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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 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 2006 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., Nagamachi A., 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 and/or treatment of anti-tumor drugs, supporting this data. In the Kasumi knock-down cells by siRNA show high radio-senseitivity, high ratio of sister chromatid exchange, and background level phosphorylation of histone H2AX, all of which suggested that these gene products involved in the repair of DNA-damage or its related cell death control. Since we established Miki+/- and -/- mice, we are now analyzing sensitivity to radiation and anti-tumor drugs of these mice. On the other hand, Miki localizes to Golgi apparatus in the interphase, centrosomes and mitotic spindles in mitosis. Downregulation of Miki causes impaired maturation and separation of centrosomes, resulting in severe mitotic defect including loss of spindle tension, rosette formation of chromosomes and chromosome scattering. Consequently, cells undergo pre-anaphase arrest, or form bi-, tri- or even multi-nuclear cells with micronuclei, which are characteristic to MDS. Poly(ADP-ribosyl)ation of Miki by tankyrase-1 is required for binding to spindles. Abnormal mitosis and nuclear morphology is observed in bone marrow pictures of chimeric mice harboring hematopoietic progenitors originated from Miki$^{+/-}$ ES cells. These data suggest that *Miki* contributes to the development of MDS associated with monosomy 7.

2. Analysis of expression control of the Bim gene.

Matsui, H., 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, gelshift 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 cochaperons such as Hsp40, Hip, CHIP, and Bag-4. Ras pathways likely contributed the regulation of cochaperones through yet unknown mechanism.

3. Analysis of transcriptional control of the *survivin* gene.

Aki D., Matsui H., Inaba T., Kurosawa 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 CHR/transcription factor complex that was reduced by induction of E2A-HLF. We are palnning to isolate this transcription factor (theoretically *trans*-repressor).

4. 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.

5. 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 established EC cells expressing β1-adrenergic receptor at a reduced level 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. Niimi, H.[*1], Harada, H.[*1], Harada, Y.[*2], Ding ,Y.[*1], Imagawa, J.[*1], Inaba, T., Kyo, T.[*3], Kimura, A.[*1] ([*1] Department of Hematology/Oncology, [*2] International Radiation Information Center, [*3] Department of Internal Medicine, Hiroshima Red Cross Hospital and Atomic Bomb Survivors Hospital, Hiroshima) Hyperactivation of the RAS signaling pathway in myelodysplastic syndrome with AML1/RUNX1 point mutations. **Leukemia** 20: 635-644, 2006

2. Minoda, Y.[*1], Saeki, K.[*1], Aki, D., Takaki, H.[*1], Sanada, T.[*1], Koga, K.[*1], Kobayashi, T.[*1], Takaesu, G.[*1], Yoshimura, A.[*1] ([*1] Division of Molecular and Cellular Immunology, The Medical Institute of Bioregulation, Kyushu University) A novel Zinc finger protein, ZCCHC11, interacts with TIFA and modulates TLR signaling. **Biochem. Biophys. Res. Commun**. 344: 1023-1030, 2006

3. Inukai T.[*1], Hirose K.[*1], Inaba T., Kurosawa, H.[*2], Hama A.[*3], Inada, H.[*4], Chin, M.[*5], Nagatoshi, Y.[*6], Ohtsuka, Y.[*7], Oda, M.[*8], Goto, H.[*9], Endo, M.[*10], Morimoto, A.[*11], Imaizumi, M.[*12], Kawamura, N.[*13],

Miyajima, Y.[*14], Ohtake, M.[*15], Miyaji, R.[*16], Saito, M.[*17], Tawa, A.[*18], Yanai, F.[*19], Goi, K.[*1], Nakazawa, S.[*1], Sugita, K.[*1] ([*1] Dept. of Pediatrics, University of Yamanashi, Faculty of Medicine, [*2] Dept. of Pediatrics, Dokkyo Medical University, [*3] Department of Pediatrics, Nagoya University Graduate School of Medicine, [*4] Department of Pediatrics, Kurume University School of Medicine, [*5] Department of Pediatrics, Nihon University School of Medicine, [*6] Section of Pediatrics, National Kyushu Cancer Center, [*7] Div. of Cellular Therapy, Institute of Medical Science, University of Tokyo, [*8] Department of Pediatrics, Okayama University Medical School, [*9] Department of Pediatrics, Yokohama City University School of Medicine, [*10] Department of Pediatrics, Iwate Medical University School of Medicine, [*11] Department of Pediatrics, Kyoto Prefectural University of Medicine Graduate School of Medical Science, [*12] Department of Hematology and Oncology, Miyagi Children's Hospital, [*13] Department of Pediatrics, Osaka Rosai Hospital, [*14] Section of Pediatrics, Anjo Kosei Hospital, [*15] Department of Pediatrics, Sendai City Hospital, [*16] Department of Pediatrics, University of Occupational and Environmental Health School of Medicine, Kitakyushu, [*17] Department of Pediatrics and Adolescent, Juntendo University School of Medicine, [*18] Section of Pediatrics, National Hospital Organization Osaka National Hospital, [*19] Department of Pediatrics, Fukuoka University School of Medicine) Hypercalcemia in childhood acute lymphoblastic leukemia: frequent implication of PTHrP and E2A-HLF from translocation 17;19. **Leukemia** 21:288-96, 2006.

4. Matsui, H,. Asou, H., Inaba, T. Cytokines direct the regulation of Bim mRNA stability by Heat shock cognate protein 70. **Mol. Cell** 25: 99-112, 2007

5. Toshiya Inaba Apoptosis Molecular Biology for Regenerative Medicine pp.36-51,2006, Corona sya (in jpn)

6. Toshiya Inaba Regulatory system for Apoptosis Miwa's Hematology pp.103-107, 2006, Bunkodo (in jpn)

7. Toshiya Inaba Isolation of genes responsible for 7q deletion Annual Review 2007 (Ketsueki) pp.95-101, 2007, Chugai igakusya(in jpn)

B. Meetings

1. Asou, H., Ozaki, Y., Matsui, H., Aki, D., Takemura, Y., Oosugi, M[*1]., Inaba, T. ([*1] Div of Oncology, The Institute of Medical Science, The University of Tokyo) Isolation and characterization of candidate myeloid tumor suppressor genes from the commonly deleted region of chromosome 7q. The 65th Annual Meeting of the Japanese Cancer Association, Yokohama, September 28-30, 2006.

2. Matsui, H., Inaba, T., Asou, H. Regulation of mRNA stability by HSC70 in the normal and abnormal hematopoiesis. The 65th Annual Meeting of the Japanese Cancer Association, Yokohama, September 28-30, 2006.

3. Inaba, T., Asou, H. Isolation and characterization of candidate myeloid tumor suppressor genes from the commonly deleted region of chromosome 7q. The 68th Annual Meeting of Japanese Society of Hematology and The 48th Annual Meeting of Japanese Society of Clinical Hematology (Symposium), Fukuoka, October 6-8, 2006.

4. Matsui, H., Asou, H., Inaba, T. Regulation of mRNA stability by HSC70 in the normal and abnormal

hematopoiesis. The 68th Annual Meeting of Japanese Society of Hematology and The 48th Annual Meeting of Japanese Society of Clinical Hematology, Fukuoka, October 6-8, 2006.

5. Takahashi, K.*1, Goi, K.*1, Satou, H.*1, Honna, H.*1, Kuroda, I.*1, Hirose, K. *1, Akahane, K. *1, Nemoto, A*1, Furuichi, Y. *1, Inukai, T*1, Sugita, K*1, Nakazawa, S*1, Inaba, T., Matsui, H., Koyama, T.*2 (*1 Dept. of Pediatrics, University of Yamanashi, Faculty of Medicine,*2 Saitama Social Insurance Hospital) Mechanism of Flt-3 inhibitor PKC412-induced apoptosis in 11q23 translocated leukemic cell lines. The 68th Annual Meeting of Japanese Society of Hematology and The 48th Annual Meeting of Japanese Society of Clinical Hematology, Fukuoka, October 6-8, 2006.

6. Watanabe, N. *1, Kitaura, J. *1, Harada, H. *2, Ono, R. *1, Komeno, Y. *1, Satou, H.*4, Inaba, T., Nakajima, H.*3, Nosaka, T.*1 (*1 Dev. Of Cellular Therapy, The Institute of Medical Science, The University of Tokyo, *2 Dept. of Clinical and Experimental Oncology, *3 The Institute of Medical Science, The University of Tokyo, *4 The Institute of Medical Science Medical Genome, The University of Tokyo) AML1 point mutation induces MDS/AML in mouse BMT model. The 68th Annual Meeting of Japanese Society of Hematology and The 48th Annual Meeting of Japanese Society of Clinical Hematology, Fukuoka, October 6-8, 2006.

7. Zhang, X.*1, Inukai, T. *1, Akahane, K. *1, Hirose, K. *1, Kuroda, I*1, Honna, H. *1, Goi, K. *1, Uno, K. *1, Yagita, H. *2, Okumura, K. *2, Inaba, T., Kurosawa, H. *3, Endo, M. *4, Goto, H. *5, Kagami, K. *1, Sugita, K. *1, Nakazawa, S. *1 (*1 Dept. of Pediatrics, University of Yamanashi, Faculty of Medicine, *2 Juntendo University, Graduate School of Medicine, *3 Dept. of Pediatrics, Dokkyo Medical University, *4 Dept. of Pediatrics, Iwate Medical University, *5 Dept. of Pediatrics, Yokohama City University School of Medicine) TRAIL-sensitivity of t(17;19)-positive acute lymphoblastic leukemia. The 68th Annual Meeting of Japanese Society of Hematology and The 48th Annual Meeting of Japanese Society of Clinical Hematology, Fukuoka, October 6-8, 2006.

8. Hirose, K.*1, Inukai, T.*1, Akahane, K.*1, Zhang, X.*1, Kuroda, I.*1, Honna, H.*1, Goi, K.*1, Inaba, T., Kurosawa, H.*2, Endo, M.*3, Goto, H.*4, Kagami, K.*1, Sugita, K.*1, Nakazawa, S.*1(*1 Dept. of Pediatrics, University of Yamanashi, Faculty of Medicine, *2 Dept. of Pediatrics, Dokkyo Medical University, *3 Dept. of Pediatrics, Iwate Medical University, *4 Dept. of Pediatrics, Yokohama City University School of Medicine) Over-expression of LMO2 gene in t(17;19)-ALL by E2A-HLF fusion transcription factor. The 68th Annual Meeting of Japanese Society of Hematology and The 48th Annual Meeting of Japanese Society of Clinical Hematology, Fukuoka, October 6-8, 2006.

9. Asou, H., Matsui, H., Ozaki, Y., Takemura, T., Nagamachi, A., Aki, D., Oosugi, M.*1, Inaba, T. (*1 Div. of Oncology, The Institute of Medical Science, The University of Tokyo) Abnormal mitosis and genetic instability induced by downregulation of candidate myeloid tumor suppressor genes isolated from the Long arm of chromosome 7. 48th Annual Meeting of the American Society of Hematology, Orlando, FL, December 9-12, 2006.

C. Others

1. Hiroya Asou, Toshiya Inaba: Characterization of genes isolated from chromosome-arm 7q frequently deleted in AML and MDS. Annual Meeting of the Development of new age therapy for MDS, the Ministry of Health,

Labor and Welfare of Japan, Tokyo (2006.7.14) (2007.1.26) (R, G)

2. Toshiya Inaba: Characterization of genes isolated from chromosome-arm 7q frequently deleted in AML and MDS. Kawasaki Medical School (2006.11.24)

3. 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, Kyoto (2006.11.17)