Division of Clinical and Experimental Oncology
Department of Molecular Oncology & leukemia Program Project

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The main purpose of our department is to elucidate the molecular mechanisms of leukemogenesis. We are trying to identify key factors that contribute to leukemogenesis from clinical samples, and also from basic research that is focused on the cytokine-dependent and ER-stress induced cell death. The latter work includes application of apoptosis regulatory genes to bioengineering. Elucidation of the mechanisms in radiation-associated leukemia is one of the most important study in our laboratory. The final goal of our study is to obtain basic data for development of new therapeutical approaches for leukemia by using molecular and cellular biology, as well as by establishing and analyzing gene-targeting mice, which has been performed based on a collaboration with Department of Developmental Oncology.

In order to complete these missions, we continued Leukemia Program Project, as a project center in Hiroshima University in collaboration with Departments of Hematology and Oncology, Pediatrics as well as Biochemistry. Our work is also deeply involved in the COE project, which is driven mainly by RIRBM.

The research projects in this laboratory, which were carried out in 2005 and are being planned for the following years are summarized as follows.

1. Isolation of tumor suppressor genes from micro-deleted region in chromosome-arm 7q in myeloid leukemia.

Asou H., Ozaki Y., Matsui H., Takemura Y., Aki D., Inaba T., Honda H. (Dept. of Developmental Oncology)

Purpose: Isolation of tumor-suppressor genes from the frequently deleted region in the chromosome-arm 7q in de novo AML, MDS and therapy-related AML. Biochemical and biological characterization will follow after isolation of candidate genes.

Methods & Results: To isolate tumor suppressor genes in the chromosome-arm 7q, we extracted DNA from leukemia samples stocked in RIRBM, and made probes for array-CGH by long-distance PCR technique. We made 268 probes each of which has about 5kb in length spanning in chromosome 7q21.3-7q31.1.3 and does not contain repetitive sequences. We identified candidate genes TITAN, Kasumi, and MIKI, and started to characterize them by using gene-targeting technology and by conventional molecular and cellular biological technique. These three genes exist in the genome of vertebra alone and deeply involved one another in the evolution. Because none of these three gene products has known functional motifs,
we tried to determine their function through elucidation of subcellular localization and their binding partner proteins. Kasumi and Titan bind to the Ku70/Ku80/DNA-PK complex, which play critical roles in the non-homologous end joining (NHEJ) repair system of double stranded DNA break. Kasumi and Titan translocate from cytoplasm to nucleus by radiation, supporting this data. On the other hand, MIKI binds to spindles in the prophase and metaphase of mitosis, and downregulation of MIKI expression by si-RNA resulted in arrest in the prophase, suggesting its critical roles in M phase.

Matsui, T., Takemura Y., Inaba, T.

**Purpose:** Identification of molecular mechanisms that regulate the expression of Bim gene, which encodes a BH3-only cell death activator and plays critical roles in hematopoiesis.

**Methods & Results:** Expression control systems were analyzed by DNase hypersensitivity assay, reporter analysis, gel shift assay, nuclear run off assay, and experiments monitoring the half life of mRNA. By reporter assay, we isolated cis-elements, which upregulate (several hundreds base pairs upstream of the transcriptional initiation site) or downregulate (in the first intron) the transcriptional efficiencies. Unexpectedly, the silencer sequence in the first intron is cytokine independent. Moreover, nuclear run off assay suggested that the expression of Bim is not controlled by transcriptional mechanisms, but by the regulation of the half-life of its message. Therefore, Bim mRNA is likely regulated by modification of mRNA degradation. In vitro assay, the half-life of Bim mRNA was elongated by cytokines. We found that this is mediated by heat shock cognate protein (Hsc70), which binds and stabilizes the mRNA. The binding potential of Hsc70 to mRNA is turned out to be regulated by cochaperones such as Hsp40, Hip, CHIP, and Bag-4. Ras pathways likely contributed the regulation of cochaperones through yet unknown mechanism.

3. Roles of mutated c-Kit in t(8;21)-positive AML.
Asou H., Inaba T.

**Purpose:** Point mutations in the kinase domain of c-Kit is frequently associated with t(8;21)-positive AML. We studied whether c-Kit mutations affect the clinical courses of these patients. We also tested the sensitivity of imatinib mesylate (Gleevec®) on leukemia cells harboring both the t(8;21) and a c-Kit mutation that has been controversial for years.

**Methods & Results:** We detected kinase domain c-Kit mutations in 12 (25.5%) of 47 patients with t(8;21)-AML. Although complete remission was achieved in 11 (91%) patients by standard chemotherapy, they had a high relapse rate and significantly poor prognosis. We investigated effects of imatinib on t(8;21)-leukemia cells with c-Kit mutations using the Kasumi-1 cell line and fresh leukemia cells isolated from untreated patients. Imatinib inhibited autophosphorylation of mutated c-Kit in Kasumi-1 and fresh leukemia cells. Subsequently, imatinib induced cell cycle arrest and apoptosis in the Kasumi-1 cells or induced cell death in fresh leukemia cells at the standard concentration (0.1 μM). Our results suggest that imatinib would be effective in eliminating minimal residual disease in these patients.

4. Analysis of transcriptional control of the survivin gene.
Aki D., Matsui T., Inaba T., Kurossawa H. (Dokkyo Med. School)

**Purpose:** E2A-HLF chimeric transcription factor, which is produced as a result of 17;19 chromosomal translocation in ALL patients, induces survivin, an antiapoptotic factor. We analyzed its mechanism.

**Methods & Results:** We observed that survivin expressed throughout the cell cycle in t(17;19)-positive leukemia cells, in spite of the well-known fact that this gene usually shows G2/M-specific expression. Reporter assay revealed that a cis-element called cell cycle homology region (CHR) is involved in this abnormal regulation. Indeed, gel shift assay detected an E2A-HLF-inducible transcription factor, which binds to CHR. In addition, Ikaros, which plays as a key regulator of
T-cell development, appears to be involved in the regulation of survivin expression.

5. Induction of the proapoptotic Bim gene by Crem

Matsui H., Inaba, T., Ohama K (Univ. of Ryukyus)

**Purpose:** Our previous study revealed that UV downregulates Bim expression. We tried to elucidate its molecular mechanisms.

**Methods & Results:** We found a UV-dependent cis-regulatory element in the first intron of the *Bim* gene by reporter assay. Gel shift analysis revealed that Crem, a bZIP transcription factor, binds to the *cis* element. Unexpectedly, Crem was downregulated by UV. Biological significance of these findings are now under investigation.

6. Development of a real-time, ultra-high sensitive whole cell sensor system for hormones and cytokines

Matsui H, Inaba, T., Mabuchi, K. (Univ. of Tokyo)

**Purpose:** Development of a real-time, ultra-high sensitive whole cell sensor system for hormones and cytokines using cultured cardiomyocytes differentiated from embryonic carcinoma (EC) cells.

**Methods & Results:** EC cells differentiate to cardiomyocytes that pulsate in culture dish in an adrenalin concentration-dependent fashion. We started to develop a real-time, ultra-high sensitive whole cell sensor system for adrenalin by monitoring pulse ratio. In order to eliminate background caused by bio-active substances other than adrenalin, we are trying to downregulate β1-adrenergic receptor using si-RNA technique in differentiated cardiomyocytes, in which pulse ratio was not affected by adrenalin. To monitor the concentration of hormones and cytokines other than adrenalin, we are planning to make artificial fusion receptors that contain both ligand-binding region of receptors for hormones or cytokines, and the cytoplasmic domain of the β1-adrenergic receptor.

A. Original Papers

1. Inukai T., Inaba T., Dang J., Kuribara R., Ozawa K., Miyajima A., Wu W., Look AT., Arinobu Y., Matsui H., Akashi K., Mabuchi, K. (Univ. of Tokyo, Department of Pediatrics, Yamanashi School of Medicine, Pediatric Oncology Department, Dana-Farber Center Institute, Division of Genetic Therapeutics, Center for Molecular Medicine, Jichi Medical School, Institute of Molecular and Cellular Bioscience, University of Tokyo, Kyusyu University, Aichi Cancer Center) TEF, an anti-apoptotic bZIP transcription factor related to the oncogenic E2A-HLF chimera, inhibits cell growth by downregulating expression of the common β chain of cytokine receptors. *Blood*, 105: 4437-4444, 2005 (I)


Yamanashi Y. *1, Yoshimura A. *2 (*1Department of Cell Regulation, Tokyo Medical and Dental University, *2Division of Molecular and Cellular Immunology, Medical Institute of Bioregulation, Kyushu University) Control of cell fate through regulation of mRNA half-life by cytokines. The Center for Child Health and Development, Clinical Hematology, Yokohama, September

5. Aki D., Mashima R. *1, Saeki K. *1, Minoda Y. *1, Yamauchi M. *1, Yoshimura A. *1 (*1Division of Molecular and Cellular Immunology, Medical Institute of Bioregulation, Kyushu University) Modulation of TLR signaling by the C-terminal Src kinase (Csk) in macrophages. Genes Cells. 10, 357-368, 2005 (I)

6. Ohishi M. *1, Matsumura Y. *1, Aki D., Mashima R. *1, Taniguchi K. *1, Kobayashi T. *1, Kukita T. *2, Iwamoto Y. *2, Yoshimura A. *1 (*1Division of Molecular and Cellular Immunology, Medical Institute of Bioregulation, Kyushu University, *2Oral cellular and Molecular Biology, Department of Oral Biological Sciences, Faculty of Dental Sciences, Kyushu University, *3Department of Orthopedic Surgery, Faculty of Medicine, Kyushu University) Suppressor of Cytokine signaling-1 and-3 regulate osteoclastogenesis in the presence of inflammatory cytokines. J. Immunol. 174, 3024-3031, 2005 (I)

7. Niimi H. *1, Harada H. *1, Harada Y. *2, Ding Y. *1, Imagawa J. *1, Inaba T., Kyo T. *3, Kimura A. *1 (*1Dept. of Hematology and Oncology, *2International Radiation Information Center, *3Dept. of Internal Medicine, Hiroshima Red Cross Hospital) Hyperactivation of the RAS signaling pathway in myelodysplastic syndrome with AML1/RUNX1 point mutations. Leukemia, in press 2006 (I)

B. Meetings


5. Akahane, K. *1, Inukai, T. *1, Nemoto, A. *1, Honma, H. *1, Goi, K. *1, Sugita, K. *1, Nakazawa, S. *1, Kiyokawa, N. *1, Fujimoto, J. *2, Goto, H. *1, Endo, M. *4, Inaba, T. (*1Dept. of Pediatrics, Yamanashi School of Medicine, *2National Center for Child Health and Development, *3Dept. of Pediatrics, Yokohama City University, *4Dept. of Pediatrics, Iwate Medical University) The E2A-HLF fusion gene derived from the t(17;19) in childhood ALL induces CD33


10. Saeki K., Fukuyama S., Aki D., Kobayashi T., Yoshimura A. (1st Division of Molecular and Cellular Immunology, Medical Institute of Bioregulation, Kyushu University) Analysis of Raftlin KO mice on the lymphocyte. The 28th Annual Meeting of the Molecular Biology Society of Japan, December 7-10.


C. Others


2. Toshiya Inaba: Characterization of genes isolated from chromosome-arm 7q frequently deleted in AML and MDS. Tokyo Medical and Dental University, Tokyo (2005. 9. 16) (G)

3. Toshiya Inaba: Characterization of genes isolated from chromosome-arm 7q frequently deleted in AML and MDS. Tokyo Hematological Seminar (2005. 9. 21) (G)

4. Hiroya Asou, Toshiya Inaba: Characterization of genes isolated from chromosome-arm 7q frequently deleted in
AML and MDS. Annual Meeting of the Translocation-related Genes in Hematological Malignancies Research Committee, the Ministry of Health, Labor and Welfare of Japan, Tokyo, (2005. 10. 28) (G)

5. Hiroya Asou, Toshiya Inaba: Characterization of genes isolated from chromosome-arm 7q frequently deleted in AML and MDS. Annual Meeting of the Late Effects for Atomic Bomb Research Committee, the Ministry of Health, Labor and Welfare of Japan, Hiroshima, (2006. 2. 17) (R, G)