Division of Clinical and Experimental Oncology
Department of Hematology and Oncology

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(March 31, 2006)

Department of Hematology and Oncology has carried out the clinical, hematological and experimental studies on the late effects of atomic bomb-radiation survivors. Focus has been made on clarifying the pathophysiological mechanism and establishing the treatment procedures for hematological disorders such as leukemia, myeloproliferative disorders, myelodysplastic syndrome, malignant lymphoma, multiple myeloma, aplastic anemia and other anemias and thrombocytopenia. The main research subjects being conducted are summarized as follows.

1) Hematopoiesis and immune function in atomic bomb survivors.
2) Diagnosis and treatment of leukemia, myeloproliferative disorders, myelodysplastic syndrome, multiple myeloma, malignant lymphoma and other hematological malignancies.
3) Basic and clinical studies on aplastic anemia, idiopathic thrombocytopenic purpura, and hemolytic anemia.
4) Basic and clinical study of hemostasis and thrombosis.
5) Proliferation, differentiation and cell death of normal and malignant hematopoietic cells.

The Science Promotion Fund from the Ministry of Education, Science, Sports and Culture provided Grant-in Aids for studies on the following subjects: 1) Myelodysplastic syndrome (MDS) and leukemia in the residents near former USSR nuclear test site in Semipalatinsk (Prof. A. Kimura), 2) Study of AML1 point mutations in myelodysplastic syndrome (Dr. H. Harada). Subjects supported by a Grant from the Ministry of Health and Labor of the Japanese Government were 1) Cooperative studies on the investigation of intractable hematopoietic disorders by Prof. A. Kimura (Chief: Dr. M. Ozawa), 2) Cooperative studies on the molecular biological investigations on the hematologic and immune disorders of the atomic bomb survivors by Prof. A. Kimura (Chief: Dr. E. Tahara), 3) Establishment of clinical care system for HIV disease by Prof. A. Kimura (Chief: Dr. S. Kimura), 4) Survey on drug resistance of HIV from newly infected patients by Prof. A. Kimura (Chief: Dr. W. Sugiura). Each Japan Leukemia Research Fund and 45th Radiation Effects Association Research Fund provided grant for Oncogenesis of MDS/AML with AML1 point mutation to Dr. H. Harada. Tsuchiya Foundation offered grants for 1) Identification of proteins responsible for signal transduction from collagen receptor-__integrin to Dr. T. Katsutani, 2) Study of bone marrow stroma cells for the development of efficient stem cell transplantation to Dr. K. Mihara, 3) Pathogenesis of MDS/AML with AML1 point mutation to Dr. H. Harada. Ryokufukai offered grants for Imatinib-resistance and its overcome in chronic myelogenous leukemia to Dr. T. Ito.

Our department has managed an outpatient clinic and inpatient ward of Hematology Oncology Department in Hiroshima University Hospital in cooperation with the staff of the Hiroshima University Hospital, (Chief of the Outpatient Clinic: Dr. H. Tanaka and Chief of the Inpatient Ward: Dr. H. Harada).

Prof. A. Kimura, Dr. H. Tanaka and Dr. H. Hyodo conducted lectures of Hematology for the 3rd year students at the Medical School and Dental School of Hiroshima University. Drs. S. Katsutani, H. Harada, K. Mihara, T. Shimomura, A. Sakai, H. Hyodo, H. Tanaka and Prof. A. Kimura provided practical trainings for the 5th and 6th year medical students at outpatient clinic and inpatient ward. Trainings for junior residents were also provided.

Drs. Y. Takimoto (Chief of Internal Medicine, Hiroshima-Nishi Medical Center), T. Kyo (Chief of Internal Medicine, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital) and N. Sasaki (Chief of Clinical Pathology, Kure Kyosai Hospital), were invited to the Department of Hematology Oncology as part time lecturers.

The summary of the clinical results in 2005 (Jan. ~ Dec.) is as follows:
1) Of the 1,350 cases (676 male and 674 female) at the outpatient clinic, 1,127 had hematological disorders (112 leukemias, 60 myeloproliferative disorders, 57 myelodysplastic syndromes, 254 malignant lymphomas, 84 multiple myelomas, 48 aplastic anemias, 189 thrombocytopenias and others), and the rest had non hematological diseases.
2) Of the 357 cases of inpatients, there were 335 hematological disorders (87 leukemias, 1 myeloproliferative disorder, 33 myelodysplastic syndromes, 112 malignant lymphomas, 35 multiple myelomas, 10 aplastic anemias, 11 other type of anemias, 2 neutropenias, 11 thrombocytopenias, 9 hemorrhagic diathesis, 24 other diseases) and 22 non hematological diseases.
3) Twenty-five cases died including 7 leukemias, 0 myeloproliferative disorders, 2 myelodysplastic syndrome 9 malignant lymphomas, 2 multiple myelomas, and 2 aplastic anemias. Autopsies were performed in 3 out of the 25 deaths (autopsy rate: 12.0%).

Thanks were given to the staff of International Radiation Information Center, who operated the computer Fujitsu PRIMERGY N800.
1. Mechanism of anti-proliferative action of interferon (IFN) on CML

Tanaka, H., Ito, T., Kimura, A.

**Purpose:** Interferon (IFN) has been widely used as an anti-proliferative drug for malignant diseases such as multiple myeloma and chronic myelogenous leukemia (CML). We aim to clarify the mechanisms of IFN action on these hematological malignancies.

**Results:** We focused on IFN-induced TRAIL. We checked serum soluble TRAIL (sTRAIL) in CML patients. We could detect sTRAIL in CML patients, almost the same levels with normal individuals. sTRAIL increased during IFN therapy, and they increased after IFN therapy compared to those before therapy. TRAIL mRNA increased by *in vitro* and *in vivo* IFN treatment in neutrophils in CML patients. TRAIL mRNA in CD34-positive cells also increased after IFN therapy. Neutrophils cultured with IFN secreted sTRAIL in the medium at a dose dependent and time dependent manner.

**Future plan:** We check whether the sTRAIL secreted from neutrophils after IFN stimulation is capable of killing Jurkat cells and CD34-positive cells in normal individuals or CML patients.

2. Mechanism of imatinib-resistance of CML

Tanaka, H., Ito, T., Kimura, A.

**Purpose:** Clarification of the mechanisms of resistance to imatinib in CML. Results: We established imatinib-highly sensitive CML cell line, and imatinib-resistant cell line. This mechanism was BCR-ABL-independent. Some drugs, FTIs, IFN, TRAIL, Zoledronate, HSP90 inhibitor, HDACI, Src inhibitors etc, were tested to know whether these can overcome the resistance. We found out that Src inhibitors and Zoledronate could overcome the resistance with alone or in a synergistic manner with imatinib. Lyn mRNA and protein expression was extremely high in the resistant line, confirmed by Western blotting including phosphorylation status. We introduced siRNA of Lyn to the resistant line, and found out that abolishment of Lyn suppressed the growth and induced apoptosis, indicating that Lyn has really important functions for the growth to the resistant line.

3. Genetic pathway in molecular pathogenesis of myelodysplastic syndrome (MDS) with AML1 point mutations

Harada, H., Harada, Y., Ding, Y., Imagawa, J., Niimi, H., Kyo, T., Inaba, T., Kimura, A. (International Radiation Information Center, Hiroshima City Asa Hospital, Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital, Department of Molecular Oncology)

AML1 mutations have been reported to be frequent in myelodysplastic syndrome (MDS) patients, especially those with refractory anemia with excess blast (RAEB), RAEB in transformation (RAEBt), and AML following MDS (defined these categories as MDS/AML). Although AML1 mutations are suspected to play a pivotal role for developing MDS/AML, acquisition of additional genetic alterations is necessary. We analyzed gene alterations in patients with AML1 mutations, comparing with those without AML1 mutation, and detected specific gene alterations in AML1-mutated MDS/AML patients. Now we are introducing both mutants of these genes and AML1 mutants into hematopoietic stem cells, and analyzing biological changes of these cells. Furthermore, we are going to inject these cells into mice and check development of MDS. Using this approach, we are able to clarify the molecular pathogenesis of MDS.

4. Myelodysplastic syndrome (MDS) among atomic-bomb survivors and radiation-exposed residents near the Semipalatinsk nuclear test site

Harada, H., Harada, Y., Zharlyganova, D., Ding, Y., Kyo, T., Hoshi, M., Kimura, A. (International Radiation Information Center, Dept. Radiation Physics, Hiroshima Red Cross Hospital and Atomic-bomb Survivors Hospital)
Increased risk of myelodysplastic syndrome (MDS) has been shown in atomic-bomb survivors. We showed that AML1 point mutations were found in half of MDS patients among atomic-bomb survivors in Hiroshima. To clarify the molecular mechanism of radiation-induced MDS, we are performing mutation analysis of genes including transcriptional factors and tumor suppressors in the patients with MDS in atomic bomb survivors and radiation-exposed residents near the Semipalatinsk nuclear test site.

5. Molecular mechanism of secondary myelodysplastic syndrome (MDS) / acute leukemia (AML)

Harada, H., Harada, Y., Ding, Y., Niimi, H., Kimura, A. (International Radiation Information Center)

Secondary MDS or AML arise after chemotherapy or radiation exposure for other malignancies. We and others have shown that high frequency of AML1 point mutation or AML1-related translocation was observed in patients with secondary MDS or AML. To clarify the molecular mechanism of secondary MDS or AML, we are trying to detect AML1 gene alteration induced by radiation or anti-cancer drug in human hematopoietic stem cells purified from cord blood cells.

6. Myelodysplastic syndrome (MDS) in relation to Radiosensitivity project.

Niimi, M., Tanaka, H., Ban, S., Imai, T., Kyo, T., Noda, M., Kimura, A. (Frontier Research Center, National Institute of Radiological Sciences, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, Hiroshima City Hospital)

We are conducting the cooperative research project with the National Institute of Radiological Science to understand the high incidence of MDS among A bomb survivors and to clarify the mechanism of high sensitivity of MDS cells to radiation. The main research is focusing on the cDNA array analysis of bone marrow CD34 cells as well as lymphocytes from MDS patients among A bomb survivors and non-A bomb survivors. This may differentiate the molecular mechanism of the MDS genesis between them, and can apply for the development of treatment.

7. Epidemiological study of MDS in A bomb survivors of Hiroshima and Nagasaki.

Kimura, A., Kodama K., Tomonaga, M., Dohy, H. (Department of Epidemiology, RERF, Department of Hematology, Nagasaki University, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital)

The Radiation Effects Research Foundation (RERF) research project on epidemiological study of MDS in A bomb survivors has been conducted.

8. Maturation of myeloma cells correlates with sensitivity to chemotherapeutic agents.

Sakai, A., Kuroda, Y., Okikawa, Y., Munemasa, S.,Katayama, Y., Kimura, A.

We analyzed both morphological and phenotypic findings of myeloma cells before and after chemotherapy in 21 patients with multiple myeloma. The morphological analysis was based on the Greipp classification, and the phenotypic analysis was performed by three-color flow cytometry using the CD38 plasma gating method (Marrow plasma 38). Flow cytometry using a combination of MPC1, CD49e and CD45 supported the morphological findings of the myeloma cells. Treatment with VAD (three or four cycles) was effective in reducing total myeloma cells, but the proportion of immature myeloma cells increased after this treatment. However, the immature myeloma cells were reduced by HD-Mel followed by ASCT. HD-CPA treatment for stem cell harvesting did not show an effect on residual immature myeloma cells after VAD. Also, thalidomide was not effective in reducing immature myeloma cells. These results suggest that VAD (three or four cycles) plus HD-Mel followed by ASCT is a reasonable treatment for multiple myeloma, and that Marrow plasma 38 analysis is a useful method for monitoring the response of multiple myeloma to chemotherapy.
9. Clinical research of stem cell transplantation

Hyodo, H., Sakai, A., Harada, H., Tanaka, H., Takata, N., Kimura, A. (1) Division of Blood Transfusion Service, Hiroshima University Hospital

Purpose: Autologous peripheral blood stem cell transplantation (Auto-PBSCT) combined with high dose chemotherapy is golden standard for relapsed malignant lymphoma, recently. And PBSCT combined with high dose L-PAM is widely used for high-risk multiple myeloma.

We’ll try to create much more effective clinical application of auto-PBSCT. Allogeneic stem cell transplantation is applied to hematological malignancies and aplastic anemia. Hiroshima University Hospital has been recognized for unrelated BMT hospital and cord blood transplantation (CBT) since 2004.

We try to study for infection prophyrax in febrile neutropenia to establish evidence based medicine.

Result: Forty-eight Auto-PBSCTs were performed for 41 cases of hematological malignancy and solid tumors. Twenty-four patients are alive. The overall survival in malignant lymphoma is 15/21 (71%), multiple myeloma 7/8 (87.5%), solid tumors 1/6 (16.7%) and acute leukemia 0/6 (0%). In acute leukemia and solid tumors this therapy can not recognize long survivor.

Nineteen cases of Allo-PBSCT have been performed since 1996 in our department. There were 2 CML in chronic phase, 8 acute leukemia, 2 MDS, 3 APLA, 1 myeloma, 1 lymphoma and 2 solid tumor. Eleven patients are alive with disease free, while eight patients were died due to recurrence and cytomegalovirus virus infection. We examine VNTR method for monitor chimerism as a marker of engraftment. We had experience of 2 UBMT and 1 CBT. Three patients are disease free survivor. We have established the system of PBSC collection, processing, transportation and cryopreservation by cooperation with Division of Blood Transfusion Service, Hiroshima University Hospital.

We will apply to regeneration medicine, too.

10. Development of targeting therapy in hematological malignancies

Mihara, K., Kimura, A.

1. Establishment of the target cells for therapy to myelodysplastic syndrome (MDS)

MDS is a group of clonal hematopoietic disorders characterized by dysplasia and susceptibility to leukemia. Since the disease entity includes quite heterogeneous pathogeneses, the clinical course and prognosis are highly variable. The International Prognostic Scoring System (IPSS) has achieved international acceptance to estimate prognosis in patients with MDS. However, IPSS score is not necessarily enough to evaluate the patient for the purpose of choosing intervention therapies. It would be helpful for the clinical treatment of patients with MDS to establish molecular markers that clearly reflect the disease progression. Since the most critical event for the progression of MDS and prognosis of patients with MDS is susceptibility to acute leukemia, we focused on molecular mechanisms supporting leukemic stem cells (LSCs). Polycomb group genes (PcG) contribute to the epigenetic regulation of DNA and critically maintain the activity of stem cells. BMI-1, which is a member of PcG, is required to regulate the adult self-renewing hematopoietic and LSCs. We therefore examined the positivity of BMI-1 expression in CD34+ cells from patients with MDS by flow cytometry to test whether BMI-1 would be a novel biomarker that is well correlated with the disease progression and prognosis of the patients and BMI-1-expressing cells might be targeting cells for therapy. We reported the expressions of BMI-1 in CD34+ cells from MDS patients were positively correlated with the prognosis and progression of the patients (Blood). We are developing the targeting therapy against BMI-1-expressing leukemic stem cells.
11. Study of thrombosis and hemostasis.

Katsutani, S., Sugihara, S., Kimura, A.

Platelet adhesion to subendothelium at sites of vascular injury is a critical initial step in hemostasis. Integrin α2β1 is a member of the integrin family of heterodimeric molecules that mediate both cell-cell adhesion and adhesion between cells and the extracellular matrix, and act as a major collagen receptor in platelets through the I-domain located at the top of α2. Patients who lack integrin α2β1 have several bleeding manifestations, indicating that integrin α2β1 has an important role in hemostasis, although this is being challenged as in mice knocking out of the integrin has only moderate effects.

On the other hand, another platelet integrin αIIbβ3, known as a fibrinogen receptor, is present in a non-active form on resting platelets and becomes activated or turns into the high affinity state when platelets are stimulated by several agonists. Thorough investigation of integrin αIIbβ3 activation has revealed that the integrin is involved in both signal transduction both from inside to outside (inside-out signal) and from outside to inside (outside-in signal). Several proteins were found to interact with the cytoplasmic tails of integrin αIIbβ3 but the precise mechanisms leading to signal transduction are not yet fully resolved. Using yeast two-hybrid technology, we will find some proteins that can interact with cytoplasmic domain of α2β1, and elucidate the signal transduction pathway through integrins.

12. International scientific joint research on late radiation effects of former USSR nuclear test in Semipalatinsk.

Hyodo, H., Kimura, A.

We are conducting international scientific joint research work on the late effects of radionuclear exposure regarding leukemias and myelodysplastic syndromes (MDS). The radiation exposure-pattern in residents of Semipalatinsk area is different from that in Atomic bombing in Hiroshima. The exposure was chronic and was both internal and external. We received from the main hospitals bone marrow smears of leukemia and MDS. In last four years we have been involved in the project of Japan International Cooperation Agency to establish the health check-up system for residents near Semipalatinsk area. This screening system is expected to find radiation-related MDS and leukemia among the residents.

List of Contributions

A. Original Papers


4. Harada, H., Harada, Y.*, Kimura, A. (*International Radiation Information Center) : Point mutations in the AML1/...


13. Kawaguchi, H. (*1, Hayashi, H. (*1, Mizuno, N. (*1, Fujita, T. (*1, Hasegawa, N. (*1, Shiba, H. (*1, Nakamura, S. (*1, Hino, T. (*1, Yoshino, H. (*1, Tanaka, H., Kimura, A., Tsuji, K. (*2, Kato, Y. (*2, Kurihara, H. (*1 (*¹Dept. Periodontal Medicine, Division of Frontier Medical Science, Graduate School of Biomedical Sciences, ²Dept. Environmetrics and Biometrics, Two Cells Co. Ltd., ³Dept. Dental and Medical Biochemistry, Division of Molecular Medical Science, Graduate School of Biomedical Sciences): Cell transplantation for periodontal diseases -A novel periodontal tissue regenerative therapy using bone marrow mesenchymal stem cells. Clinical Calcium 15(7): 1197-1202, 2005. (in Jpn.)


B. Meeting Presentations

4/7-9

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5/12-15


6/4


9/24


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10/21


11/12


11/12-13

11/14

11/15-17

12/1-3


34. Miyake, K. *1, Fujita, K. *2, Unei, H. *2, Tomita, T. *2, Tayama, Y. *1, Fujii, T. *3, Takata, N. *3, Kimura, A., Kihira, K. *2


10/12-13


38. Iwamoto, S. *, Mihara, K., Imai C. *, Campana, D. * (*Hematology-Oncology and Pathology, St. Jude Children's Research Hosp., Memphis, TN. USA) : L-serine produced by mesenchymal cells in the bone marrow microenvironment is a trophic factor for hematopoietic progenitor cells. (Blood 106(11): 175a, 2005.)

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43. Kawaguchi, H. *1, Hayashi, H. *1, Mizuno, T. *1, Iwata, N. *1, Fujita, T. *1, Hasegawa, N. *1, Nakamura, S. *1, Hino, T. *1, Shiba, H. *1, Yoshino, H. *1, Tanaka, H., Kimura, A., Igarashi, A. *2, Tsuji, K. *2, Kato, Y. *2,3, Kurihara, H. *1 (*1Division of Frontier Medical Science, Graduate School of Biomedical Sciences, *2Two cells Co.Ltd., *3Division of Molecular Medical Science, Graduate School of Biomedical Sciences) : Periodontal therapy by transplantation of bone marrow mesenchymal stem cells. 5th Annual Congress of the Japanese Society for Regenerative Medicine, Okayama, 2006.

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(R), (A), (G) and (C) are reports on the study using Radiation Experiments, Animal Experiments, Gene Technology Facilities and Studies established at the International Radiation Information Center, respectively. (I) indicates reports printed in the scientific journals listed in Current Contents.