Research Hospital

Department of Advanced Medical Science
先端診療部

Professor	Naohide Yamashita, M.D., Ph.D.	教　授	医学博士	山　下　直　秀
Lecturer	Takashi Nakaoka, M.D., Ph.D.	講　師	医学博士	中　岡　隆　志
Project Lecturer	Hitomi Nagayama, M.D., D.M.Sc.	特任講師	医学博士	長　山　人　三
Clinical Associate	Hideki Ohno, M.D., Ph.D.	助　教	医学博士	大　野　秀　樹
Clinical Associate	Naoyuki Iso-O, M.D., Ph.D.	助教(併任)	医学博士	磯　尾　直　之
Clinical Associate	Naoyuki Takahashi, M.D., Ph.D.	助教(併任)	医学博士	高　橋　直　之

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) Adoptive transfer of allogeneic umbilical cord blood-derived cytotoxic lymphocytes, (2) Analysis of the gradient expression of genes in human colonic mucosa, (3) Analysis of the effect of Helicobacter pylori eradication on non-ulcer patients, (4) Analysis of the role of Dnm3os, a non-coding RNA in skeletal development, (5) Analysis on the mechanisms of cardiac outflow tract development and (6) Analysis of immune-related microRNA expression in human cord blood and adult peripheral blood cells upon proinflammatory stimulation.

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

Nagayama H., Fujita S., et al.

We have intensively investigated the possibility of adoptive transfer of allogeneic umbilical cord blood-derived cytotoxic T lymphocytes (CTLs) for the treatment of hematological malignancies and solid tumors. Cryopreserved or fresh umbilical cord blood was used as the source of CTLs. Some combination of T cell growth factors and antigen-specific stimulation were utilized for the massive expansion of CTLs. Flow cytometric analysis, HLA-restricted tetramers and cytoplasmic interferon-γ stain reveals the cellular differentiation with functional maturation of ex vivo expanded CTLs. (manuscript in preparation) Now we are claiming the international patent of this method from the University of Tokyo (PCT/LP2010/63181) and will be exposed later.

2. 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. We speculate that the gradient expression of genes in human colonic mucosa might be related to the disease development and progression. We evaluated the expression levels of genes throughout the GI tract and in other tissues by micro array and northern blot analysis. As a result of these analyses, some genes showed the expression gradient to increase to-

ward the distal colon. We are currently investigating the expression changes of these genes in human intestinal diseases.

3. Analysis of the effect of *Helicobacter pylori* eradication on non-ulcer patients

Ohno H et al.

Recent reports showed that eradication of *Helicobacter pylori* (Hp) has prophylactic effect on the development of gastric cancer. The guideline of the Japanese Society for Helicobacter Research strongly recommended the eradication therapy for all Hp-positive patients including non-ulcer patients. Therefore, we set up outpatient clinic for the eradication therapy to prevent Hp associated disease such as gastric cancer. But it is unclear that Hp eradication therapy can improve gastrointestinal symptoms of non-ulcer patients. We are investigating the long-term effects of Hp eradication on non-ulcer patients.

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

Nakaoka T. et al.

Dnm3os, a non-coding RNA, 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 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.

5. 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. 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.

6. Analysis of immune-related microRNA expression in human cord blood and adult peripheral blood cells upon proinflammatory stimulation

Takahashi N. et al.

Cord blood (CB) transplantation has advantages in terms of incidence and severity of acute graft-versus-host disease (GVHD), while it has disadvantages in terms of infection. To elucidate the molecular mechanism underlying the immune response of CB-derived cells during acute GVHD and infection following CB transplantation, we examined expression of 69 immune-relating microRNAs (miRNAs) and 11 protein-coding mRNAs in $CD4^{+}$, $CD8^{+}$, and $CD14^{+}$ cells of CB and adult peripheral blood (APB) upon proinflammatory stimulation. Under basal condition, 20 miRNAs showed differential expression between CB and APB, for 8 of which the expression appeared to be regulated by MYC that was overexpressed in CB-derived $CD4^{+}$ and $CD8^{+}$ cells. Upon IFN-γ stimulation, 9 miRNAs changed in expression mainly in $CD14^{+}$ CB cells, while only 2 miRNAs changed in expression in $CD14^{+}$ CB cells upon LPS stimulation. IFN-γ and LPS up-regulated anti-inflammatory/anti-proliferative genes in $CD14^{+}$ CB cells. These results suggest that the mechanisms regulating expression of such immune-relating miRNAs and mRNAs in $CD14^{+}$ CB cells are much more sensitive to proinflammatory stimuli than those in APB $CD14^{+}$ cells, which appears to be related to the poor immunoreactivity of $CD14^{+}$ CB cells.

Publications

Nagayama H, Matsumoto K, Isoo N, Ohno H, Takahashi N, Nakaoka T, Shinozaki M, Watanabe M, Inoue Y, Nagamura F, Oyaizu N and Yamashita N. Gastrointestinal bleeding during anti-angiogenic peptide vaccination in combination with gemcitabine for advanced pancre-

atic cancer. *Clin J Gastroenterol* 2010; 3: 307-317.

Narumoto O. Horiguchi K. Horiguchi S. Moriwaki Y. Takano-Ohmuro H. Shoji S. Misawa H. Yamashita N. Nagase T. Kawashima K. Yamashita N (2010) Down-regulation of secreted lymphocyte antigen-6/urokinase-type plasminogen activator receptor-related peptide-1 (SLURP-1), an endogenous allosteric alpha7 nicotinic acetylcholine receptor modulator, in murine and human asthmatic conditions. *Biochem Biophysl Res Commun* 398: 713-8

Matsumoto K, Nagamura F, Ogami Y, Yamashita N, Kamibeppu K. (2010) Difficulties of Nursing Staff Involved in Phase 1 Oncology Trials in Japan. *Cancer Nursing* (Internatl J Cancer Care). 印刷中

Research Hospital

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

Professor	Arinobu Tojo, M.D., D.M.Sc.	教　授	医学博士	東　條　有　伸
Associate Professor	Satoshi Takahashi, M.D., D.M.Sc.	准教授	医学博士	高　橋　　聡
Associate Professor	Kaoru Uchimaru, M.D., D.M.Sc.	准教授	医学博士	内　丸　　薫
Assistant Professor	Jun Ooi, M.D.	助　教		大　井　　淳
Assistant Professor	Koichiro Yuji, M.D., D.M.Sc.	助　教	医学博士	湯　地　晃一郎
Assistant Professor	Nobuhiro Ohno, M.D.	助　教		大　野　伸　広
Assistant Professor	Nobuhiro Tsukada, M.D., D.M.Sc.	助　教	医学博士	塚　田　信　弘
Assistant Professor	Seiko Kato, M.D., D.M.Sc.	助　教	医学博士	加　藤　せい子
Assistant Professor	Ai Kotani, M.D., D.M.Sc.	助　教	医学博士	幸　谷　　愛
Assistant Professor	Seiichiro Kobayashi, M.D., D.M.Sc.	助　教	医学博士	小　林　誠一郎
Project Assistant Professor	Toshiro Kawakita, M.D.	特任助教		河　北　敏　郎

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 350 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 novel therapeutic agents including tyrosine kinase inhibitors for Philadelphia-chromosome positive leukemias, RI-conjugated or non-conjugated anti-CD20 monoclonal antibodies for B cell lymphoma and a 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. Unrelated cord blood transplantation after myeloablative conditioning in adults with advanced myelodysplastic syndromes.

Sato A, Ooi J, Takahashi S, Tsukada N, Kato S, Kawakita T, Tojo A

We analyzed the disease-specific outcomes of adult patients with advanced myelodysplastic syndrome (MDS) treated with cord blood transplantation (CBT) after myeloablative conditioning. Between August 1998 and June 2009, 33 adult patients with advanced MDS were treated

with unrelated CBT. The diagnoses at transplantation included refractory anemia with excess blasts (n=7) and MDS-related secondary AML (sAML) (n=26). All patients received four fractionated 12 Gy TBI and chemotherapy as myeloablative conditioning. The median age was 42 years, the median weight was 55?kg and the median number of cryopreserved nucleated cells was 2.51×10^7 cells per kg. The cumulative incidence of neutrophil recovery at day 50 was 91%. Neutrophil recovery was significantly faster in sAML patients (P=0.04). The cumulative incidence of plt recovery at day 200 was 88 %. Plt recovery was significantly faster in CMV seronegative patients (P<0.001). The cumulative incidence of grade II-IV acute GVHD (aGVHD) and extensive-type chronic GVHD was 67 and 34%, respectively. Degree of HLA mismatch had a significant impact on the incidence of grade II-IV aGVHD (P=0.021). TRM and relapse at 5-years was 14 and 16%, respectively. The probability of EFS at 5 years was 70%. No factor was associated with TRM, relapse and EFS. These results suggest that adult advanced MDS patients without suitable related or unrelated BM donors should be considered as candidates for CBT.

2. The impact of steroid use as a GVHD treatment or prophylaxis within 100 days after CBT

Kawakita T, Tsukada N, Takahashi S, Ooi J, Kato S, Tojo A

The incidence of severe graft-versus-host disease (GVHD) in cord blood transplantation (CBT) is generally low, but still exists. In our institute, we use cyclosporine (CsA) and short term methotrexate (MTX) as GVHD prophylaxis and minimally use steroid to avoid infection or infection-related complications. In this study, we retrospectively analyzed the clinical data to clarify the impact of steroid use to the outcome of CBT. PATIENTS: We have performed 140 CBT after myeloablative conditioning using CsA with short term MTX as GVHD prophylaxis for adults at IMSUT between August 1998 and October 2008. The median age was 39 (range, 16-55) years and the median number of cryopreserved nucleated CB cells was 2.38 (range, 1.21-5.69)$\times10^7$/kg. Although 82 of 140 patients (59 %) suffered from grade II-IV aGVHD, only 31% patients received steroid after CBT. Steroid was used in 17 patients (12%) as a treatment for mainly GVHD and the dosage of prednisolone in the treatment group were 2 mg/kg (n=7), 1 mg/kg (n=8), and 0.5mg/kg (n=2). Twenty-six patients (19%) changed CsA to steroid because of intolerability (20: renal dysfunction, 4: encephalopathy, 2: others) and received 1mg/kg (n=4) or 0.5mg/kg (n=22) (alternative group). Overall survival in 5 years were 78% in the non-steroid use group, 71% in the treatment group, however 45% in the patients with alternative steroid use. The intolerability of CsA within 100 days after CBT seems to be a significant poor factor. We should modify the procedures including post-transplant immune modulation in such patients.

3. Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan

Uchimaru K

Definitive risk factors for the development of adult T-cell leukemia (ATL) among asymptomatic human T-cell leukemia virus type I (HTLV-1) carriers remain unclear. Recently, HTLV-1 proviral loads have been evaluated as important predictors of ATL, but a few small prospective studies have been conducted. We prospectively evaluated 1218 asymptomatic HTLV-1 carriers (426 males and 792 females) who were enrolled during 2002 to 2008. The proviral load at enrollment was significantly higher in males than females (median, 2.10 vs 1.39 copies/100 peripheral blood mononuclear cells [PBMCs]; $P<.001$), in those 40 to 49 and 50 to 59 years of age than that of those 40 years of age and younger ($P=.02$ and .007, respectively), and in those with a family history of ATL than those without the history (median, 2.32 vs 1.33 copies/100 PBMCs; $P=.005$). During follow-up, 14 participants progressed to overt ATL. Their baseline proviral load was high (range, 4.17-28.58 copies/100 PBMCs). None developed ATL among those with a baseline proviral load lower than approximately 4 copies. Multivariate Cox analyses indicated that not only a higher proviral load, advanced age, family history of ATL, and first opportunity for HTLV-1 testing during treatment for other diseases were independent risk factors for progression of ATL.

4. Monitoring CMV pp65 antigen during chemotherapy for adult T-cell leukemia/lymphoma

Isobe M, Ohno N, Ohfuchi-Tsuda M, Kobayashi S, Yuji K, Uchimaru K, Tojo A

Chemotherapy of Adult T-cell leukemia (ATL) patients is frequently complicated with opportunistic infection due to defective cellular immu-

nity. In immunosuppressive state, reactivation of Cytomegarovirus (CMV) infection is one of the most important complications. CMV pp65 antigen is routinely monitored in the patients after stem cell transplantation for early detection of reactivation of CMV infection but there is little information about CMV pp65 antigen during the chemotherapy for ATL. We determined CMV pp65 antigen in the patients with ATL who were treated with intensive combination chemotherapy. Eleven patients with aggressive ATL (9 with acute type ATL, 2 with lymphoma type ATL) were treated in our hospital during September 2008 and April 2010. Median age of the patients was 58 years old (28～69 years old) and median follow up time is 119 days (61～358 days). Eight patients were treated with LSG15 but 2 of them became refractory to the therapy and received mEPOCH thereafter. Three other patients were refractory to chemotherapy and died by ATL. Nine of the patients became positive for CMV pp65 antigen during chemotherapy and treated with gancyclovir (GCV). Six of the 9 patients treated with GCV became positive for CMV antigen after cease of GCV treatment but CMV antigen decreased by induction of GCV again. None of the patients developed CMV-related disease. In conclusion, CMV antigenemia is highly frequent complication in the patients with ATL during chemotherapy and our data suggested that monitoring of CMV antigen and pre-emptive therapy for CMV could prevent progression into CMV-related disease.

5. Aberrant CD3/CD7 expression in CD4+ T cells of HTLV-1 carriers

Ohno N, Kobayashi S, Isobe M, Tsuda M, Uchimaru K, Tojo A

Outpatient clinic for HTLV-1 carriers is set once a week in our department and about 200 HTLV-1 carriers visited there this year. Discrimination between asymptomatic carriers and indolent ATL patients such as smoldering type-ATL depends on morphological examination of peripheral blood films according to Shimoyama's criteria but it is sometimes confusing due to morphological diversity of ATL cells. We reported that ATL cells could be clearly discriminated as $CD3^{dim}/CD7^{low}$ population using Flow cytometry (FACS). To investigate that CD3/CD7 expression pattern can detect clonal expansion of HTLV-1 infected cells in the peripheral blood of HTLV-1 carrier, we analyzed CD3/CD7 expression on CD4+ T cells in 35 HTLV-1 carriers using multi-color FACS. In some HTLV-1 carriers, $CD3^{dim}/CD7^{dim}$ population was expanded compared with non-HTLV-1 infected healthy control. HTLV-1 proviral load was significantly increased in these carriers who exhibited expanded $CD3^{dim}/CD7^{dim}$ population by FACS analysis. Morphologically this $CD3^{dim}/CD7^{dim}$ population sorted using multi-color FACS showed nuclear abnormality. Inverse long PCR detected major HTLV-1 infected clone in some of these cases. These results suggested that CD3/CD7 expression analysis using our multi-color FACS system in HTLV-1 carriers could be useful for detection of high risk carriers for ATL progression.

6. Intensive chemotherapy followed by stem cell transplantation for aggressive ATL patients.

Isobe M, Tsuda M, Ohno N, Kobayashi S, Yuji K, Uchimaru K, Tojo A

Adult T-cell leukemia/lymphoma (ATL) is one the intractable T cell malignancies. Mean survival time for aggressive ATL patients treated by LSG15 protocol, which is one of the most effective chemotherapeutic regimens, is only 13 months and overall survival at 3 years is 24 percent. Several studies showed that stem cell transplantation (SCT) including reduced intensity one was promising for the treatment of aggressive ATL. We treated 20 aggressive ATL patients (17 acute type, 3 lymphoma type; 8 males and 12 females) by intensive combination chemotherapy and perform stem cell transplantation for feasible patients. The median age of the patients was 59 years (range 28-75). Two cases were refractory to chemotherapy and died by ATL. Two cases were complicated with infection and died in partial remission. One patient had no indication for stem cell transplantation because of the age over 70 years. Thirteen patients already received stem cell transplantation (1 myeloablative and 12 non-myeloablative SCT). Disease status at SCT was as follows: 2 in complete remission, 9 in partial remission, 2 in progressive disease. Nine of the patients who received SCT remain alive in complete remission at between 48 days and 2057 days (median 434 days) after SCT. Two patients who received SCT in progressive disease died because of early relapse at 48 days and 104 days after SCT. These results suggested promising therapeutic effect of SCT for aggressive ATL except for the patient in progressive disease.

7. Acute promyelocytic leukemia with adult Down syndrome

Tsuda M, Isobe M, Kobayashi S, Yuji K, Ohno N, Uchimaru K, Tojo A

Down syndrome (DS) is associated with increasing risk of acute myeloid leukemia (AML). The most common subtype of AML described in patients with DS is acute megakaryocytic leukemia (AMKL)., which occur in younger than 4 years of age. The occurrence of acute promyelocytic leukemia (APL) is extremely rare in DS patients. Indeed, only one case has been reported as APL with adult DS. We here report a 23 year-old male with DS diagnosed as having APL. The patient was referred with pancytopenia and pneumonia . He had no history of transient abnormal myelopoiesis at newborn period. His leukemic cells were CD13+, CD33+, CD34−, with t(15; 17), +21 on cytogenetic analysis and expressed PML/RAR-chimeric mRNA. He showed the complication of disseminated intravascular coagulation on admission. All-trans retinoic acid (ATRA) was started immediately. As WBC count increased regardless of starting ATRA treatment, we added a reduced dose of chemotherapy modified form AML-D05 protocol for AML with DS, in combination with ATRA because AML patients with DS had been reported to have a high cure rate when treated with reduced dose chemotherapy. He went into cytogenetic complete remission after induction therapy, and molecular complete remission during consolidation chemotherapy. This case is valuable since APL is very uncommon in DS patients. Our experience showed that less intensive chemotherapy seemed to be effective even on APL in DS patients.

Publications

Sato A, Ooi J, Takahashi S, Tsukada N, Kato S, Kawakita T, Yagyu T, Nagamura F, Iseki T, Tojo A, Asano S. Unrelated cord blood transplantation after myeloablative conditioning in adults with advanced myelodysplastic syndromes. Bone Marrow Transplant, 2010 Apr 19. [Epub ahead of print]

Kim DW, Banavali SD, Bunworasate U, Goh YT, Ganly P, Huang H, Irving I, Jootar S, Goh HG, Koh LP, Li W, Naoe T, Ng SC, Purushotaman V, Reksodiputro H, Shih LY, Tang JL, Tojo A, Wang J, Wong R. Chronic myeloid leukemia in the Asia-Pacific region: current practice, challenges and opportunities in the targeted therapy era. Leuk Res. 34: 1459-71, 2010

Iwanaga M, Watanabe T, Utsunomiya A, Okayama A, Uchimaru K, Koh KR, Ogata M, Kikuchi H, Sagara Y, Uozumi K, Mochizuki M, Tsukasaki K, Saburi Y, Yamamura M, Tanaka J, Moriuchi Y, Hino S, Kamihira S, Yamaguchi K, and for the Joint Study on Predisposing Factors of ATL Development investigators. Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan. Blood 116: 1211-1219, 2010

Research Hospital

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

Head, Lecturer	Takeshi Fujii, M.D. D.M.Sc.		科長(講師)	医学博士	藤井　毅
Professor	Aikichi Iwamoto, M.D., D.M.Sc.		教　授	医学博士	岩本愛吉[*1]
Assistant Professor	Tomohiko Koibuchi, M.D., D.M.Sc.		助　教	医学博士	鯉渕智彦

【兼任】
[*1]Division of Infectious Diseases, The Advanced Clinical Research Center
（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 2010, 66 new patients with HIV infection have visited or been admitted to our hospital and 491 patients in total are currently under our clinical management. The total number of in-patients with HIV-infection during 2010 was 35, and about 10 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, Syoichi Shimizu, Kentaro Imai, Eisuke Adachi, Toshiyuki Miura[1], Hitomi Nakamura[1], Michiko Koga[1], Tadashi Kikuchi[1], Takashi Odawara, and Aikichi Iwamoto[1]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center

66 new patients with HIV-1 infection visited our hospital this year (from January 1 to December 31, 2010), and 491 patients in total are under medical management in our outpatient clinic. The total number of HIV-infected inpatients during 2010 was 35. 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

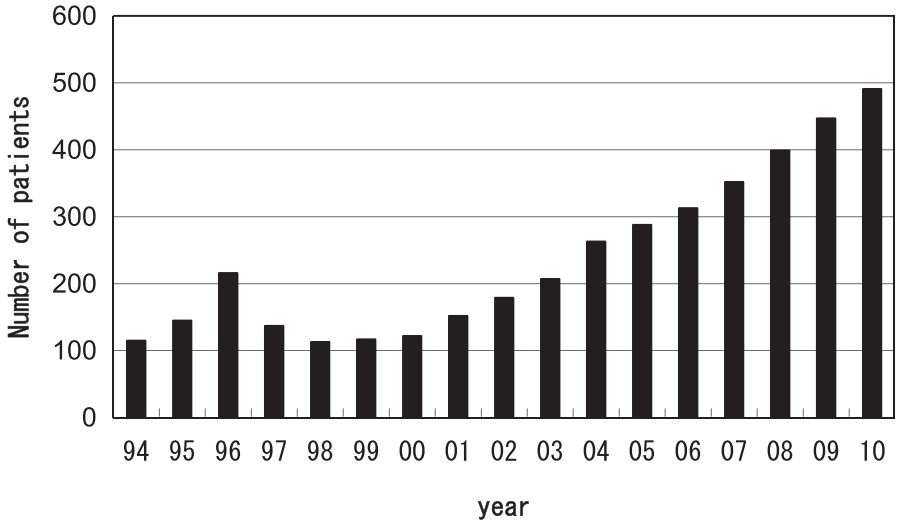

Figure 1. Number of HIV-infected outpatients in IMSUT Hospital

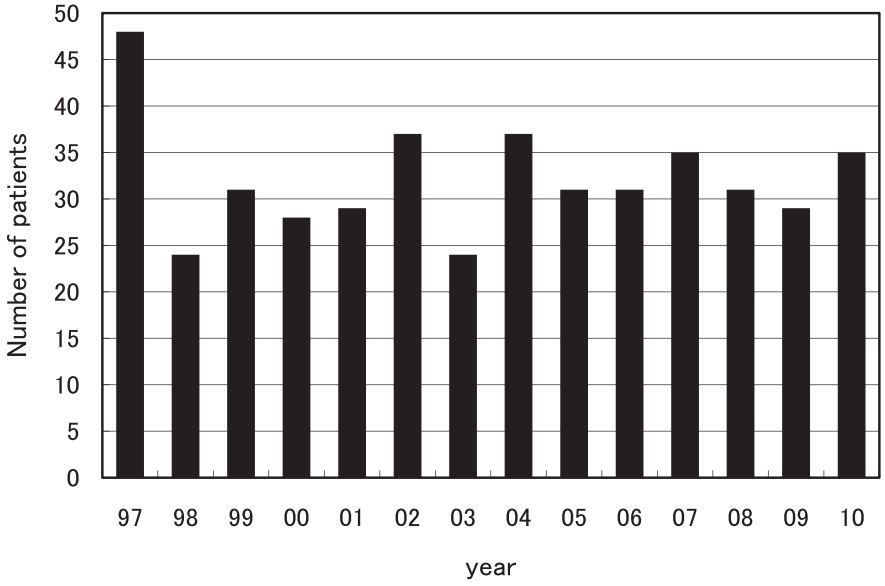

Figure 2. Number of HIV-infected inpatients in IMSUT Hospital

introduction of highly active anti-retroviral therapy (HAART) which effectively suppresses the replication of HIV. Anti-retroviral therapy has been introduced to around 360 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, Tadashi Kikuchi[1], Syoichi Shimizu, Kentaro Imai, Eisuke Adachi, Hitomi Nakamura[1], Michiko Koga[1], Toshiyuki Miura[1], and Aikichi Iwamoto[1]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center

Dozens of important medicines essential for treatment of tropical or parasitic diseases are not licensed in Japan. For instance, artesunate and injectable quinine for falciparum malaria, injectable metronidazole for amebiasis, pyrimethamine and sulfadiazine for toxoplasmosis, etc. are not licensed. Research Group on Chemotherapy of Tropical Diseases, Research on Publicly Essential Drugs and Medical Devices, Grant from the Ministry of Health, Labour and Welfare had been established to cope with this situation. We are the central medical institution of the research group importing and providing these or-

Table 1: List of Orphan Drugs

Generic Name	Brand name	Indication
Chloroquine sulfate	Avloclor	Malaria
Atovaquone-proguanil	Malarone	Malaria
Artemether-lumefantrine	Riamet	Malaria
Artesunate (oral, rectal)	Plasmotrim	Malaria
Injectable quinine	Quinimax	Malaria
Primaquine phosphate	Primaquine	Malaria (anti-relapse agent)
Injectable metronidazole	Metronidazole	Amebiasis
Paromomycin	Humatin	Amebiasis (luminal agent)
Triclabendazole	Egaten	Fascioliasis
Nitazoxanide	Alinia	Cryptosporidiosis (immunocompromised)
Sodium stibogluconate	Pentostam	Leishmaniasis
Miltefosine	Impavido	Leishmaniasis
Suramin	Germanin	African trypanosomiasis
Melarsoprol	Arsobal	African trypanosomiasis
Eflornithine	Ornidyl	African trypanosomiasis
Nifurtimox	Lampit	American trypanosomiasis
Sulfadiazine	Sulfadiazine	Toxoplasmosis
Pyrimethamine	Daraprim	Toxoplasmosis
Propamidine eye drop	Brolen Eye Drop	Acanthamoeba keratitis

phan drugs if needed, and colleting clinical data. This year, we imported and stored 19 orphan drugs (table1) and distributed required ones to 25 designated hospitals in all over Japan.

Also we have clinics for overseas travelers. This year, more than hundred of overseas travelers 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, dengue, typhoid fever, amebiasis, post-exposure prophylaxis of rabies and so on.

3. Comprehensive preoperative evaluation of patients with hemophiliac arthropathy

Tomohiko Koibuchi, Takeshi, Fujii, Syoichi Shimizu, Kentaro Imai, Eisuke Adachi, 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, Syoichi Shimizu, Kentaro Imai, Eisuke Adachi, Toshiyuki Miura[1], Hitomi Nakamura[1], Michiko Koga[1], Tadashi Kikuchi[1], 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.

5. Hepatitis B virus co-infection in HIV-infected Patients in IMSUT hospital

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

The rate of Hepatitis B virus (HBV) co-infection is higher in HIV-infected patients compared with that in the general population because of common transmission routes. The percentage of HBV surface antigen (HBsAg) positive HIV-infected patients in IMSUT hospital is around 5%. On the other hand, HBsAg positive rate in Japanese population is estimated 1.0%. HBV co-infection of HIV-infected patients accelerates development of cirrhosis. Prevention of HBV infection is therefore essential for HIV-infected patients. However, HIV-infected patients respond poorly to HBV vaccination even if the patients' CD4 T-cell count is high. We recently reported the low response rates of HBV vaccination in HIV-infected Japanese patients; out of 12 patients received HBV vaccination at standard schedule (0-, 1-, 6-month) in IMSUT hospital, only one was a responder (HBsAb $\geqq$10 IU/L). Our data support that the strategy of improving the efficacy of HBV vaccination in HIV-infected patients is urgently needed.

6. Pulmonary nocardiosis caused by Nocardia exalbida complicating Pneumocystis pneumonia in a HIV-infected patient

Kentaro Imai, Tomohiko Koibuchi, Takeshi, Fujii, Toshiyuki Miura[1], Hitomi Nakamura[1], Michiko Koga[1], Tadashi Kikuchi[1], Aikichi Iwamoto[1], Katsukiyo Yazawa[2] and Tohru Gonoi[2]: [1]Division of Infectious Diseases, The Advanced Clinical Research Center, [2]Medical Mycology Research Center, Chiba University, Chiba, Japan

A 47-year-old male with optimally-controlled type-2 diabetes mellitus and chronic hepatitis B was admitted to a local hospital for a 1-week history of cough and high-grade fever. He was diagnosed with Pneumocystis pneumonia (PCP) and Klebsiella pneumonia from a chest radiograph and sputum. Simultaneously, he was found to have HIV infection with CD4 count of 76/μl. Despite alteration of treatment secondary to the development of allergic reaction to sulfamethoxazole-trimethoprim (TMP-SMX), the patient was able to complete a three-week therapy for PCP after switching to pentamidine isetionate. After treatment of PCP, he was referred to our hospital for the initiation of anti-HIV therapy. He presented with recurrent high-grade fever for a few days prior to his initial visit, which subsequently led to his admission. Chest CT showed the enlargement of previously identified infiltrate in left upper lung field, and the sputum culture upon admission was positive for Gram-positive branching rods; the organism was later identified as Nocardia exalbida. Due to suflamide allergy, the patient was treated with imipenem (IMP) and amikacin (AMK) intravenously for seventeen days, followed by garenoxacin (GRNX) orally for six months without any adverse effects. The chest infiltrate resolved completely, and he remains stable without relapse eight months after the completion of the therapy. Pulmonary nocardiosis should be considered as a differential diagnosis of recurring pneumonia in immunocompromised patients, especially in HIV-infected individuals. Oral administration of GRNX following IMP and AMK can be as an alternative therapy to TMP-SMX in cases of pulmonary nocardiosis caused by N. exalbida.

7. Immuune response of pandemic 2009 H1N1 influenza vaccine in HIV-1 infected patients

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

Poor immunogenicity to seasonal influenza

vaccine was reported in HIV-1 positive populations, owing to their impaired functions of B-cells. However there are few reports in HIV-1 positive populations about immunogenicity of pandemic 2009 H1N1 influenza vaccine, which is antigenically so different from seasonal H1N1 that little immune protection exists in human populations. We aimed to determine the immune response of pandemic 2009 H1N1 influenza vaccine in HIV-1 positive populations. 103 HIV-1 infected outpatients of the hospital affiliated to IMSUT and 17 healthy controls have received a single dose of split pandemic H1N1 influenza (A/ California/ 7/ 2009) vaccine and serum samples were taken before and 2 month after vaccination for neutralizing antibody (NT) analysis. Before vaccination, 19 (18.4%) patients and 3 (17.6%) healthy controls had NT titer $\geq$1: 16. At 2 month after vaccination, NT titers of 51 (49.5%) patients and 10 (58.8%) healthy controls had increased at least fourfold. Geometric mean titer (GMT) are increased 4.4 at prevaccination to 19.2 at 2 month after vaccination in HIV-1 infected patients and 4.3 to 18.8 in healthy controls. The differences of neither response rate nor GMT are statistically significant. This study is collaborated with Division of Virology, Department of Microbiology and Immunology.

Publications

1. Imai K, Koibuchi T, Kikuchi T, Koga M, Nakamura H, Miura T, Gonoi T, Yazawa K, Iwamoto A, and Fujii T, Pulmonary nocardiosis caused by Nocardia exalbida complicating Pneumocystis pneumonia in a HIV-infected patient. J Infect Chemother. *in press*
2. Tasaka S, Tokuda H, Sakai F, Fujii T, Tateda K, Johkoh T, Ohmagari N, Ohta H, Araoka H, Kikuchi Y, Yasui M, Inuzuka K, Goto H. Comparison of clinical and radiological features of pneumocystis pneumonia between malignancy cases and acquired immunodeficiency syndrome cases: a multicenter study. Intern Med. 49: 273-81, 2010
3. Kakugawa T, Mukae H, Hishikawa Y, Ishii H, Sakamoto N, Ishimatsu Y, Fujii T, Koji T, Kohno S. Localization of HSP47 mRNA in murine bleomycin-induced pulmonary fibrosis. Virchows Arch. 456: 309-15, 2010
4. Liu J, Wu P, Gao F, Qi J, Kawana-Tachikawa A, Xie J, Vavricka CJ, Iwamoto A, Li T, Gao GF. Novel immunodominant peptide presentation strategy: a featured HLA-A*2402 restricted CTL-epitope stabilized by intrachain hudrogen-bonds from SARS-CoV nucleocapsid protein. J Virol. 84: 11849-11857, 2010.
5. Zhu D, Kawana-Tachikawa A, Iwamoto A, Kitamura Y. Influence of polymorphism in dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin-related (DC-SIGNR) gene on HIV-1 trans-infection. Biochem Biophys Res Commun, 393: 598-602, 2010.
6. Koga, M., Kawana-Tachikawa, A., Heckerman, D., Odawara, T., Nakamura, H., Koibuchi, T., Fujii, T., Miura, T., and Iwamoto, A. Transition of impact of HLA class I allele expression on HIV-1 plasma virus loads at a population level over time. Microbiol. Immunol. 54: 196-205, 2010. Apr 2010.
7. Sun Y, Liu J, Yang M, Gao F, Zhou J, Kitamura Y, Gao B, Tien P, Shu Y, Iwamoto A, Chen Z, Gao GF. Identification and structural definition of H5-specific CTL epitopes restricted by HLA-A*0201 derived from the H5N1 subtype of influenza A viruses. J Gen Virol. 91: 919-930, 2010 Apr 2010.
8. Kondo, N., Miyauchi, K. Meng, F. Iwamoto, A. and Matsuda, Z. Conformational changes of the HIV-1 envelope protein during membrane fusion were inhibited by the replacement of its membrane-spanning domain. J. Biol. Chem. 285: 14681-14688, 2010. May 7, 2010.
9. Yu, L., Aoki, C., Shimizu, Y., Shimizu, K., Hou, W., Yagyu, F., Wen, X., Oshima, M., Iwamoto, A., Gao, B., Liu, W., Gao, GF., and Kitamura, Y. Development of a simple system for screening anti-hepatitis C virus drugs utilizing mutants capable of vigorous replication. J. Virol Methods 2010. in press.
10. Miyauchi, K., Curran, A.R., Long, Y., Kondo, N., Iwamoto, A., Engelman, D.M., and Matsuda, Z. The membrane-spanning domain of gp41 plays a critical role in intracellular trafficking of the HIV envelope protein. Retrovirology 2010, 7: 95. doi:10.1186/1742-4690-7-95. E- published, November 13, 2010.
11. Liu, S., Kondo, N., Long, Y., Xiao, D., Iwamoto, A., and Matsuda, Z. Membrane topology analysis of HIV-1 envelope glycoprotein gp41. Retrovirology. MS: 1060899922421288 in Press.
12. Miura, T., Z.L. Brumme, M.A. Brockman, P. Rosato, J. Sela, C.J. Brumme, F. Pereyra, D.E. Kaufmann, A. Trocha, B.L. Block, E.S. Daar, E. Connick, H. Jessen, A.D. Kelleher, E. Rosenberg, M. Markowitz, K. Schafer, F. Vaida, A. Iwamoto, S. Little, and B.D. Walker. Impaired replication capacity of acute/early vi-

ruses in persons who become HIV controllers. J Virol 84: 7581-91
13. Nakamura H, Miyazaki N, Hosoya N, Koga M, Odawara T, Kikuchi T, Koibuchi T, Kawana-Tachikawa A, Fujii T, Miura T, Iwamoto A. Long-term successful control of super-multidrug-resistant human immunodeficiency virus type 1 infection by a novel combination therapy of raltegravir, etravirine, and boosted-darunavir. J Infect Chemother. 2010 Jun 30. [Epub ahead of print]

Research Hospital

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

Associate Professor Kohichiro Tsuji
Clinical Associate Yasuhiro Ebihara

准教授 医学博士 辻 浩一郎
助 教 医学博士 海老原 康 博

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, Shinji Mochizuki[1], Kohichiro Tsuji; [1]Division of Stem Cell Processing, Center for Stem Cell Biology and Regenerative Medicine

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, Shinji Mochizuki[1], Atsushi Manabe[2], Yuji Zaike[3]; [2]St. Luke's International Hospital, [3]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, Shinji Mochizuki[1], Yoshitoshi Ohtsuka[4], Atsushi Manabe[2], Yuji Zaike[3], 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

Yasuhiro Ebihara, Shinji Mochizuki[1], Satoshi Takahashi[5], Kohichiro Tsuji; [5]Division of Molecular Therapy, Advanced Clinical Research Center

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

Yasuhiro Ebihara, Shinji Mochizuki[1], Hideyuki Takedani[6], Tokiko Nagamura-Inoue[7], Shigeyuki Wakitani[7], Arinobu Tojo[5], Hiromitsu Nakauchi[8], Kohichiro Tsuji; [6]Department of Joint Surgery, Research Hospital, [7]Department of Cell Processing and Transfusion, Research Hospital, [8]Department of Orthopedic Surgery, Osaka City University Graduate School of Medicine, [9]Division of Stem Cell Therapy, Center for Stem Cell Therapy and Regenerative Medicine

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.

Publications

Nagamachi, A.*, Phyowai, H.*, Ma, F.*, Miyazaki, K., Yamasaki, N., Kanno, M., Inaba, T., Honda, Z., Okuda, T., Oda, H., Tsuji, K., Honda, H. A 5′untranslated region containing the IRES element in the Runx1 gene is required for angiogenesis, hematopoiesis and leukemogenesis in a knock-in mouse model. Dev Biol 345: 226-236, 2010. *co-first author

Funayama, K., Saito-Kurimoto. Y., Ebihara, Y., Shimane, Y., Nomura, H., Tsuji, K., Asano, S. Adhesion-mediated self-renewal abilities of Ph+ blastoma cells. Biochem Biophys Res Commun 396: 193-198, 2010.

Ma, F., Yang, W., Tsuji, K. Regenerative medicine based on human ES and iPS cells. In Stem Cells in Medicine. Edited by Isobe K (Transworld Research Network, Kerala), in press.

Ma, F., Yang, W., Ebuhara, Y., Tsuji, K. Generation of blood cells from human embryonic stem cells and their possible clinical utilization. In Embryonic Stem Cells, INTECH, in press.

Ma, F., Gu, Y., Nishihama, N., Ebihara, Y., Tsuji, K. Differentiation of human embryonic and induced pluripotent stem cells in coculture with murine stromal cells. In Lineage-specific differentiation of human embryonic and induced pluripotent stem cells methods and protocols. edited by Marton PJ, Ye K, Jin S (Humana Press, New York), in press.

Ma, F., Gu, Y., Ebihara, Y., Tsuji, K. Generation of blood cells from human ES cells and embryonic hematopoiesis. J Pediatr Biochem, in press.

Iwatsuki-Horimoto, K., Horimoto, T., Tamura, D., Kiso, M., Kawakami, E., Hatakeyama, S., Ebihara, Y., Koibuchi, T., Fujii, T., Takahashi, K., Shimojima, M., Sakai-Tagawa, Y., Ito, M., Sakabe, S., Iwasa, A., Takahashi, K., Ishii, T., Gorai, T., Tsuji, K., Iwamoto, A., Kawaoka, Y. Sero-prevalence of pandemic (HINI) 2009 influenza A virus among schoolchildren and their parents in Tokyo, Japan. J Clinic Microbiol, in press.

Tsuda, M., Ebihara, Y., Mochizuki, S., Uchimaru, K., Tojo, A., Tsuji, K. Reduced dose chemotherapy for acute promyelocytic leukemia with adult Down syndrome. Br J Haematol, in press.

Research Hospital

Department of Rheumatology and Allergy
アレルギー免疫科

Professor	Chikao Morimoto, M.D., D.M.Sc.	教　授	医学博士	森　本　幾　夫
Associate Professor	Hirotoshi Tanaka, M.D., D.M.Sc.	准教授	医学博士	田　中　廣　壽
Assistant Professor	Osamu Hosono, M.D., D.M.Sc.	病院講師	医学博士	細　野　　治
Project Assistant Professor	Satoshi Iwata, M.D., D.M.Sc.	特任講師	医学博士	岩　田　哲　史
Assistant Professor	Hiroshi Kawasaki, M.D., D.M.Sc.	助　教	医学博士	河　崎　　寛
Assistant Professor	Noritada Yoshikawa, M.D., D.M.Sc.	助　教	医学博士	吉　川　賢　忠
Assistant Professor	Kei Ohnuma, M.D., D.M.Sc.	助　教	医学博士	大　沼　　圭

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. Hopefully we will perform phase I/II clinical trial utilizing humanized CD26 mAb for the treatment of the above diseases, such as malignant mesothelioma and other CD26 positive malignant tumors soon.

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), IL-6 blocking therapy (tocilizumab) and costimulatory signal blocking therapy (abatacept) 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 another anti-CD26 mAb (9C11) which recognizes the epitope different 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-Prkdcscidil2 rgtm1Sug/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 (Department of Rheumatology and Allergy), Noriaki Shimizu, Takako Maruyama, Chikao Morimoto (Division of Clinical Immunology).

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. Our recent achievement is now been applied in clinical settings in the Research Hospital.

a. Glucocorticoid receptor (GR) project

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

(i) Development of Dissociating Ligand for the GR

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

gory 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.

(ii) 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.

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

We performed target gene identification and clarification of their biological significance in cardiac muscles and skeletal muscles.

1. The elucidation of tissue-specific target genes of GR action is difficult, since the GR overlaps functionally with the mineralocorticoid receptor (MR) at the level of ligand-binding specificity, and most metabolically active organs, including the heart, express substantial levels of both GR and MR. Endogenous glucocorticoid-namely, cortisol in humans and corticosterone (COR) in rodents-binds to both the GR and the MR with comparable affinity. In the absence of 11β-hydroxysteroid dehydrogenase 2, which converts the glucocorticoid to inactive metabolites, the intramyocardial concentration of glucocorticoid reflects the free concentration in plasma, which is 1,000-fold higher than that of the mineralocorticoid aldosterone (ALD). Therefore, it seems likely that glucocorticoid rather than mineralocorticoid occupies the MR and influences the proinflammatory response after myocardial infarction. Thus, it is crucial to clarify the GR-specific target genes independently of the functional redundancy with MR. Recently, we performed DNA microarray analysis to evaluate the changes in gene expression profiles in neonatal rat cardiomyocytes after stimulation with COR, the GR-selective agonist cortivazol (CVZ), or ALD. Unexpectedly, we found that the expression of genes that encode 2 key enzymes in a common pathway of prostaglandin biosynthesis were upregulated by glucocorticoids via the GR in cardiomyocytes: phospholipase A2 group IVA (Pla2g4a; encoding cytosolic calcium-dependent phospholipase A2 [cPLA2]), which belongs to the class of cPLA2s that preferentially cleave arachidonic acid from membrane phospholipids; and prostaglandin-endoperoxide synthase 2 (Ptgs2; encoding COX2), which converts arachidonic acid into PGH2. Importantly, ALD did not have similar stimulatory effects on these genes. The induction of Pla2g4a and Ptgs2 by GR is specific for cardiomyocytes, since GR has been shown to transrepress the activation of these proinflammatory genes in most cells. Therefore, we sought to investigate the major types of prostanoids produced in cardiomyocytes after exposure to glucocorticoids and to clarify the roles of these products in cardiac physiology. Among the genes for PGH2 isomerases, expression of Ptgds, which encodes lipocalin-type prostaglandin D synthase (L-PGDS), was selectively upregulated by a GR-specific ligand. Consistent with this result, PGD2 was the most prominently induced prostaglandin by GR-specific ligand stimulation of cultured cardiomyocytes and in vivo hearts. Using isolated Langendorff-perfused hearts and cultured cardiomyocytes, we demonstrate that the activation of L-PGDS-mediated production of PGD2 was crucial for the cardioprotection against ischemia/reperfusion conferred by glucocorticoid-GR signaling. Our results suggest what we believe to be a novel interaction between glucocorticoid-GR signaling and the arachidonic acid cascade-mediated cardiomyocyte survival pathway.
2. Muscle comprises ~40% of body mass and contributes not only to the structure and movement of the body but also to nutrient storage and supply. In adult mammals, skeletal muscle hypertrophy/atrophy is characterized by an increase/decrease in the size (as opposed to the number) of individual myofibers, respectively. The control of muscle mass is believed to be determined by a dynamic balance between anabolic and catabolic proc-

esses (Hoffman and Nader, 2004). Mammalian target of rapamycin (mTOR) is a crucial component of the anabolic machinery for protein synthesis. mTOR consists of two complexes: mTORC1, which includes Raptor, signals to S 6K and 4E-BP1, controls protein synthesis, and is rapamycin sensitive; and mTORC2, which includes Rictor, signals to Akt, and is rapamycin insensitive. mTORC1 integrates four major signals: growth factors, energy status, oxygen, and amino acids, especially branched-chain amino acids (BCAA). Prototypically, insulin/IGF-1 activates mTOR via the PI3K-Akt pathway. It is currently considered that mTORC1, and not mTORC2, is essential for the maintenance of muscle mass and function. Protein degradation in skeletal muscle cells is essentially mediated by the activity of two conserved pathways: the ubiquitin-proteasomal pathway and the autophagic/lysosomal pathway (Sandri, 2008). The ubiquitin-proteasomal pathway is responsible for the turnover of the majority of soluble and myofibrillar muscle proteins. The activity of this pathway is markedly increased in atrophying muscle due to the transcriptional activation of a set of E3 ligase-encoding genes, e.g., atrogin-1 and MuRF1 (Glass, 2003, Sandri et al., 2004). Autophagy also plays an important role in the degradation of skeletal muscle, and is indicated to be a consequence of an ordered transcriptional program involving a battery of genes, e.g., LC3 and Bnip3 (Mizushima et al., 2008). These positive and negative pathways are balanced in a highly coordinated manner for the determination of myofiber size and total muscle volume; however, distortion of this balance with a relative increase in degradation results in the generalized decrease of myofiber size and muscle atrophy (Hoffman and Nader, 2004). Pioneering studies demonstrated that muscle atrophy is a result of active processes that are transcriptionally controlled through the expression of a particular gene set; the forkhead box O (FoxO) transcription factors are common components of a number of atrophy models and act as critical liaison molecules for protein degradation and autophagy via the transcriptional regulation of, for example, atrogin-1, MuRF1, LC3, and Bnip3 (Mammucari et al., 2007; Sandri et al., 2004; Stitt et al., 2004; Zhao et al., 2007). In clear contrast, it is evident that each disease has proper signaling pathways to FoxOs and that other components of the cellular machinery often participate in the progression of atrophy (Moresi et al., 2010; Suzuki et al., 2007). Therefore, for the development of therapies against muscle atrophy, it should be addressed how the transcriptional program triggered by a particular atrophy pathway is orchestrated and how the balance of muscle protein synthesis and degradation is distorted in each disease.

Typically, glucocorticoid-induced muscle atrophy is characterized by fast-twitch type II glycolytic muscle fiber loss with reduced or no impact on type I fibers. The mechanism of such fiber specificity is yet unknown. Previous reports suggested that the glucocorticoid-GR system has anti-anabolic and catabolic effects and promotes degradation via the induction of a set of genes including atrogin-1, MuRF1, and myostatin (Menconi et al., 2007; Schakman et al., 2008). Although the involvement of FoxO transcription factors is reported in the gene regulation of atrogin-1 and MuRF1 under the presence of excess glucocorticoids (Sandri et al., 2004; Stitt et al., 2004), the biochemical role of GR in the transcriptional regulation of muscle tissue has not yet been determined. Therefore, we investigated how GR-mediated gene expression coordinately modulates anti-anabolic and catabolic actions to understand the functional coupling of metabolism and volume regulation in muscle.

We identified REDD1 and KLF15 genes as direct targets of GR. REDD1 is known to be induced by various stressors, including glucocorticoid, and to inhibit mTOR activity via the sequestration of 14-3-3 and the increase of TSC1/2 activity (Wang, et al., 2006; DeYoung et al., 2008). We clearly identified the functional GRE via the promoter analysis of REDD1 gene. On the other hand, KLF15 is a recently discovered transcription factor that is involved in several metabolic processes in skeletal muscle; e.g., KLF 15 transcriptionally upregulates the gene expression of branched-chain aminotransferase 2 (BCAT2), a mitochondrial enzyme catalyzing the first reaction in the catabolism of BCAA to accelerate BCAA degradation and alanine production in skeletal muscle (Gray et al., 2007). Moreover, phenotypic analysis of cardiac-specific KLF 15 knockout mice revealed marked left ventricular hypertrophy, indicating the negative regulatory role of KLF15 on muscle mass (Fisch et al., 2007). We here demonstrated that KLF15 participates in muscle catabolism via the transcriptional regulation of atrogin-1 and MuRF1. Moreover, KLF15 affects mTOR through BCAA degradation and negatively modulates myofiber size. mTOR activation inhibits GR-mediated transcription by suppressing GR recruitment onto target genes, strongly suggesting a mutually exclusive crosstalk between mTOR and GR. Pharmacological activation of mTOR with BCAA attenuated GR-mediated gene expression, leading to the substantial restoration of muscle

in glucocorticoid-treated rats. We, therefore, indicate the critical importance of the interaction of GR and mTOR in the regulation of metabolism-volume coupling in skeletal muscle.

III. Clinical Trial

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

We have participated a phase I/2a clinical trial of humanized anti-NGF mAb (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 osteoarthritis 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 finished 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.

V. Participation in Medical Genome Science Program and Global COE Program "Center of Education and Research for the Advanced Genome-Based Medicine"

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

We participate in Medical Genome Science Program/Global COE Program. The courses are "Introduction of Medicine and Medical Ethics" and "Experience and Practice of Medicine", especially arranged for non-M.D. students. The former is a series of lectures drawing outline of Medicine (history, internal medicine, surgery, nursing, nutrition, medication, translational research, and medical ethics), 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 Department of Radiology, Laboratory Medicine, Blood Transfusion, Surgical Center, Nursing Quarters, as well as the patient round in Department of Hematology/Oncology and Department of Advanced Medical Science/Infectious Disease and Applied Immunology/Rheumatology and Allergy. We especially thank all the people working in the Research Hospital of the Institute of Medical Science.

Publications

Iwata S and Morimoto C. BCAR1 (p130Cas). Targeted Proteins Database. In press (2011).

Shimizu N, Yoshikawa N, Ito N, Maruyama T, Suzuki Y, Takeda S, Nakae J, Tagata Y, Nishitani S, Takehana K, Sano M, Fukuda K, Suematsu M, Morimoto C, and Tanaka H. Crosstalk between glucocorticoid receptor and nutritional sensor mTOR in skeletal muscle. Cell Metab. 13:170-182, 2011.

Ohnuma K, Hosono O, Dang NH, Morimoto C. Dipeptidyl peptidase in autoimmune pathophysiology. In Ramesh G. (ed.), Advances in Clinical Chemistry. (Elsevier Publishing). Vol. 53, 2011.

Ghani FI, Yamazaki H, Iwata S, Okamoto T, Aoe K, Okabe K, Mimura Y, Fujimoto N, Kishimoto T, Yamada T, Xu CW, Morimoto C. Identification of cancer stem cell markers in

human malignant mesothelioma cells. Biochm Biophys Res Commun. 404:735-42, 2011.
Yamazaki H, Morimoto C. Dang NH (edition). Stem cell property for T-cell malignancy. Research Signpost. T-cell malignancies: recent developments and novel therapeutic approaches, pp 197-202, 2010.
Arimoto-Miyamoto K, Kadowaki N, Kitawaki T, Iwata S, Morimoto C, Uchiyama T. Optimal stimulation for CD70 induction on human monocyte-derived dendritic cells and the importance of CD70 in naive CD4(＋) T-cell differentiation. Immunology 130:137-49, 2010.
Otsuki N, Dang NH, Kumagai E, Kondo A, Iwata S, Morimoto C. Aqueous extract of Carica papaya leaves exhibits anti-tumor activity and immunomodulatory effects. J Ethnopharmacol. 127:760-7, 2010.
Ohnuma K, Hosono O, Katayose T, Yoshikawa N, Kawasaki H, Fujii T, Oyaizu N, Tanaka H, Morimoto C. Microscopic polyangiitis initiated with liver dysfunction, calf pain and fever of unknown origin. Rheumatol Int. 30: 1651-6, 2010.
Takasawa W, Ohnuma K, Hatano R, Endo Y, Dang NH, Morimoto C. Inhibition of dipeptidyl peptidase 4 regulates microvascular endothelial growth induced by inflammatory cytokines. Biochem Biophys Res Commun. 401: 7-12, 2010.
Havre PA, Dang LH, Abe M, Urasaki Y, Ohnuma K, Morimoto C, Dang NH. The role of CD26 in T-cell biology and T-cell malignancies. In Nam H. Dang (ed.), T-Cell Malignancies: Recent Developments and Novel Therapeutic Approaches. Research Signpost. 37-54, 2010.
大沼圭，森本幾夫．作用機序からみた分子標的薬～モノクローナル抗体．日本臨床．68：1841−1847，2010．

Research Hospital

Department of Applied Genomics
ゲノム診療部

Professor	Yoichi Furukawa M.D., Ph.D.	教 授	医学博士	古 川 洋 一(併任)
Professor	Yusuke Nakamura M.D., Ph.D.	教 授	医学博士	中 村 祐 輔(併任)
Professor	Yoshinori Murakami M.D., Ph.D.	教 授	医学博士	村 上 善 則(併任)
Assistant Professor	Naoyuki Takahashi M.D., Ph.D.	助 教	医学博士	高 橋 直 之(併任)

Our department was established for the application of human genome information in clinics. As a research project, we are working on the development of a diagnostic system for Lynch syndrome or hereditary non-polyposis colorectal cancer (HNPCC). In our earlier study, we took a part of genetic testing in the collaborative research project, "Registration and diagnosis of Japanese HNPCC patients". Using the data obtained in the project, we are now developing an algorithm to identify those who are at a high risk to Lynch syndrome. To apply the system, we opened an outpatient clinic for hereditary colorectal cancer patients.

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

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

Hereditary non-polyposis colorectal cancer (HNPCC) is an autosomal dominant hereditary disease accompanied by tumors arising mainly in the colon and other HNPCC-associated organs, such as stomach, renal pelvis, and endometrium. We earlier performed genetic analyses of *MSH2*, *MLH1*, and *MSH6*, three responsible genes for HNPCC, as a collaborative project of registration and diagnosis of Japanese HNPCC patients conducted by Japanese Study Group for Colorectal Cancer. A total of 131 familial colorectal cancer patients who fulfilled the modified Amsterdam's II criteria were registered, and the frequency of HNPCC in registered patients with colorectal cancer was determined. For genetic diagnosis, we analyzed the three responsible genes by PCR-direct sequencing and Multiplex Ligation-dependent Probe Amplification. As a result, we identified pathogenic mutations in 69 of 131 cases. These mutations included missense and nonsense mutations, small insertions and deletions, and gross genetic alterations including large deletions and duplications. The analysis identified alterations not only in exons but also in introns.

We have developed a mini-gene assay system to examine genetic alterations associated with disrupted splicing, and proved that this system is useful for the characterization of variants in introns as well as exons. Using this system, two genetic alterations were diagnosed as pathogenic mutations because both alterations resulted in either exon-skipping or activation of cryptic-splicing.

We have been analyzing cases that do not harbor any pathogenic mutations in the three responsible genes but contain large deletions in the *TACSTD* (*Ep-CAM*) gene.

Challenges of increasing the sensitivity of diagnosis and finding people at a genetically high-risk to this disease are ongoing.

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 Institute of Medical Science Hospital, we provide genetic counseling to clients who want to have counseling. Clients include those who are anxious about genetic issues associated with hereditary diseases. In 2010, we had a total of 24 cases including Lynch syndrome, familial breast cancer, Peutz Jeghers syndrome, familial polyposis of the colon, and neurofibromatosis. In the counseling, we appropriately provided information of these diseases and took psychological care of the clients in collaboration with clinical psychologists. Genetic testing was performed in three cases. The genetic counseling has been carried out to assist the realization of genetic data into clinic.

Publications

1. Yamamoto K, Ishida T, Nakano K, Yamagishi M, Yamochi T, Tanaka Y, Furukawa Y, Nakamura Y, and Watanabe T. SMYD3 interacts with HTLV-1 Tax and regulates sub-cellular localization of Tax. Cancer Science 2010 in press.
2. Stefanou N, Papanikolaou V, Furukawa Y, Nakamura Y, and Tsezou A. Leptin as a critical regulator of hepatocellular carcinoma development through modulation of human telomerase reverse transcriptase. BMC Cancer. 10: 442, 2010.
3. Yamaguchi K, Sakai M, Shimokawa T, Yamada Y, Nakamura Y, and Furukawa Y, C 20orf20 (MRG-binding protein) as a potential therapeutic target for colorectal cancer. Br. J. Cancer 102: 325-31, 2010.
4. Nozawa H, Yamada Y, Muto Y, Endo J, Asakage M, Oka T, Furukawa Y, and Arai M. Double primary adenocarcinomas of the jejunum and descending colon with lung metastases presenting rare immunohistochemical phenotypes: a case report. Eur J Gastroenterol Hepatol. 22: 228-33, 2010.

Research Hospital

Department of Radiology
放射線科

Associate Professor Shigeru Kiryu, M.D., D.M.Sc.
Assistant Professor Makoto Watanabe, M.D., D.M.Sc.

准教授 医学博士 桐 生 茂
助 教 医学博士 渡 辺 慎

The Department of Radiology works in general diagnostic radiology, neuroradiology, clinical nuclear medicine, and radiation therapy. For clinical imaging, we have a multi-detector row CT scanner, high-field MRI unit, and hybrid gamma camera system. We perform all examinations of CT, MRI, angiography, and nuclear medicine, and official reports on all the examinations are made by board-certified radiologists. Clinical studies are conducted in collaboration with other departments and other institutions. We also investigate the technical aspects of molecular imaging in intact small animals for its application to preclinical studies using optical imaging system and MRI.

Effect of anesthesia and hypothermia on the hepatic kinetics of Gd-EOB-DTPA: Evaluation using MRI of conscious mice

Shigeru Kiryu, Yusuke Inoue[1], Makoto Watanabe, and Kuni Ohtomo[2]: [1]Department of Diagnostic Radiology, Kitasato University School of Medicine and [2]Department of Radiology, Graduate School of Medicine, University of Tokyo.

MRI of small animals is usually performed under anesthesia, and thus may be affected by physiological changes caused by anesthesia, such as hypothermia. We developed a method for body MRI of conscious mice and investigate the effect of isoflurane anesthesia and hypothermia on the hepatic kinetics of a hepatobiliary contrast agent gadoxetate disodium (Gd-EOB-DTPA). Conscious or anesthetized mice were restrained on a holder, and the rectal temperature was measured serially. Serial MRI of the liver was performed after intravenous injection of Gd-EOB-DTPA with or without temperature control. The temperature dropped rapidly in anesthetized mice beside the MR unit. The decline was less prominent in conscious mice. The temperature decreased less in anesthetized mice and remained constant in conscious mice in the RF coil. The washout of Gd-EOB-DTPA was slower in anesthetized hypothermic mice than in conscious normothermic mice. Warmed anesthetized mice showed faster washout, and cooled conscious mice showed delayed washout. Severer hypothermia in anesthetized mice resulted in weaker initial enhancement and slower washout. By separately manipulating the presence or absence of anesthesia and hypothermia, we demonstrated that washout of Gd-EOB-DTPA was delayed under hypothermia, regardless of anesthesia. Serial body MRI of conscious mice was feasible and allowed the evaluation of kinetics of a contrast agent, while excluding the possible effects of anesthesia.

Quantitative Effect of Reducing Body Thickness on Visualizing Murine Deep Abdominal Lymph Nodes by In Vivo Fluorescence Reflectance Imaging

Yusuke Inoue[1], Makoto Watanabe, Shigeru Kiryu, and Kuni Ohtomo[2]

Scattering and absorption in the tissues are major problems for in vivo imaging based on a fluorescence reflectance imaging technique. We evaluated the quantitative relationship between body thickness and fluorescent signals from a deep abdominal source in intact mice. Mice were injected with quantum dots (peak emission, 800 nm) into the right rear footpad, and fluorescent signals from the iliac lymph node located deeply in the abdomen were assessed by fluorescence reflectance imaging. Stepwise compression of the mouse abdomen to reduce the body thickness was attained using a homemade simple device. The iliac node signals were weak and diffuse without compression but became stronger and more localized with decreasing body thickness. Using excitation light of approximately 710 nm wavelength, the lymph node/background contrast increased about 16 times with a 4 mm reduction in body thickness. Contrast enhancement was more evident using shorter wavelength excitation light. Overlying tissues profoundly affect signals from a deep source in fluorescence reflectance imaging. Our simple compression method may contribute to quantitatively assessing deep fluorescent sources.

Lymph Drainage from the Mammary Glands in Mice: A Magnetic Resonance Lymphographic Study with Gadofluorine M

Fugeng Sheng[3], Yusuke Inoue[1], Shigeru Kiryu, Makoto Watanabe, and Kuni Ohtomo[2]: [3]Department of Radiology, Affiliated Hospital of The Academy of Military Medical Sciences, Beijing, China.

We investigated to determine the capability of MR lymphography using gadofluorine M and to demonstrate normal lymph drainage from the mammary glands in mice. Three mice were intradermally injected with gadofluorine M near the papilla of the right fifth mammary gland, and subsequently underwent serial MR imaging to determine the appropriate method for assessment of the lymphatic pathway. MR lymphography was performed in 10 mice for the five right mammary glands to assess lymph drainage from each gland. After intradermal injection near the right fifth papilla, high signal intensities representing lymph drainage were clearly demonstrated in the right inguinal and right proper axillary lymph nodes in all mice. The contrast between the lymph node and adjacent muscles was highest 10 minutes after injection and was still evident at 30 minutes. The lymph pathways from the five right mammary glands were successfully revealed, and no contralateral lymph nodes received lymph flow. Although variations in lymph drainage patterns from the first and second mammary glands existed among mice, injection in the third, fourth, and fifth glands gave consistent results. Lymphatics from the third gland drained exclusively into the proper axillary lymph node, and those from the fourth and fifth glands drained into the inguinal and proper axillary nodes. MR lymphography with gadofluorine M allows noninvasive visualization of lymph drainage from the mammary glands in healthy mice.

Timing of imaging after d-luciferin injection affects the longitudinal assessment of tumor growth using in vivo bioluminescence imaging.

Yusuke Inoue[1], Shigeru Kiryu, Makoto Watanabe, Arinobu Tojo[4], and Ohtomo K[2]: [4]Division of Molecular Therapy, Advanced Clinical Research Center, University of Tokyo.

The peak signal or the signal at a predetermined, fixed time point after D-luciferin injection may be used for the quantitative analysis of in vivo bioluminescence imaging. We repeatedly performed sequential bioluminescence imaging after subcutaneous injection of D-luciferin in mice bearing subcutaneous tumors. The peak time in each measurement became shorter early after cell inoculation, presumably due to gradual establishment of intratumoral vasculature, and reached a plateau of about 10 min on day 10. Although the correlation between the signal at a fixed time point and the peak signal was high, the signal at 5 or 10 min normalized for the peak signal was lower for earlier days, which caused overestimation of tumor growth. The time course of the signals after D-luciferin injection may vary with time after cell inoculation, and this variation should be considered when determining the imaging protocol for quantitative bioluminescence tumor monitoring.

Publications

Kiryu, S., Inoue, Y., Masutani Y., Haishi, T., Yoshikawa, K., Watanabe, M., and Ohtomo, K. Distortion correction in whole-body imaging of live mice using a 1-T compact magnetic resonance imaging system. Jpn. J. Radiol. In press

Sheng, F., Inoue, Y., Kiryu, S., Watanabe, M., and Ohtomo, K. Interstitial Magnetic Resonance Lymphography in Mice with Gadopentetate Dimeglumine and Gadoxetate Disodium. J. Magn. Reson. Imaging. In press

Tomizawa, N., Komatsu, S., Akahane, M., Torigoe, R., Kiryu, S., and Ohtomo, K. Influence of hemodynamic parameters on coronary artery attenuation with 320-detector coronary CT angiography. Eur. J. Radiol. In press

Inoue, Y., Watanabe, M., Kiryu, S., and Ohtomo, K. Quantitative Effect of Reducing Body Thickness on Visualizing Murine Deep Abdominal Lymph Nodes by In Vivo Fluorescence Reflectance Imaging. J. Fluoresc. In press

Sheng, F., Inoue, Y., Kiryu, S., Watanabe, M., and Ohtomo, K. Lymph Drainage from the Mammary Glands in Mice: A Magnetic Resonance Lymphographic Study with Gadofluorine M. Acad. Radiol. In press

Akai, H., Kiryu, S., Matsuda, I., Satou, J., Takao, H., Tajima, T., Watanabe, Y., Imamura, H., Kokudo, N., Akahane, M., and Ohtomo, K. Detection of hepatocellular carcinoma by Gd-EOB-DTPA-enhanced liver MRI: Comparison with triple phase 64 detector row helical CT. Eur. J. Radiol. In press

Tomizawa, N., Komatsu, S., Akahane, M., Torigoe, R., Kiryu, S., and Ohtomo, K. Relationship between beat to beat coronary artery motion and image quality in prospectively ECG-gated two heart beat 320-detector row coronary CT angiography. Int. J. Cardiovasc. Imaging. In press

Matsuda, I., Hanaoka, S., Akahane, M., Sato, J., Komatsu, S., Inoh, S., Kiryu, S., Yoshioka, N., Ino, K., and Ohtomo, K. Adaptive statistical iterative reconstruction for volume-rendered computed tomography portovenography: improvement of image quality. Jpn. J. Radiol. 28: 700-6, 2010.

Inoue, Y., Kiryu, S., Watanabe, M., Tojo, A., and Ohtomo, K. Timing of imaging after d-luciferin injection affects the longitudinal assessment of tumor growth using in vivo bioluminescence imaging. Int. J. Biomed. Imaging. 2010: 471408, 2010.

Tajima, T., Takao, H., Akai, H., Kiryu, S., Imamura, H., Watanabe, Y., Shibahara, J., Kokudo, N., Akahane, M., and Ohtomo, K. Relationship between liver function and liver signal intensity in hepatobiliary phase of gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid-enhanced magnetic resonance imaging. J. Comput. Assist. Tomogr. 34: 362-6, 2010.

Inoue, Y., Masutani, Y., Kiryu, S., Haishi, T., Watanabe, M., Tojo, A., and Ohtomo, K. Integrated imaging approach to tumor model mice using bioluminescence imaging and magnetic resonance imaging. Mol. Imaging. 9: 163-72, 2010.

Furukawa, R., Akahane, M., Yamada, H., Kiryu, S., Sato, J., Komatsu, S., Inoh, S., Yoshioka, N., Maeda, E., Takazawa, Y., and Ohtomo, K. Endometrial stromal sarcoma located in the myometrium with a low-intensity rim on T2-weighted images: report of three cases and literature review. J. Magn. Reson. Imaging. 31: 975-9, 2010.

Tomizawa, N., Akai, H., Akahane, M., Ino, K., Kiryu, S., and Ohtomo, K. Prepancreatic postduodenal portal vein: a new hypothesis for the development of the portal venous system. Jpn. J. Radiol. 28: 157-61, 2010.

Inoue, Y., Kiryu, S., Watanabe, M., Oyaizu, N., and Ohtomo, K. Fluorescence lymph node mapping in living mice using quantum dots and a compression technique. J. Fluoresc. 20: 599-606, 2010.

Kiryu, S., Inoue, Y., Yoshikawa, K., Shimada, M., Watanabe, M., and Ohtomo, K. Diet and gastrointestinal signal on T1-weighted magnetic resonance imaging of mice. Magn. Reson. Imaging. 28: 273-80, 2010.

桐生茂，佐原力三郎，井上優介，館野円，大友邦．痔瘻のMRI．画像診断．秀潤社　30：854−862，2010．

渡辺慎，大友邦．カラー図譜．今日の治療指針2010年版．医学書院，10−30，2010

Research Hospital

Department of Surgery (Gastrointestinal and Breast Surgery)
外科(主として、大腸・胃・食道・乳腺領域)

Associate Professor	Masaru Shinozaki, M.D., Ph.D.		准教授	医学博士	篠崎　大
Lecturer	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

The principal goal of our department is to provide surgical service of malignancy and inflammatory bowel disease and to develop and conduct clinical research and clinical trials in early stages (Phase I and II) on patients at the Research Hospital. We have also been offering diagnostic and therapeutic endoscopy, including upper and lower gastrointestinal endoscopic examinations. Between January and May, 2010, Dr. Shinozaki led the entire Department. As of June, 2010, the Department of Surgery was divided to two groups. With cordial cooperation of three fellows (Dr. Yasuhiro Mizuno, Dr. Junko Takei, and Dr. Mitsuru Matsukura), we retrieved our activity in patient care and clinical research.

1. Summary of surgical treatment in 2010

Masaru Shinozaki, Giichiro Tsurita, Keisuke Hata, Yasuhiro Mizuno, Junko Takei, Mitsuru Matsukura

We performed various surgical operations (Table). 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 number.

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 have 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. In this point of view, Dr. Sameshima and Dr. Kawamura are willing to help us when laparoscopic colorectal surgery is undergone. Recently, breast cancer has become a particular field only for highly specialized physicians bearing extreme knowledge. Although Dr. Yoneyama conducted this field until March, 2010, and he wished to continue, it was also interfered. Dr. Sanuki took over the out-patient clinic and assisted our breast cancer operations as of June, 2010.

2. Summary of endoscopic examination in 2010

Giichiro Tsurita, Keisuke Hata, Masaru Shinozaki, Yasuhiro Mizuno, Junko Takei, Mitsuru Matsukura

Under the cooperation with Department of Advanced Medical Science, we performed 599 (27% increase compared with the number last year) upper gastrointestinal endoscopies and 520 (27% increase) 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. Our three fellows (Y.M., J.T., and M.M) learned gastrointestinal endoscopic technique and have been making great progress.

3. Clinical Research.

A. The role of micro RNA and its relation to carcinogenesis in inflammatory bowel disease

Keisuke Hata, Masaru Shinozaki, Junko Takei, Yasuhiro Mizuno, Giichiro Tsurita

Recently, micro RNA (miRNA) had been known to play a crucial role in post-transcriptional regulation. In inflammatory bowel disease (IBD), its etiology has not been revealed yet. However, interaction between mucosa and intraluminal bacteria and immunological response are speculated to be included at least in the pathophysiology. There may be a possibility that abnormality in miRNA is involved. Furthermore, the patients with IBD are subject to suffer from colorectal cancer due to chronic inflammation. We investigate the possible relation between miRNA and carcinogenesis.

B. The comparison between step biopsy and target biopsy at surveillance colonoscopy in long-standing ulcerative colitis: a randomized control study

Keisuke Hata, Masaru Shinozaki, Junko Takei, Yasuhiro Mizuno, Giichiro Tsurita

Patients with long-standing ulcerative colitis have increased risk of colorectal cancer. The prognosis of symptomatic patients is extremely poor, and surveillance colonoscopy with multiple biopsies is recommended for such patients. However, the methods of biopsies are different between the Western countries and Japan: Numerous (more than 32) blind biopsies are recommended in the former countries, whereas target biopsied with restricted number are favored in Japan. Although the detection rates of dysplasia, which is known as a precancerous lesion as well as a marker of invasive cancer, seem to be substantially similar, when studies were executed separately, no direct comparison has been attempted yet. We started to include three patients, who were randomized to the two groups, without any complications.

C. Cohort study for individualized postoperative adjuvant chemotherapy using pyrimidine analog in stage III colon cancer.

Giichiro Tsurita, Masaru Shinozaki, Keisuke Hata, Junko Takei, Yasuhiro Mizuno

Pyrimidine analog is the basic drug of colorectal cancer. However, the relationship between enzymatic profile of CRC concerning the metabolism of pyrimidine analog and the effect on the survival has not been revealed yet. Therefore, we conducted a prospective study where the activity of representative enzymes of pyrimidine analog, e.g. thymidylate sythetase, is measured in the postoperative patients who receive adjuvant chemotherapy. Enrollment to this study had begun.

4. Clinical research under development

We have been seeking for new projects in partnership with basic research departments in the Institute.

5. Clinical trials under development

In 2010, Antibody and Vaccine Center has been developed at the Institute. We are planning to administer cancer related peptides under various situations to draw maximal effects.

Publications

Shinozaki M, Hata K, Matsukura M, Mizuno Y, Takei J, Tsurita G. Portal vein thrombosis and pulmonary artery thromboembolism after laparoscopic colectomy. Minim Invasive Ther Allied Technol. 2010 Nov 24. [Epub ahead of print]

Mizuno Y, Narimatsu H, Kishi Y, Kodama Y, Murashige N, Yuji K, Matsumura T, Kami M. Structural problems of medical news reports in newspapers: a verification of news reports on an incident of mass nosocomial Serratia infection. J Infect Chemother. 2010; 16(2): 107-12.

Table. Surgical procedures performed in 2010

Cancer/Neoplasm		Jan.-May	Jun.-Dec.
Stomach	Total gastrectomy	1	2
Colorectum	Right hemicolectomy	1	6
	Partial resection		2
	Left hemicolectomy	1	1
	(Laparoscopic		1)
	Sigmoidectomy		4
	(Laparoscopic		2)
	Anterior resection	1	2
	(+Partial colectomy		1)
	Low anterior resection	2	6
	Abdominoperineal resection	1	1
	Transverse colostomy	1	
	Subtotal colectomy		1
Liver	Hepatectomy of S2+S3	1	
	Partial hepatectomy	1	
Bile duct	Pancreaticoduodenectomy (PD)	1	
	Pyrolus preserving PD	1	
Breast	Partial mastectomy	2	1
	Mastectomy		2
	Axillary nodes dissection	1	
Lymph node swelling	Open biopsy	1	
	Lymph node sampling		1
Gastrointestinal stromal tumor	Excision	2	
Others			
Gallbladder stone	Laparoscopic cholecystectomy	2	3
	Cholecystectomy	2	1
Ulcerative colitis	Proctectomy (IACA)		1
	Subtotal colectomy	2	2
	Proctocolectomy (IAA)		2
Crohn's disease	Right hemicolectomy	1	
	Ileocecal resection	1	
	Partial colectomy+seton drainage	1	1
	Stricture plasty		1
	Gastrojejunostomy	1	
	Biopsy at perianal region		1
Ileus	Resection of small bowel	1	1
	Adhesiotomy		1
Stoma	Closure	1	1
Appendicitis	Appendectomy	1	
Inguinal hernia	Repair	5	2
Hemorrhoids	Hemorrhoidectomy		4
Anal fistula	Lay open		2
Primary hyperparathyroidism	Resection of left parathyroid	1	
Total		37	52

Research Hospital

Team Violet, Department of Surgery
外科（主に肝臓，胆のう，膵臓）

Lecturer	Akihiko Itoh, M.D.	講　師		伊　藤　精　彦
Professor	Hideaki Tahara, M.D., D.M.Sc.	教　授	医学博士	田　原　秀　晃
Assistant Professor	Akira Kanamoto, M.D., D.M.Sc.	助　教	医学博士	金　本　　彰

We have been engaged in the surgical treatment of solid tumors and the immunotherapy of various malignancies. We have also been offering diagnostic services, including endoscopic examination on upper and lower intestines. One of the goals of our team is to provide evidence-based standard therapies including surgery, chemo-therapy, and radiation for cancer patients. However, additional emphasis has been put on the development of the novel immunological and gene therapies in intimate collaboration with the Division of Bioengineering, Advanced Clinical Research Center and Core Facility for Therapeutic Vectors of Research Hospital. We have conducted multiple early-phase clinical trials (Phase I and II) for cancer patients at Research Hospital utilizing its fundamental functions enabling clinical research of high quality.

I. Summary of surgical treatment and other procedures performed in 2010

Akihiko Itoh, Akira Kanamoto, Hideaki Tahara,

Team Violet has been newly established in an independent group of the Department of Surgery on June 1st of 2010. This team has been treating the patients with the diseases of gastrointestinal organs having the focus on, but not limited to, hepato-biliary system and pancreas.

Surgical operations have been performed by the team personnel on 42 cases under general anesthesia and spinal or epidural and/or local anesthesia in 2010. As shown in Table 1, major operations were performed in 26 patients with malignant diseases and in 16 patients with benign diseases.

II. Clinical trials for cancer patients using immunologic approaches

a. Phase I/IIa clinical trial of melanoma vaccine using gp100 derived peptides restricted to HLA-A*2402.

Akira Kanamoto, Marimo Sato*, Akihiko Ito, Hideaki Tahara: *Division of Bioengineering, Advanced Clinical Research Center, IMSUT

Epitope peptides derived from gp100, a melanoma associated antigen, are used for the cancer vaccine to treat the patients with advanced malignant melanoma. We have previously performed phase I clinical trial that six patients with stage IV melanoma were immunized with a vaccine consisting of HLA-A*2402-restricted epitope peptide derived from gp100 melanoma differential antigen (gp100-int4: VYFFLPDHL) emulsified with incomplete Freund's adjuvant

Table 1. Major operations performed in 2010

Malignant Diseases		Benign Diseases	
hepato-biliary organs and pancreas			
Hepatectomy	3	Cholecystecyomy	4
Extended chelecystectomy	1		(laparoscopic; 3)
Pancreatico-duodenectomy	2	Splenectomy	1
Miscellaneous	11		
Stomach			
Gastrectory	2		
Gastrojejunostomy	1		
Colon and rectum			
colo-rectal resection	3		
Retroperitoneum			
Tumor resection	2		
Miscellaneous			
LN biopsy	1	Miscellaneous procedures	11
TOTAL	26		16

(IFA). No related adverse effects without grade I toxicity were observed in these patients. In two patients (Patient 2 and 3), vitiligo was observed after vaccination.

Based on the data of the phase I trial using gp 100 derived peptides, a phase I/IIa clinical trial of similar melanoma vaccine were performed. HLA-A*2402-restricted gp100 derived peptide (gp100-int4) was used with IFA and low dose interleukin (IL-2) in order to augment for anti-tumor immunity. Our goals in this clinical trial were to examine safety and clinical efficacy of the treatment. Immune responses associated with the peptide vaccination were also examined. We have enrolled and treated 30 melanoma patients in total as of 2010. The protocol was well tolerated, and no cardiac, hematological, hepatic, or renal toxicities related to this treatment were noted. Immunological monitoring was performed to determine IFN-gamma production and analyze A24/gp100 multimer staining using PBMC stimulated with gp100-int4 peptide. The ELISPOT assays on the PBMCs taken from some enrolled patients showed significant increase of the IFN-gamma producing cell after vaccination in response to the *in vitro* stimulation with gp100-int4 peptide. With A24/gp100 multimer examination, increment of the frequencies of T-cell population positive for both A24/gp100-multimer and CD8 staining was noted in some patients after vaccination. Additional analysis on immunological responses are currently in the process of examination in Division of Bioengineering, Advanced Clinical Research Center.

b. Gene therapy using DCs transfected with IL-12 genes

Akira Kanamoto, Marimo Sato*, Hisako Katano, Takafumi Nakamura**, Akihiko Ito, Hideaki Tahara: *Division of Bioengineering, Advanced Clinical Research Center, IMSUT, **Core Facility for Therapeutic Vectors, Research Hospital, IMSUT**

We have been involved in development of cancer immunotherapy using dendritic cells (DCs) manipulated to induce better immune responses. Our strategies include the usage of agents to induce desirable maturation of DCs in culture of DCs to have better function in situ. In order to obtain DCs suitable for the vaccination with class I-restricted melanoma-associated antigen gp100, we have been using monocyte-derived DCs stimulated with OK-432 and prostaglandin E2 (OK-P-DCs) in previous study. We have shown that OK-P-DCs have phenotypic characteristics of matured DCs, ability to successfully induce antigen specific CTLs in vitro, and capability to migrate (Sato M et al, Cancer Sci. 2003). Based on these preclinical results, we initiated phase I clinical protocol to treat stage IV melanoma patients with OK-P-DCs pulsed with gp100 epitope peptide restricted to HLA-A*2402. In the study described above, we have evaluated peptide-specific immunological responses in the enrolled patients using the methods established for the analysis of PBMCs. All the patients enrolled have well tolerated the treatment with no serious adverse events related to the treatment. The migration of the administered OK-P-DCs pulsed with gp100 was confirms with the imaging for the radio-labeled DCs in the patients. Significant immune responses to gp100 were detected as early as 2 weeks after the 1st injection of OK-P-DCs pulsed with gp100 in all patients.

Based on the information of our own, we are now in the process of initiating cancer gene therapy using intra-tumoral administration of dendritic cells transfected to secrete IL-12. To facilitate this clinical trial, a clinical grade adenoviral vector carrying IL-12 genes was produced by CFTV. Such reagent has been tested for cGMP compliance. Once it has been confirmed to have a required quality, we will initiate the process to formally test the effects of this strategy in The Research Hospital as a Phase I clinical trial.

Publications

1. Baba T, Sato-Matsushita M, Kanamoto A, Itoh A, Oyaizu N, Inoue Y, Kawakami Y, Tahara H. Phase I clinical trial of the vaccination for the patients with metastatic melanoma using gp100-derived epitope peptide restricted to HLA-A*2402 J Translational Medicine 8: 84 (12 pages), 2010
2. Kutsuna, N., Yamazaki S, Kaiga T, Inagaki. Y, Hayashi Y, Okada S, Kanamoto A, and Takayama T.: Partial MHC matched Donor CD8＋ T-Cells are Indispensable to Switch Splenocytic Chimera. Journal of Surgical Research (in press 2011)
3. Yamano T, Watanabe S, Hasegawa H, Suzuki T, Abe R, Tahara H, Nitta T, Ishimaru N, Sprent J, and Kishimoto H. Ex-vivo expanded DC induce donor-specific central and peripheral tolerance and prolong the acceptance of donor skin allografts. Blood (in press, 2011)
4. Hikichi M, Kidokoro M, Haraguchi T, Iba H, Shida H, Tahara H & Nakamura T. MicroRNA regulation of vaccinia virus for enhanced oncolytic activity and reduced pathogenicity in oncolytic virotherapy. Molecular Therapy (in press, 2011)

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 2009, there are 73 surgical treatments for hemophilia (50 for hemophilia A, 11 for hemophilia B, 11 for hemophilia with inhibitor and 1 for deficiency factor VII patient).

In 2010, we were performed 19 surgical treatments (16 for hemophilia A, 2 for hemophilia B, and 1 hemophilia with inhibitors); 10 total joint arthroplasties, 3 arthroscopic synovectomies and 6 other surgical treatments.

Publications

1) Takedani, H., Kawahara, H. and Kajiwara, M. Major orthopaedic surgeries for haemophilia with inhibitors using rFVIIa. Haemophilia. 16: 290-295, 2010.
2) Takedani, H. Continuous infusion during total joint arthroplasty in Japanese haemophilia A patients: comparison study among two recombinants and one plasma-derived factor VIII. Haemophilia. 16: 740-746, 2010.

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	Reiko Shibata, M.D.	助　教	医学士	柴　田　玲　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

1) 今村佐知子，鎭西美栄子，山田芳嗣．腹腔鏡補助下大腸切除術後に発症した上肢末梢循環障害症例および腕神経叢麻痺症例とその後の対策．麻酔　59(12)，1494–1497，2010

2) 鎭西美栄子．ヒト免疫不全ウイルス(HIV)のキャリアである．麻酔科トラブルシューティングA to Z．高崎眞弓，河本昌志，川真田樹人，岡本浩嗣編集．文光堂．P 226–229，2010

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, Hatsuko 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 at Research Hospital

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 2010, we assisted the conduct of 10 protocols. Details of the protocols are summarized in Table 1. Table 2 shows the number of patients enrolled into clinical trials at RH in 2010.

Table 1.

Number of protocol	Started in 2010	Continuation before 2010	Total
TR	0	2	2
Clinical trials from pharmaceutical companies	0	4	4
Multi-center studies	0	4	4
	0	10	10

Table 2.

Number of patients	Enrolled in 2009	Continuation before 2009	Total
TR	0	1	1
Clinical trials from pharmaceutical companies	2	3	5
Multi-center studies	4	8	12
	6	12	18

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

Kazufumi Matsumoto, Makiko Tajima, 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. Education and training for ethics board members: Is e-learning the solution?

Makiko Tajima, Fumitaka Nagamura.

The Japanese Ministry of Health, Labour and Welfare recently revised the Ethical Guidelines for Clinical Studies. The revised guidelines require education and training for members of a research ethics committee (REC). Some training programs on ethics are offered in an e-learning format for added convenience to learners. E-learning, also called online learning, web-based learning, or distance learning, has potential to be an effective training tool. We conducted a literature and internet search to assess the feasibility of e-learning in REC member training. E-learning is suitable for studying bioethical principles as well as laws and regulations relevant to clinical research. It is also useful to share criteria for protocol review and to update information on science and technology. E-learning is especially effective when the same training courses are offered repeatedly and when members are unable to assemble at the same location and same time due to geographical and temporal limitations. E-learning materials are shared among REC members, faculty, staff, and students, and may be open to the public. Disadvantages of e-learning include a high dropout rate due to lack of social interaction and the requirement for additional human and financial resources. To overcome these disadvantages, conventional methods such as lectures, workshops, and on-the-job training (OJT) should be incorporated into the training program. E-learning that includes the use of learning networks, such as online discussion forums, may provide results similar to those of workshops and OJT.

E-learning can be a valuable tool for REC member training. Combining different types of training media, or blended learning, is recommended to conduct effective training.

Publications

Matsumoto K, Nagamura F, Ogami Y, Yamashita N, Kamibeppu K. Difficulties of Nursing Staff Involved in Phase 1 Oncology Trials in Japan. Cancer Nur (in press)

Nagayama H, Matsumoto K, Isoo N, Ohno H, Takahashi N, Nakaoka T, Shinozaki M, Watanabe M, Inoue Y, Nagamura F, Oyaizu N, Yamashita N. Gastrointestinal bleeding during the anti-angiogenic peptide vaccination in combination with gemcitabine for advanced pancreatic cancer. *Clin J Gastroenterol* 3(6); 307-17, 2010.

Sato A, Ooi J, Takahashi S, Tsukada N, Kato S, Kawakita T, Yagyu T, Nagamura F, Iseki T, Tojo A, Asano S. Unrelated cord blood transplantation after myeloablative conditioning in adults with advanced myelodysplastic syndromes. *Bone Marrow Transplant* (in press)

Chi HT, Vu HA, Iwasaki R, Nagamura F, Tojo A, Watanabe T, Sato Y. Detection of exon 12 type A mutation of NPM1 gene in IMS-M2 cell line. *Leu Res* 34(2); 261-2, 2010.

松本和史，長村文孝，藤原紀子，尾上裕子，上別府圭子，山下直秀　看護系大学院におけるトランスレーショナルリサーチ看護学の教育プログラムの構築　看護教育　51(8)　696–700，2010.

田嶋麻紀子，長村文孝　倫理審査委員研修におけるeラーニングの位置づけ　臨床評価　(印刷中)

長村文孝　トランスレーショナルリサーチにおける医学と心理学の連携　心理学ワールド50回記念誌　(印刷中)

長村文孝　臨床安全情報(有害事象)が原因で中止となった薬剤の分析　月刊ファームステージ　(印刷中)

長村文孝　FDAの今後の展開　医薬品・医療機器FDA申請・査察対応集　情報機構　(印刷中)

長村文孝　国内外がん診療ガイドラインから見る今後の標準療法予測と治療薬開発戦略　月刊ファームステージ　5；12–16，2010.

長村文孝　がん患者のこころに寄り添うためにサイコオンコロジーの基礎と実践　大木桃代編　39–42，57–60，2010.

Research Hospital

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

Professor Arinobu Tojo, M.D., D.M.Sc.
Lecturer Tokiko Nagamura-Inoue, M.D., D.M.Sc.

教 授 医学博士 東 條 有 伸
講 師 医学博士 長 村 登紀子

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:

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

We have explored the umbilical cord as a new mesenchymal stem cells (MSC) source. We studied the differentiation ability of cord-derived MSC including osteocytic, chondrocytic, adipocytic, and also hepatic cell differentiation.

4. Institute of Medical Science, University Tokyo Cell Resource Center (IMSUT-CRC):

Nagamura-Inoue T, Miki Yuzawa, 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. In 2010, we developed and changed the name 'Room for Clinical Cellular Technology (RCCT)' to 'IMSUT-CRC' as for more functional broad units for cell therapy.

Publications

1. Miki Yuzawa, Tokiko Nagamura-Inoue, Ikuo Ishige, Kazuo Ogami, Tomoki Tamura, Atsuko Takahashi, Hideki Kodo, Satoru Yamaguchi, and Arinobu Tojo, Time from cord blood collection to processing and temperature influence the quality of mononuclear cell products isolated using a density-gradient protocol., The Japan Society of Transfusion Medicine and Cell Therapy. (日本輸血・細胞治療学会誌), in press, 2010
2. Hishizawa M, Kanda J, Utsunomiya A, Taniguchi S, Eto T, Moriuchi Y, Tanosaki R, Kawano F, Miyazaki Y, Masuda M, Nagafuji K, Hara M, Takanashi M, Kai S, Atsuta Y, Suzuki R, Kawase T, Matsuo K, Nagamura-Inoue T, Kato S, Sakamaki H, Morishima Y, Okamura J, Ichinohe T, Uchiyama T. Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study. Blood. 2010 Aug 26; 116: 1369-76. Epub 2010.
3. Isoyama K, Oda M, Kato K, Nagamura-Inoue T, Kai S, Kigasawa H, Kobayashi R, Mimaya J, Inoue M, Kikuchi A, Kato S; Japan Cord Blood Bank Network.Long-term outcome of cord blood transplantation from unrelated donors as an initial transplantation procedure for children with AML in Japan. Bone Marrow Transplant. 45: 69-77, 2010

Research Hospital

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

Professor	Hideaki Tahara, M.D., D.M.Sc.		教授(室長)	医学博士	田 原 秀 晃(併)
Project Associate Professor	Takafumi Nakamura, Ph.D.		特任准教授	生命科学博士	中 村 貴 史
Project Assistant Professor	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 2010 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[1], Hisako Katano[1], Akira Kanamoto[2], Marimo Sato-Matsusita[2], Hideaki

Tahara[1,2]: [1]CFTV, [2]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[1], Takafumi Nakamura[1], Akira Kanamoto[2], Marimo Sato-Matsusita[2], Hideaki Tahara[1,2]: [2]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[3], Yasushi Ino[3], Takafumi Nakamura[1], Hisako Katano[1], Hideaki Tahara[1]: [3]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[4], Marimo Sato-Matsusita[2], Takafumi Nakamura[1], Hisako Katano[1], Hideaki Tahara[1,2]: [4]Department of Orthopedics, Graduate School of Medicine, Osaka City University

In collaboration with Kawasaki Heavy Industries, Inc., we are 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 (2007-2010), and Advanced Clinical Research Center of IMSUT.

Publications

Meng X, Nakamura T, Okazaki T, Inoue H, Takahashi A, Miyamoto S, Sakaguchi G, Eto M, Naito S, Takeda M, Yanagi Y and Tani K: Enhanced Antitumor Effects of an Engineered Measles Virus Edmonston Strain Expressing the Wild-type N, P, L Genes on Human Renal Cell Carcinoma. Mol Ther. 18: 544-551, 2010

Katoh M, Kazuki Y, Kazuki K, Kajitani N, Takiguchi M, Nakayama Y, Nakamura T and Oshimura M: Exploitation of the interaction of measles virus fusogenic envelope proteins with the surface receptor CD46 on human cells for microcell-mediated chromosome transfer. BMC Biotechnol. 10: 37, 2010

Takenobu H, Shimozato O, Nakamura T, Ochiai H, Yamaguchi Y, Ohira M, Nakagawara A, Kamijo T: CD133 suppresses neuroblastoma cell differentiation via signal pathway modification. Oncogene 30: 97-105, 2011

中村貴史．麻疹ウイルスによるがん治療法の開発．臨床とウイルス38：197–205，2010．

Research Hospital

Department of Laboratory Medicine
附属病院　検査部

Associate Professor　Naoki Oyaizu, M.D., Ph.D.
Assistant Professor　Naouki Isoo, M.D., Ph.D.

准教授，部長　医学博士　小柳津　直　樹
助　教　医学博士　磯　尾　直　之

The Department of Laboratory Medicine consists of eight divisions-clinical physiology, hematology, biochemistry, serology, bacteriology, molecular diagnosis and pathology, and a division of flow cytometry analysis. This Department engages in the laboratory analysis and gives diagnosis of clinical materials in the hospital. We also have recently established a new laboratory, which verifies the safety of biomedical reagents under GMP standard, is now officially named the "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 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 standards.

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 accepting 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. Molecular analysis of the hematological neoplasms

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 molecule; AML1-MTG8, PML-RARA gene expression; JAK2, FLT3/ITD mutational analysis; and 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 stem 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.

GMP-based facility of laboratory analysis
Equipments of the Laboratory of TR verification

Publications

1. Baba T, Sato-Matsushita M, Kanamoto A, Itoh A, Oyaizu N, Inoue Y, Kawakami Y, Tahara HPhase I clinical trial of the vaccination for the patients with metastatic mela-

noma using gp100-derived epitope peptide restricted to HLA-A*2402., *J Transl Med.* 16; 8: 84, 2010
2. Fluorescence lymph node mapping in living mice using quantum dots and a compression technique. Inoue Y, Kiryu S, Watanabe M, Oyaizu N, Ohtomo K. *J Fluoresc.*: 599-606, 2010.
3. Ohnuma K, Hosono O, Katayose T, Yoshikawa N, Kawasaki H, Fujii T, Oyaizu N, Tanaka H, Morimoto C. Microscopic polyangiitis initiated with liver dysfunction, calf pain and fever of unknown origin. Rheumatol Int. 30: 1651-6, 2010..