activity in its extracellular region that is preferentially expressed on memory T cells. The soluble form of CD26 (sCD26) is present in serum and recombinant soluble CD26 can enhance peripheral blood T cell proliferation induced by the recall antigen. We demonstrated that CD26 binds Caveolin-1 on antigen presenting cells, and that following CD26-caveolin-1 interaction on recall antigen-loaded monocytes, caveolin-1 is phosphorylated, with linkage to NF-κB activation, followed by upregulation of CD86. In addition, reduced caveolin-1 expression on monocytes inhibits CD26-mediated CD86 upregulation and abrogates CD26 effect on recall antigen-induced T cell proliferation, and immunohistochemical studies revealed an infiltration of CD26+ T cells in the sublining region of rheumatoid synovium and high expression of caveolin-1 in the increased vasculature and synoviocytes of the rheumatoid synovium. Taken together, these results strongly suggest that CD26-caveolin-1 interaction plays a role in the upregulation of CD86 on recall antigen-loaded monocytes and subsequent engagement with CD28 on T cells, leading to antigen-specific T cell activation such as the T-cell-mediated antigen-specific response in rheumatoid arthritis (RA).

Currently we are focusing on the translational research of utilization of anti-CD26 monoclonal antibody (mAb) as well as recombinant soluble CD26 for treatment of malignant tumors, immune-mediated disorders and immune defi-

ciency diseases. In this regards, the phase I/II clinical trial utilizing humanized CD26 mAb for the treatment of malignant mesothelioma and other CD26 positive malignant tumors has been already started at Gustave Roussy Institute in Paris.

a. Clinical significance of soluble CD26/DPPIV in various disease conditions

(i) Soluble CD26/DPPIV in autoimmune and other immune-mediated disorders

Our previous studies demonstrated that CD26-caveolin-1 interaction plays a role in the upregulation of CD86 on recall antigen-loaded monocytes and subsequent engagement with CD28 on T cells, leading to antigen-specific T cell. Possible substrates of CD26/DPPIV include several critical cytokines and chemokines. CD26 could modulate function of several cytokines and chemokines such as RANTES (CCL5), SDF-1α (CXCL12) and glucagons-like peptide 1 (GLIP-1) through its DPPIV enzyme activity. We have shown that the DPPIV enzyme activity of plasma sCD26 was low in HIV-1-infected individuals, and was inversely correlated with HIV-1 RNA, and that the in vitro addition of recombinant sCD26 could enhance purified protein derivative-induced lymphocyte proliferation. These DPPIV enzyme activity of sCD26 in HIV-1-infected individuals contributes to the immunopathogenesis of HIV infection. Furthermore, we have shown that serum levels of sCD26 and its specific DPPIV activity were significantly decreased in SLE and were inversely correlated with SLE disease activity index score, but not with clinical variables or clinical subsets of SLE. Serum levels of sCD26 may be involved in the pathophysiology of SLE, and appear to be useful as a new disease activity measure for SLE.

We have examined sCD26 and its specific DPPIV activity in serum of patients with inflammatory bowel diseases (IBD), such as Crohn's disease or ulcerative colitis in collaboration with Gastrointestinal Unit, School of Medicine, Keio University. The DPPIV activity was reduced in patients with IBD and was significantly lower in patients with Crohn's disease compared to with ulcerative colitis ($P < 0.05$). We are analyzing clinical significance of sCD26/DPPIV using clinical data. We have also measured sCD26/DPPIV levels in sera and synovial fluid from patients with RA and found significant decrease of serum sCD26 and its specific DPPIV activity. These findings indicate that CD26 may be potentially important for the pathophysiology of IBD and RA. Furthermore, we have investigated autoantibodies against CD26 in serum using ELISA and Western blotting methods. We have not found anti-CD26 autoantibody which could reduce DPPIV activity so far. We plan to examine the effect of TNF-α blocking therapy (infliximab, etanercept, adalimumab) on serum levels of sCD26/DPPIV in patients with RA and its clinical significance.

(ii) Soluble CD26/DPPIV in malignancies associated with asbestos exposure

CD26/DPPIV is able to cleave selected biological factors to alter their functions and regulates topoisomerase II α level in hematologic malignancies, affecting sensitivity to doxorubicin and etoposide. Expressed on various tissues, CD26 is involved in the development of certain human cancers. We have shown CD26 is highly expressed on the cell surface of malignant mesothelioma and that a newly developed humanized anti-CD26 mAb has an inhibitory effect on malignant mesothelioma cells in both in vitro and in vivo experiments.

We examined sCD26 and its specific DPPIV activity in serum and pleural effusion of patients with asbestosis in collaboration with Okayama Rosai Hospital. Serum levels of sCD26 and its specific DPPIV activity was significantly reduced in patients with both malignant mesothelioma and primary lung cancer associated with asbestos exposure compared to patients with pleural plaque. As there seems to be a relationship between pleural CD26/DPPIV and prognosis in mesothelioma, we have done the measurement of more samples and serial studies to confirm their clinical significance. We are now analyzing their clinical significance, especially their association with prognosis of mesothelioma.

(iii) Effect of humanized anti-CD26 mAb on measurement of soluble CD26/DPPIV

In our ELISA for measuring soluble CD26/DPPIV we used two different mouse anti-CD26 mAbs (5F8, 1F7) which could not interfere each other. Administration of our newly developed humanized anti-CD26 mAb might form immune complex with sCD26, which is possible to interfere measurement of serum sCD26. We confirm the interference of humanized anti-CD26 mAb with anti-CD26 mAb (1F7) for detecting sCD26 in our ELISA. Therefore, instead of 1F7 we selected the additional anti-CD26 mAb (9C11) which recognize the different epitope from humanized anti-CD26 mAb and 5F8. In clinical trial utilizing humanized CD26 mAb we could measure serum sCD26/DPPIV without interference of administered anti-CD26 humanized mAb.

b. CD26-based molecular target therapy for graft-versus-host disease in hematopoietic stem cell transplantation

Graft-versus-host disease (GVHD) remains a major cause of morbidity and mortality in allogeneic hematopoietic stem cell transplantation (alloHSCT). In GVHD, mature donor T cells that accompany the stem cell graft attack recipient tissues, especially the skin, liver, gastrointestinal tract, and lung. Therefore, all patients undergoing alloHSCT receive GVHD prophylaxis to impair T cell function; however, treatment to prevent GVHD can be deleterious since mature donor T cells play a critical role in mediating reconstitution of the adaptive immune system. Recipients of alloHSCT are thus at great risk for infections, particularly when prolonged immunosuppression is required for treatment of GVHD. Although the role of CD26/DPPIV in GVHD needs to be studied in more detail, treatment with a murine antibody against human CD26 was reported to have an effect in patients with steroid-resistant acute GVHD following alloSCT (Bacigalupo A., et al., Acta Haematol 1985: 73: 185, de Meester, et al., Immunobiology 1993: 188: 145). To examine the efficacy of CD26-targeting therapy in GVHD more profoundly, we established mouse GVHD model using human peripheral blood lymphocytes (huPBL) (xenograft GVHD mouse model; x-GVHD). After NOD/LtSz-scid or NOD/Cg-Prkdcscidil2rgtm1Sug/Jic mice were injected with appropriate numbers of huPBL, mice show symptoms of GVHD such as loss of weight, loss of hair, deterioration of activity, and thinning of ear pads. Histopathological examination revealed that CD3+CD26+ human lymphocytes were infiltrated in the skin, intestinal mucosa, salivary gland, lung and liver of the x-GVHD mice. In this mouse model, humanized anti-CD26 monoclonal antibody (mAb) was injected two weeks later of onset of x-GVHD, and the symptoms of GVHD were improved after ten injections of humanized anti-CD26 mAb. Moreover, x-GVHD was observed to be suppressed when humanized anti-CD26 mAb was prophylactically administered. Taken together, it may be possible that the full therapeutic potential of alloSCT will be realized by approaches that aim to minimize GVHD by targeting CD26-mediated T cell regulation.

II. Therapeutically targeting transcription factors

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

We are interested in the mechanism of eukaryotic 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 regulations 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 cer-

tain 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 7SK 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.

III. Clinical Trial

Osamu Hosono, Kei Ohnuma, Noritada Yoshikawa, Hiroshi Kawasaki, Hirotoshi Tanaka, Chikao Morimoto

We have participated a phase I/2a clinical trial (Osamu Hosono as principal investigator in our hospital) and a post marketing survey (Humira: adalimumab). The phase 1/2a clinical trial is a randomized, placebo-controlled, double blind, multicenter study of the safety, tolerability, efficacy and pharmacokinetics in Japanese patients with o.steoarthritis of the knee. We registered 10 osteoarthritis patients and finished 4 cases for evaluation (6 cases were dropped out).

We have participated post marketing survey of biologics for treatment of rheumatoid arthritis (Remicade, Enbrel and Humira) so far. The post marketing survey of Humira will finish in 2010. We are going to participate SECURE (Safety of Biologics in Clinical Use in Japanese Patients with Rheumatoid Arthritis in the Long Term) study.

IV. Case Reports

Osamu Hosono, Kei Ohnuma, Noritada Yoshikawa, Hiroshi Kawasaki, Hirotoshi Tanaka, Chikao Morimoto (Department of Rheumatology and Allergy), Naoki Oyaizu (Department of Laboratory Medicine)

Microscopic polyangiitis initiated with liver dysfunction, calf pain and fever of unknown origin.

We report a case of microscopic polyangiitis (MPA), presenting onset with a spiking fever, liver/biliary dysfunction without jaundice and calf pain without elevation of serum creatine phosphokinase. During 1 month of careful examinations for initial diagnosis, the patient developed renal dysfunction and pulmonary hemorrhage. Based on the results of positive MPO-ANCA, renal and pulmonary involvements, the patient was diagnosed with MPA and treated with high-dose prednisolone and oral cyclophosphamide. Soon after initiation of the treatment, symptoms such as fever, calf pain, liver/biliary dysfunction and renal dysfunction disappeared with decrease of MPO-ANCA titer to the normal level.

IV. Contribution to Medical Genome Science Program

Satoshi Iwata (Division of Clinical Immunology), Noritada Yoshikawa, Kei Ohnuma, Hiroshi Kawasaki, Osamu Hosono, Hirotoshi Tanaka, and Chikao Morimoto.

We have made contribution to Medical Genome Science Program, the Institute of Medical Science, the University of Tokyo. Our mainly involving courses are, "Introduction of Medicine and Medical Ethics" and "Experience and Practice of Medicine", especially arranged for non-M.D. students of the program. The former is a series of lectures drawing outline of Medicine (past, present & future), and the latter is a weekly practice aiming to make attendants to get experienced in practical Medicine while rounding at the Research Hospital of the Institute of Medical Science. The attendants are supposed to visit a variety of divisions in the hospital, such as Radiology, Laboratory Medicine, Blood Transfusion, Surgical Center, Nursing Quarters, as well as the patient round by Department of Hematology/Oncology, and by Department of Advanced Medical Science/Infectious Disease and Applied Immunology/Rheumatology and Allergy. We especially thank all the people in the Research Hospital of the Institute of Medical Science, who participate in "Introduction of Medicine and Medical Ethics" and "Experience and Practice of Medicine".

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Our department was established for the development of genome-based medicine toward human cancer. As a collaborative project of registration and diagnosis of Japanese HNPCC patients conducted by Japanese Study Group for Colorectal Cancer, we earlier performed genetic analyses of MSH2, MLH1, and MSH6, three responsible genes for HNPCC. The data was used for the development of better diagnostic strategy to HNPCC.
In addition to the research project, we are in charge of outpatient clinic for genetic counseling in Research Hospital, IMSUT. In 2009, a total of 19 counseling and one genetic testing were given to the clients in the clinic.

1. Evaluation of pathogenicity of mutations in the splice-acceptor/donor sites of HNPCC-responsible genes

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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 the collaborative project. A total of 131 patients with familial colorectal cancer who fulfilled the modified Amsterdam's II criteria were registered, and the frequency of HNPCC in registered patients with colon was determined. For genetic diagnosis, we performed PCR-direct sequencing and Multiplex Ligation-dependent Probe Amplification for the three responsible genes. As a result, we identified pathogenic mutations in 69 of the 131 cases. These mutations included missense and nonsense mutations, small insertions and deletions, and gross genetic alterations including large deletions and duplications. In addition to these genetic changes, mutations in introns were also involved in the pathogenesis. However it is sometimes difficult to interpret correctly the pathogenicity of variants in exons as well as introns.

To evaluate the effect of splice-site mutations in two patients, we carried out a functional splicing assay using minigenes. Consequently, this assay showed that the mutation of c.1731＋5G＞A in MLH1 led to exon15 skipping, and that the mutation of c.211＋1G＞C in MSH2 created an activated cryptic splice-site 17-nucleotides upstream in exon1. These aberrant splicing patterns were not observed when wild type sequence was used for the assay. We also obtained concordant results by RT-PCR experi-

ments with transcripts from the patients. Furthermore, additional functional splicing assays using two different intronic mutations described in earlier studies revealed splicing alterations that were in complete agreement with the reports. These results suggested that functional splicing assay is helpful for evaluating the effects of genetic variants on splicing.

2. Genetic counseling and related activities.

Naoyuki Takahashi, Yoshinori Murakami, Yoichi Furukawa, Reiko Sada[1], Momoyo Ohki[2], Kohichiro Tsuji[3], Koichiro Yuji[4], Kisako Sato[5], Masae Ono[6], Shiro Ikegawa[7], Toshihiro Tanaka[7], Mayumi Tamari[7], Tsuyoshi Sakamoto[8], and Yusuke Nakamura[9]: [1]Division of Bioengineering, [2]Bunkyo University, [3]Department of Pediatric Hematology-Oncology, [4]Department of Hematology-Oncology, [5]Department of Nursing, [6]Department of Pediatrics, Tokyo Teishin Hospital, [7]Center for Genomic Medicine, RIKEN, [8]Department of Neurology, Jikei Medical University, [9]Laboratory of Molecular Medicine, Human Genome Center

In the genetic counseling clinic, we provided genetic counseling for clients who suffered from or had a family member(s) of hereditary diseases. Genetic diseases and related problems seen at the clinic in 2009 include hemophilia, amyotrophic lateral sclerosis, spinal and bulbar muscular atrophy, hereditary colorectal cancer, and Alzheimer disease. We also took psychological care of the clients in collaboration with clinical psychologists. In addition, genetic testing was provided.

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Research Hospital

Department of Radiology
放射線科

Associate Professor	Yusuke Inoue, M.D., D.M.Sc.	准教授	医学博士	井 上 優 介
Lecturer	Shigeru Kiryu, M.D., D.M.Sc.	講 師	医学博士	桐 生 茂
Assistant Professor	Makoto Watanabe, M.D., D.M.Sc	助 教	医学博士	渡 辺 慎

The Department of Radiology works in general diagnostic radiology, neuroradiology, clinical nuclear medicine, radiation therapy, and molecular imaging. For clinical imaging, we have a multi-detector row CT scanner, high-field MRI unit, and gamma camera. We perform all examinations of CT, MRI, angiography, and nuclear medicine, and official reports on all the examinations are made by board-certified radiologists and a nuclear medicine physician. Molecular imaging assesses molecular and cellular events in living organisms noninvasively. We investigate the technical aspects of molecular imaging in intact small animals for its application to preclinical studies.

Development of integrated imaging using bioluminescence imaging and magnetic resonance imaging

Yusuke Inoue, Yoshitaka Masutani[1], and Arinobu Tojo[2]: [1]Department of Radiology, Graduate School of Medicine, University of Tokyo and [2]Division of Molecular Therapy, Advanced Clinical Research Center, University of Tokyo.

We developed an integrated imaging, a combination of bioluminescence imaging (BLI) and magnetic resonance imaging (MRI), and applied it to longitudinal tumor monitoring in mice. Mice were fixed on a flat, transparent plate, and BLI and MRI were successively performed. After spatial coregistration was achieved semiautomatically using three small markers, the BLI image was fused with all slices of MRI. For the evaluation of the accuracy in image registration, 25 markers were attached to the mice, and integrated imaging of fluorescence imaging and MRI was performed. The results showed acceptable accuracy, although the registration error increased with increasing distance from the center of the imaging field and was larger in imaging the convex dorsal side of the mouse than in imaging the flat ventral side. Then, mice were inoculated with luciferase-expressing tumor cells subcutaneously or directly into the liver, and integrated BLI/MRI was performed repeatedly to evaluate disease progression in individual mice. Integrated BLI/MRI allowed longitudinal assessment of disease progression and facilitated detailed interpretation of imaging findings, including the anatomical localization of lesions revealed by BLI and recognition of subtle MRI abnormalities corresponding to BLI signals. Integrated BLI/MRI is indicated to be a practical method for comprehensive, longitudinal assessment of disease model mice. As a next step, we aim at incorporating distortion correction into the integrated BLI/MRI system to achieve accurate, whole-body image fusion.

Assessment of deep abdominal lymph nodes in mice using fluorescence imaging and quantum dots

Yusuke Inoue, Shigeru Kiryu, Makoto Watan-

abe, and Naoki Oyaizu[3]: [3]Department of Laboratory Medicine, Institute of Medical Science, University of Tokyo.

We investigated the technical aspects of fluorescence lymph node mapping to assess deep abdominal lymph nodes in intact mice. Quantum dots were injected subcutaneously in the rear footpads of mice, and the time course of the light signal was assessed by two-dimensional fluorescence reflectance imaging. The abdomen was compressed with transparent, colorless tape to reduce the body thickness, and fluorescence signals from the abdominal lymph nodes were compared with and without compression. Popliteal, sacral, iliac, and renal lymph nodes were commonly delineated, and the lymph node signals increased up to 3 hours. The compression of the abdomen significantly enhanced the delineation of the iliac nodes located deeply in the abdomen. They were invisible at 5 pmol without compression but visible even at 1 pmol with compression. Fluorescence imaging after local injection of quantum dots allows to delineate deep abdominal lymph nodes in addition to superficial lymph nodes in intact mice, with the aid of the simple compression technique. Now, we are studying quantitative relationship between fluorescence signals and body thickness using a dedicated compression device.

Effect of anesthesia and hypothermia on the hepatic kinetics of a hepatobiliary contrast agent for magnetic resonance imaging

Shigeru Kiryu, Makoto Watanabe, and Yusuke Inoue

Magnetic resonance (MR) imaging of small animals is usually performed under anesthesia, and thus may be affected by physiological changes caused by anesthesia, such as hypothermia. We have shown that the washout of hepatobiliary contrast agents, Gd-BOPTA and Gd-EOB-DTPA, from the liver are slower in mice anesthetized with isoflurane than in conscious mice, which may be attributable to direct action of isoflurane or associated hypothermia. We developed a method for body MR imaging of conscious mice and separately evaluated the effect of isoflurane and hypothermia on the hepatic kinetics of Gd-EOB-DTPA. Conscious or anesthetized mice were restrained on an imaging holder and underwent MR imaging serially after intravenous injection of Gd-EOB-DTPA, with or without temperature control. The washout of Gd-EOB-DTPA from the liver was slower in anesthetized hypothermic mice than in conscious normothermic mice. When anesthetized mice were warmed to normothermia, the washout was accelerated and became as fast as that in conscious normothermic mice. When cooling reduced the temperature in conscious mice similarly with anesthetized mice without temperature control, the washout was delayed, as in anesthetized hypothermic mice. By separately manipulating the presence or absence of anesthesia and hypothermia, we showed that hepatic washout of Gd-EOB-DTPA was delayed under hypothermia, independently of the use of anesthesia. Our method of MR imaging of conscious mice permits the assessment of the kinetics of a contrast agent, excluding the possible effects of anesthesia.

Distortion correction in whole-body imaging of live mice using a 1-T compact magnetic resonance imaging system

Shigeru Kiryu, Yoshitaka Masutani[1], and Yusuke Inoue

There are geometric distortions in magnetic resonance (MR) imaging, which may degrade volumetry and fusion with images acquired on other modalities. We assessed geometric distortion in MR images acquired on a compact 1-T permanent magnet unit using a small three-dimensional (3D) grid phantom and developed a distortion correction method applicable to whole-body imaging of living mice. The 3D grid phantom was imaged using a T1-weighted, 3D fast low-angle shot sequence, with various imaging parameters. Geometric distortion was evident and prominent on both peripheral sides along the axis of the coil. The effect of imaging parameters on distortion was limited, but relatively large for TE. The 3D image transformation for distortion correction was determined using the 3D phantom image data. The application of the correction decreased distortion in the 3D phantom images obtained on another day, suggesting the validity of the correction. When a two-dimensional (2D) grid phantom was imaged together with a mouse, geometric distortion was observed for the phantom. The 3D phantom-based correction reduced the distortion substantially, irrespective of the position of the mouse, indicating the applicability of the correction to whole-body mouse imaging. Our distortion correction method is expected to improve the ability of MR imaging in small animal experiments.

Lymph node mapping in living mice using magnetic resonance imaging.

Fugeng Sheng, Shigeru Kiryu, and Yusuke Inoue

Although lymph nodes are important in oncology and immunology, the lymphatic system in mice still remains to be investigated. as sites of metastatic involvement, and animal models of lymph node metastasis are being developed. Magnetic resonance (MR) imaging provides high-resolution, tomographic images and, after local injection of a contrast agent, permits the assessment of lymph drainage from the injection site. We are investigating the techniques of interstitial MR lymphography and its application to the assessment of the lymph pathway in mice. We compare the time courses of signal intensities in the lymph nodes after local injection of various contrast agents, including extracellular agents, hepatobiliary agent, and micelle-forming agents, to determine appropriate imaging techniques. The lymph nodes can be delineated using various agents; however, retention in the nodes is largely variable among agents, and the injection dose and the timing of imaging should be determined for each agent. We have developed a new method of the fusion of bioluminescence imaging and MR imaging and are extending it to the fusion of fluorescence imaging and MR imaging for detailed assessment of lymph drainage. Lymph drainage from various sites and in various mice will be examined using interstitial MR lymphography combined with fluorescence lymph node mapping.

Improvement of a compact magnetic resonance unit using a 1-T permanent magnet

Yusuke Inoue, Shigeru Kiryu, and Tomoyuki Haishi[4]: [4]MRTechnology.

Magnetic resonance (MR) imaging provides high-resolution tomographic images with no radiation exposure and is accepted as a potent tool for small animal experiments. However, high cost, low research accessibility, and difficulty in operation and image interpretation may preclude the use of MR imaging in biological experiments. A system using a permanent magnet may offer a favorable solution. It can be installed in a small room together with other devices because of its smallness and weak magnetic field leakage. The cost for installation is relatively low, and running cost is negligible. Openness of the permanent magnet system provides excellent accessibility to the sample to be imaged. Although relatively low magnetic field of a permanent magnet reduces signal-to-noise ratio, we have developed convenient imaging techniques to obtain three-dimensional images with acceptable quality in a reasonable scan time. Now, we attempt to install various imaging sequence including higher resolution sequence and T2-weighted imaging sequence. We are also creating supporting devices to facilitate imaging various mice and developing software for data acquisition and image processing to improve convenience, versatility, and image qualities. The investigation of appropriate mouse preparation is another way to sophisticate imaging techniques. Our aim is to construct a convenient MR unit suitable for biological experiments.

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Research Hospital

Department of Surgery
外科

Associate Professor	Masaru Shinozaki, M.D., Ph.D.	准教授	医学博士	篠崎　大
Professor	Hideaki Tahara, M.D., Ph.D.	教　授	医学博士	田原秀晃
Lecturer	Akihiko Itoh, M.D.	講　師		伊藤精彦
Assistant Professor	Giichiro Tsurita, M.D., Ph.D.	病院講師	医学博士	釣田義一郎
Assistant Professor	Keisuke Hata, M.D., Ph.D.	助　教	医学博士	畑　啓介
Assistant Professor	Akira Kanamoto, M.D., Ph.D.	助　教	医学博士	金本　彰

The principal goal of our department is to provide surgical service of malignancy and inflammatory bowel disease and to develop and conduct clinical trials in early stages (Phase I and II) on patients at the Research Hospital. We have been engaged in the surgical treatment of solid tumors and the immunotherapy for malignant diseases originated from various organs. We have also been offering diagnostic and endoscopic treatment services, including upper and lower endoscopic examinations.

1. Summary of surgical treatment in 2009

Masaru Shinozaki, Akihito Itoh, Giichiro Tsurita, Kimiyasu Yoneyama, Keisuke Hata, Akira Kanamoto

We performed various surgical operations. Malignancy is the leading indication for operation, followed by benign diseases, such as inflammatory bowel disease (IBD) and hernia. Among the patients with malignancy, colorectum is by far the leading organ.

In 2008, three colorectal specialists (M.S., G.T., and K.H.) came to the Department. Since then, we have experienced more IBD patients. We treated not only surgical IBD patients but also medical patients. Laparoscopic surgery for colorectal disease has been applied to selected patients. We updated operative procedures to minimize surgical site infection. Furthermore, routine intraoperative endoscopic observation after colorectal anastomosis was initiated. Intractable diseases, including malignancy and IBD, are our main target in surgical treatment and we are seeking for less invasive procedures and new strategies for patients' cure and quality of life.

2. Summary of endoscopic examination in 2009

Giichiro Tsurita, Keisuke Hata, Masaru Shinozaki, Akira Kanamoto, Masahisa Jinushi

Under the cooperation with Department of Advanced Medical Science, we performed 473 upper gastrointestinal endoscopies and 411 colonoscopies without major complications. Dr. Tsurita has been the chief of Division of Endoscopy and played the crucial role in examinations. For the patients' satisfaction, we aggressively perform endoscopic treatment and avoid operation as much as possible.

Table. Surgical procedures performed in 2009

Cancer		
Stomach	Dista gastrectomy	1
	Total gastrectomy	1
Colorectum	Ileocecal resection	1
	Right hemicolectomy	6
	(Laparoscopic	1)
	Partial resection	1
	(Laparoscopic	1)
	Sigmoidectomy	5
	Hartmann's operation	1
	Low anterior resection	5
	(+Ileocecal resection	1)
	Intersphincteric resection	2
	Abdominoperineal resection	1
	Transverse colostomy	1
Anus	Transanal excision	1
Liver	Partial hepatectomy	5
Breast	Partial mastectomy	8
Pancreas	Lymphnode sampling	1
Ovary	Oophorectomy	1
Gastrointestinal stromal tumor	Excision	1
Others		
Gallbladder stone	Laparoscopic cholecystectomy	3
Common bile duct stone	Choledocostomy	1
Ulcerative colitis	Restorative proctocolectomy	1
	Proctocolectomy	1
Crohn's disease	Ileocecal resection	1
Stoma	closure	2
Incisional hernia	Repair	1
Inguinal hernia	Repair	4
Hemorrhoids	Hemorrhoidectomy	3
Superficial mass	Excision	2
Total		61

3. Clinical trials terminated in 2009.

A. Phase I/II colorectal cancer trials

Masaru Shinozaki, Giichiro Tsurita, Keisuke Hata

We performed phase I/IIa clinical trials of colorectal cancer vaccine using cancer related peptides restricted to HLA-A*2402 or HLA-A*0201. Genome-wide exploration using cDNA microarray profiling enabled a new tumor associated antigen that can induce potent cytotoxic T-cells (CTLs) specific to tumor cells. Among them, we selected RNF43 (ring finger protein 43), TOMM 34 (translocase of outer mitochondrial membrane 34) as target peptides for HLA-A*2402. Targeting tumor specific new blood vessels also seems to be promising. Therefore, peptides derived from VEGFR1 (vascular endothelial growth factor receptor 1) and VEGFR2 were adopted for both HLA-A*2402 and HLA-A*0201

trials. A total of five patients were enrolled in either trial without severe complications.

B. Phase I/II breast cancer trial

Masaru Shinozaki, Giichiro Tsurita, Kimiyasu Yoneyama, Keisuke Hata

We performed phase I/IIa clinical trials of colorectal cancer vaccine using cancer related peptides restricted to HLA-A*2402 or HLA-A*0201. Genome-wide exploration using cDNA microarray profiling enabled a new tumor associated antigen that can induce potent cytotoxic T-cells (CTLs) specific to tumor cells. Among them, we selected TTK protein kinase as target peptide for HLA-A*2402. Targeting tumor specific new blood vessels also seems to be promising. Therefore, peptides derived from VEGFR1 (vascular endothelial growth factor receptor 1) and VEGFR2 were adopted for HLA-A*0201 trials. A total of five patients were enrolled in either trial without severe complications.

4. Clinical trials under development

We are planning to administer cancer related peptides under various situations to draw maximal effects.

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Research Hospital

Department of Joint Surgery
関節外科

Lecturer Hideyuki Takedani, M.D., D.M.Sc.

講 師 医学博士 竹 谷 英 之

Department of Joint Surgery was established in 2006. Our mission is evaluation and treatment of hemophilic arthropathy. In Japan, many hospitals are able to control bleeding for haemophilia by concentrates, however there are few hospitals focus on surgical treatments except us. Many haemophilia patients come to our department from all over Japan. We evaluate their joint condition and function roentgenographically and physiotherapeutically and decide indication of surgical treatment. Many of patients will be performed joint arthroplasties and arthroscopic synovectomy to improve their quality of life.

Surgical treatment for haemophilia

Hideyuki Takedani

From 2006 to 2008, there are 55 surgical treatments for hemophilia (39 for hemophilia A, 7 for hemophilia B and 9 for hemophilia with inhibitor).

In 2009, we were performed 18 surgical treatments (11 for hemophilia A, 4 for hemophilia B, 2 hemophilia with inhibitors and 1 for deficiency factor VII patient); 13 total joint arthroplasties, 4 arthroscopic sunovectomies and one other surgical treatment.

Publications

Takedani, H,. Kawahara, H. and Kajiwara, M. Major orthopaedic surgeries for haemophilia with inhibitors using rFVIIa. Haemophilia. in press.

Research Hospital

Surgical Center
手術部

Associate Professor	Mieko Chinzei, M.D., M.D.Sc.	准教授	医学博士	鎮　西　美栄子
Assistant Professor	Sachiko Imamura, M.D.	助　教	医学士	今　村　佐知子
Clinical Engineer	Kazuyo Shionome		臨床工学技士	塩　野　目万代

Our clinical practice and clinical studies have been focused on (1) anesthetic management in patients undergoing major surgery including joint arthroplastic surgery for hemophilia patients, (2) management of chronic intractable pain or improving the quality of life of patients with life-threatening illness (3) assessment of the impact of anesthesia and surgery on autonomic nervous activity, and (4) risk management of medical electronic devices in Research Hospital.

1. Safety in anesthetic management, especially focusing on prevention of deep vein thrombosis during total hip arthroplasty in hemophilia patients.

Management of bleeding in patients with hemophilia has improved since the development of coagulation factor substitution therapy. In almost all of the hip or knee arthroplasty, intraoperative embolism has been detected with transesophageal echocardiography (TEE). But there may have been no report on TEE findings during arthroplastic surgery in hemophilia patients. We find TEE detected variable degree of echogenic materials in right atrium (RA) during THA in hemophilia patients under continuous infusion of coagulation factor. This may suggest that we need to consider risks not only on the side of hemorrhage but embolic events for perioperative management of hemophilia patients.

2. Management of chronic intractable pain.

We organized a palliative care support team in Research Hospital for the patients suffering with intractable physical and mental pain caused by life-threatening illness and/or complications of the treatments. In patients of hematological malignancy with long treatment history, many of their illness have been diagnosed as reaction to severe stress and adjustment disorder, especially prolonged depressive reaction (F43, the ICD-10 classification of mental and behavioral disorders)

3. Assessment of the impact of anesthesia and surgery on autonomic nervous activity.

It is generally accepted that the parameters derived from power spectral analysis (PSA) of heart rate variability (HRV) can provide a non invasive measure of autonomic nervous activity. We have published several works on assessment of the impact of anesthetics on autonomic nervous activity during perioperative period using real time monitor for PSA of HRV.

4. Risk management of medical electronic devices.

We ourselves engage in preventive maintenance and care of the life support machines including instruments for mechanical ventilation

or blood purification and defibrillator. We also supervise physicians during clinical usage of these instruments. We have promoted dual-directional information system on malfunctions or incidents of the rest of medical electronic devices in this hospital in collaboration with the Division of Clinical Trial Safety Management.

Publications

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Research Hospital

Department of Clinical Trial Safety Management
附属病院・医療安全管理部

Associate Professor Fumitaka Nagamura, M.D., D.M.Sc 准教授 医学博士 長 村 文 孝

Division of Clinical Trial Safety Management (DCTSM) owes two major missions. One is the risk management of the Research Hospital (RH) and the other is the support for the conduct of clinical trials, especially for Translational Research (TR). Our roles on TR varies from the assistance for planning study design and writing protocol to the data confirmation by Case Report Form which is managed by Translational Research Coordinator (TRC) and the quality assurance of TRs by monitoring/audit. To protect the right of participants into TR and to conduct TR scientifically and ethically appropriately, we have organized TRC, which consists nurse, pharmacist, clinical laboratory technologist, dietitian, and clinical psychotherapist.

1. Risk management of Research Hospital

Fumitaka Nagamura, Hatsue Narita, Makiko Tajima

We have engaged in the risk management and the protection of medical incidents/accidents at RH. We have promoted the report system on medical incidents and accidents, and quick corresponding scheme such as "Medical Accident-Response Meeting" and "Council of Risk Management in the RH". We take place at least two seminars for staffs of RH on medical safety every year. Participation to these seminars is the obligation of workers of RH. We have created manuals on the risk management and Standard Operating Procedures (SOP) on operations of RH and revised quickly when required.

2. Assistance and oversight of Clinical trials/ TRs

Kazufumi Matsumoto, Kumiko Sumino, Noriko Fujiwara, Minako Kohno, Makiko Tajima, Fumitaka Nagamura

The assistance of TRC is indispensable for the conduct of clinical trials, especially for TR. In 2009, we assisted the conduct of 26 protocols, and three of them were newly started in this period. Details of protocols are summarized in Table 1. Table 2 shows the number of patients enrolled into clinical trials at RH in 2009.

Table 1.

Number of protocol	Started in 2009	Continuation before 2009	Total
TR	0	16	16
Clinical trials from pharmaceutical companies	2	3	5
Multi-center studies	1	4	5
	3	23	26

Table 2.

Number of patients	Enrolled in 2009	Continuation before 2009	Total
TR	10	12	22
Clinical trials from pharmaceutical companies	4	6	10
Multi-center studies	1	7	8
	15	25	40

3. The Development of the Scholastic Program for the Graduate Students of Nurses in the Area of Translational Research.

Kazufumi Matsumoto, Noriko Fujiwara, Fumitaka Nagamura.

Purpose: Translational Research (TR) is the early phase of clinical trials, which applied the developments of basic researches for patients with incurable and/or life-threatening diseases. High-educated nurses are indispensable for the conducts of TRs in terms of the protection of participants in TRs and the conducts of scientifically appropriate TRs. We developed the scholastic program for the graduate students of nurses in the area of TR.

Method: We planed and implemented the two-weeks program to foster the expert research nurse aimed at the graduate students. It consists of the lectures on the feature points of TR (e.g. ethical considerations of TR, and the role of research nurse), role-plays of Institutional Review Board and obtaining Informed Consent, case conference, and the experience of the actual operations. We evaluated the reports and the questionnaires from the students to explore the degree of their understandings and satisfactions for this program. These reports and questionnaires were analyzed in accordance with the qualitative method. Result: Six students participated in the program and we evaluated the reports and the questionnaires. Students could understand the role of research nurse and the necessary ability and organization to play this role appropriately. They were satisfied with the content and the quality of lectures and role-plays, however, the experiences of the actual operations did not meet their demands due to the less acquisition of the practical expertise. Conclusion: Generally, our program meets the demands of the students, however, the improvement of the content on the experience of the actual operations is the next issue.

4. Comparison of pivotal studies for approval of oncologic drugs in Japan and the U.S.

Fumitaka Nagamura, Makiko Tajima.

"Drug-lag" is one of the problems on drug development process in Japan, especially for life-threatening diseases such as oncologic drugs. To evaluate this problem, we analyzed pivotal studies for oncologic drugs both in Japan and the U.S., because the U.S. Food and Drug Administration has accept widely foreign data. In this study, we collected data from database of web-page of regulatory agencies of two countries, package inserts, papers on pivotal studies. In the U.S., two hundred ninety pivotal studies of 88 drugs were evaluable. One hundred twenty-one studies were conducted in the U.S>, twenty-seven were in North America, sixty-five were international outside the U.S., sixty-seven were international including the U.S.. The number of studies conducted in Japan was only three, this number is the seventh in Asian countries, and at least two studies were not included in pivotal studies due the problems of the line of indication and insufficient setting. The occupation of phase III studies among pivotal studies was 50/121 in the U.S. and 3/111 in Japan. The lack of the experience to conduct phase III studies in terms of both regulatory and institutional aspects and different standard therapy setting, would be the causes of problems in Japan.

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Research Hospital

Department of Cell Processing and Transfusion
セルプロセッシング・輸血部

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Our department is established in 1990, in order to manage the transfusion medicine and the cell processing for hematopoietic stem cell transplantation. We have cooperate with Tokyo Cord Blood Bank, whose cell processing and cryopreservation facility was established the first in IMSUT, in 1997 and transferred these function to Yotsugi Facility of Donated Blood Distribution Corporation and in 2008, we have established the research cord blood stem cell bank as IMSUT-Cell Resource Center (IMSUT-CRC) corporate with Tokyo CBB. Also we have been engaged to study for the development of various cell therapies together with other departments, as follows.

1. Expanded regulatory T cell therapy for GVHD, transplantation and autoimmune diseases.

Nagamura-Inoue T, Ogami K, Tojo A

Regulatory T cells harbored the immunosuppressive effects and were related to the pathogenesis of graft-versus-host disease (GVHD), rejection of organ transplantation and autoimmune disease. We developed the system of *ex vivo* expansion of $CD25^{+}FOXP3^{+}$regulatory T cells from the small amount of peripheral blood and also cord blood (CB), to apply the immunological therapy.

2. Research Cord Blood Stem Cell Bank (IMSUT-Cell Resource Center):

Nagamura-Inoue T, Ishige I, Yuzawa M, Tamura T, Ogami K, Tojo A

"Research Cord Blood Stem Cell Bank" (former named 'Research Stem Cell Resource Bank') was established by the support of MEXT (Ministry of Education, Culture, Sports, Science and Technology) for the development of the medicine including Regenerative Medicine and drug discovery in Japan since 2004. The research banks process CB units, which are non-conforming for clinical use, and cryopreserve and provide the frozen CB to domestic researchers for research use via RIKEN Bioresource Center.

Visit our home page http://scb.ims.u-tokyo.ac.jp/

3. Exploring mesenchymal stem cells derived from Umbilical Cord:

Ishige I, Nagamura-Inoue T, Yuzawa M, Tamura T, Tojo A

In addition to contribute the research use of cord blood banking as the regenerative leading project, we have been explored the new source, mesenchymal stem cells derived from umbilical cord including Wharton jelly, artery, and vein cooperating with the hospital.

4. Room for Clinical Cellular Technology (RCCT):

Nagamura-Inoue T, Ogami K, Tojo A

To promote the cell therapy related to translational research, RCCT has been established in 1997. Until now, the following projects had implemented; 1) Cord blood cell processing for banking (for Tokyo Cord Blood Bank and Research cord blood stem cell bank), 2) Dendritic cell therapies, 3) Regenerative therapy of alveolar bone derived from bone marrow mesenchymal cells, 4) Gene therapy for renal cancer.

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Research Hospital

Core Facility for Therapeutic Vectors
治療ベクター開発室

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Project Research Associate	Hisako Katano, D.D.S., Ph.D.		特任助教	歯学博士	片 野 尚 子

The primary function of the Core Facility for Therapeutic Vectors (CFTV) is to support clinical trials that require the genetic modification and/or ex vivo manipulation of patients' tissue or cells under current Good Manufacturing Practice (cGMP) conditions defined by FDA of USA. The CFTV is the first facility established in Japanese academia to produce genetic or cellular vectors of clinical grade. Using this facility, the adenoviral vector and herpes vector were prepared in CFTV for clinical use in 2009 and 2008 respectively.

1. Preparation of Standard Operating Procedures (SOPs)

The cGMP compliance is maintained using written SOPs prepared by ourselves. The SOPs codify all aspects of laboratory activities including facility design and operations of the personnel. The SOPs enables the staff not only to produce the reagents with high quality in the stable manners but also to help identify areas for improvement.

2. Adoption of ISO

In order to continuously improve our activities, quality management system of the CFTV has been assessed and found to be in accordance with the requirements of the quality standards detailed ISO9001:2008; in the scope of development and manufacture of cell and gene therapy products.

3. Validation of CFTV

The CFTV consists of two distinct units; 1) Vector Unit, the primary viral vector production suite which may also function as *ex vivo* transduction suite; 2) Cell Unit, cell processing suite capable of generating dendritic cells for immunotherapy and gene therapy. There are two self-contained vector production suites in the Vector Unit and two self-contained tissue culture suites in the Cell Unit. These suites are kept Class 10,000. There are many features incorporated into the design of this CFTV to minimize the risk of cross-contamination between products; i.e., unidirectional traffic flow, individual airlocks to each production suite, single-pass HEPA filtered supply air, 100 percent exhaust from the biological safety cabinets through dedicate ducts, among others. Periodical validation has been performed on the facility and the equipments in CFTV to ensure cGMP compliance.

4. Projects in CFTV

Four projects are now in progress in the CFTV.

I. Cancer gene therapy using dendritic cells transfected with IL-12 genes

Takafumi Nakamura, Hisako Katano, Akira Kanamoto*, Marimo Sato*, Hideaki Tahara:

[*]Division of Bioengineering, Advanced Clinical Research Center

・Preparation of the Clinical Lot

We have been preparing the replication-defective recombinant adenoviral vector encoding human interleukin-12, which is an immune-stimulatory cytokine. The backbone of this vector is based on the E1- and E3-deleted serotype 5 adenovirus with a modified fiber, harboring an integrin-binding CDCRGDCDC-motif within the HI-loop of its knob protein. The IL-12 genes are driven by a CA promoter (CMV-IE enhancer with the chicken β-actin promoter). The master virus seed stock (MVSS) and purified final material have been prepared following the optimization of purified method for the production of high-titer vector. The purified material is now in the process of quality examination for use of early phase trials.

II. Vaccine therapy with peptide-loaded dendritic cells for advanced melanoma

Hisako Katano, Takafumi Nakamura, Akira Kanamoto[*], Marimo Sato[*], Hideaki Tahara: [*]Division of Bioengineering, Advanced Clinical Research Center

・Preparation of Peptide-Loaded Dendritic Cells (DCs)

We have been supporting phase I clinical trials against melanoma. Based on the results of the basic research performed in Division of Bioengineering, the SOPs of the DC preparation have been written and used. The cellular reagents have been successfully prepared in the Cell Unit and offered for clinical trials without serious problems.

III. Oncolytic viral therapy using genetically engineered herpes simplex viruses for malignant brain tumors.

Tomoki Todo[**], Yasushi Ino[**], Takafumi Nakamura, Hisako Katano, Hideaki Tahara: [**]Department of Neurosurgery, Graduate School of Medicine, The University of Tokyo

・Manufacture of the viral vector

In collaboration with the research team, we have been preparing oncolytic herpes simplex virus. We have supported the establishment of the master and working cell banks of Vero cells to produce genetically engineered herpes simplex viruses. The cGMP compliant MVSS, which contains a replication-competent herpes simplex virus type 1 vector defective for the α47 gene, was successfully produced. The purified final products have been successfully prepared, approved for clinical use by the authorities and are now ready for the use in phase I clinical trial for brain cancer patients.

IV. Development of robotized cell culture system

Shigeyuki Wakitani[***], Marimo Sato[*], Takafumi Nakamura, Hisako Katano, Hideaki Tahara: [***]Department of Orthopedics, Graduate School of Medicine, Osaka City University

In collaboration with Kawasaki Heavy Industries, Inc., we have been developing robotized cell culture system which could be applied to a variety of procedures including virus production as a funded project by NEDO.

5. Financial Support

This CFTV has been supported in large by Coordination, Support and Training Program for Translational Research from Ministry of Education, Culture, Sports, Science and Technology (2007-2009), and Advanced Clinical Research Center of IMSUT.

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Research Hospital

Department of Laboratory Medicine
附属病院　検査部

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The Department of Laboratory Medicine consists of eight divisions-clinical physiology, hematology, biochemistry, serology, bacteriology, molecular diagnosis and pathology, and a division of flow cytometrical analysis. This Department engages in the laboratory analysis and gives diagnosis of clinical materials in the hospital. In addition, We have established new facility named "Laboratory of TR verification". While facilitating the ongoing translational research (TR) projects in the research hospital, the Department functions as an integrated diagnosis & monitoring laboratory that evaluates the safety and effectiveness of experimental therapeutic approaches.

Overview

Our basic research strategies include the following approaches: characterizing molecular mechanisms underlying the pathology, developing a novel method to measure the disease-defining mechanism in the clinical materials and evaluating the effectiveness of molecular-targeted therapies thereby contributing to the translational research conducted in the institute. Integrating molecular-/biochemical-based laboratory assays on the solid background of pathological examinations enables us to evaluate the effectiveness of experimental clinical trials and leads to correct experimental therapies that further promote translational research. Our department also functions as an integrated diagnosis & safety-monitoring laboratory as well as the division of quality control by examining/evaluating the safety of investigational new drugs under GMP conditions.

1. Evaluation of "proof of concept" of the TR clinical trials and validate safety of the biomedical reagents under GMP standard

Evaluation of "proof of concept" of the TR clinical trial is one of our important missions which are described as follows. In addition to this, the missions include validate the safety of the biomedical reagents such as vector constructs are cellular therapeutic materials which will be used for patients as gene therapy and cell therapy, respectively. These are critical to conduct TR clinical trials in a safe manner. We thus established a new division, which specifically aimed to fulfill this purpose under the quality of GMP regulation. We are now ready to validate the safety by conducting aseptic test, endotoxin-free test and micoplasma-free test under strictly regulated GMP facility.

2. Pathological evaluation of cancer immunotherapy

We have initiated the analysis of surgical specimen obtained from the patients under cancer immuno-therapy conducted in the research hospital. By applying sophisticated immunohistochemical techniques, we now are intensively analyzing materials from cases including GM-CSF-based gene therapy for renal cell carcinoma

and dendritic cell-based or peptide-pulsed anti-melanoma immuno-therapy. One of our goals is to evaluate the effectiveness of the therapies and to elucidate the mechanisms of anti-tumor immune response elicited by the therapy *in situ.*

3. Elucidation of immunopathological mechanisms of autoimmune-based hematological disorders

We found the presence of characteristic pathological findings in bone marrow specimen from some patients with MDS-RA, aplastic anemia, or pure red cell aplasia, which implicates that common immunopathological mechanism, may be operative in these hematological abnormalities; that is destruction of erythroid precursors by immune-based mechanisms in the bone marrow. In collaboration with the Department of Hematology, the Department of Laboratory Medicine will elucidate molecular mechanisms based on the pathological consideration to establish new disease entities and develop new therapeutic interventions.

4. Analysis of the chimeric gene expression of hematological disorder

We have initiated the analysis of bcr-abl gene expression in specimen from patients with CML and Ph1+ve ALL by real-time PCR and nested RT-PCR techniques. In addition, we sequenced the amplified products to provide information for the molecular resistance to STI571 treatment. We are expanding the target molecules to non-hematological disorder, which includes c-kit, PDGF-R genes that is associated with gastro-intestinal stromal cell tumor (GIST).

5. Developing quick & inclusive diagnosis system for infectious disease

Since the introduction of new therapeutic maneuver, host-pathogen interactions have drastically altered drawing attention. This has resulted in altered recognition and molecular interaction of infected cells with immune cells, leading to atypical pathological as well as clinical manifestations. While distinguishing infectious disease and immunological disorder calls for urgent attention, it may be difficult to achieve these tasks in some cases due to modified manifestations. To avoid such cases, it is imperative to establish a comprehensive diagnosis system of infectious disease to the earliest possible opportunity.

6. Immunopathogical analysis of hematopoietic cell transplantation

The number of allogeneic hematopoietic stem cell transplantation (HSCT), mainly cord blood transplantation, has been performed for the treatment of hematological malignancies. Graft-versus-host disease (GVHD), a life-threatening complication, occurs as a complication of allogeneic HSCT. Our prime goal is to develop a new way to detect GVHD and make an accurate evaluation of GVHD at our laboratory.

Laboratory of TR verification
GMP-based facility of laboratory analysis