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Division of Radiation and Regeneration Control
Department of Developmental Biology

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The experimental aims of this department are focused on the following two themes; “analysis of biological functions of genes using gene-engineered mice” and “basic investigation of regenerative medicine toward clinical application”.

As for gene-engineered mice, Hiroaki Honda and Kazuko Miyazaki generated several lines of transgenic and knockout/knockin mice and are analyzing their phenotypes. As for the basic investigation for regenerative medicine, Keigo Nakagawa is attempting to differentiate mouse ES (embryonic stem) cells to gastrointestinal stem cells in vitro.

Wataru Watanabe, a graduate student from Dept. of Ophthalmology and Visual Science, is analyzing transgenic mice expressing ODAG (ocular development-associated gene) under the control of a photoreceptor-specific promoter.

Tatsuya Tazaki, a graduate student from the 1st Dept. of Surgery, is analyzing conditional knockout mice lacking Cas in various tissues.

Nariaki Fujimoto is investigating the metabolism-activating and functional mechanisms of hormone- and endocrine-disrupting chemicals.

The research projects in this department are as follows:

1. Analysis of genes involved in blast crisis of chronic myelogenous leukemia using a transgenic mouse model

Honda, H., Miyazaki. K,, Yamazaki, N., Nakamura, T. (Department of Carcinogenesis, The Cancer Institute of JFCR), Oda, H. (The 2nd Department of Pathology, Tokyo Womens’ University), Wolff, L. (Leukemogenesis Section, Laboratory of Cellular Oncology, National Cancer Institute, USA)

Chronic myelogenous leukemia (CML) is a hematological malignancy of multipotential hematopoietic stem cells transformed by a chimeric protein, p210BCR/ABL. Clinically, it begins as an indolent chronic phase (CP) but inevitably progresses to a fatal blast crisis (BC). Additional genetic events would account for transition from CP to BC but underlying molecular mechanism(s) remains unknown.

We have generated transgenic mice for p210BCR/ABL that exhibit CML-like myeloproliferative disorder reproducibly. By applying retroviral insertional mutagenesis to the transgenic mice, we analyzed genes whose overexpression or inactivation contributes to BC of CML. Virus-infected p210BCR/ABL transgenic mice developed acute leukemias in a shorter period than virus-infected control littermates. We analyzed virus-integrated genes by inverted PCR and identified several interesting genes.

2．Generation and analysis of model mice for chimeric transcriptional factors isolated from human leukemias

Miyazaki, K., Honda, H., Yamazaki, N., Inaba, T., Oda, H. (The 2nd Department of Pathology, Tokyo Womens' University), Mitani, K. (Department of Hematology, Dokkyo Medical School)

In human hematological malignancies, a number of disease-specific chromosomal abnormalities have been detected. Most of these are chromosomal translocations and advances of molecular biology revealed that chimeric transcriptional factors are generated in many cases. We focus on BCR/ABL, AML1/ETO, AML1/EVI1, E2A/HLF. and E2A/PBX1 fusion proteins that are created by t(9;22), t(8;21), t(3;21), t(17;19), and t(1;19), respectively, and are developing a novel knockin system in which the chimeric genes are inducibly expressed after birth. We focused on E2A/HLF and found that acquired expression of E2A/HLF is not sufficient for developing leukemia, suggesting that secondary gene alteration would be required. To explore this process, we employed retroviral insertional mutagensis to the E2A/HLF inducible knockin mice. Virus-infected E2A/HLF inducible knockin mice developed acute leukemias and we analyzed virus-integrated genes by inverted PCR and identified several sets of transcription factors, which would collaborate with E2A/HLF to develop acute leukemias.

3．Functional analysis of a novel MBT-containing gene, Hemp, isolated from a hematopoietic stem cell library

Miyazaki, K., Honda, H., Oda, H. (The 2nd Department of Pathology, Tokyo Womens' University), Lemischka, I.R. (Department of Molecular Biology, Princeton University, USA)

Hemp (hematopoietic expressed mammalian polycomb) was isolated from a highly purified hematopoietic stem cell library and is considered to belong to polycomb gene family since it contains four repeats of MBT (malignant brain tumor) domain. To investigate its biological function, we generated mice, in which the MBT domain of Hemp was deleted. We found that homozygous mice die soon after birth, exhibit skeletal abnormalities, and show proliferation/differentiation defects of lympoid/myeloid progenitors.

4．Generation and analysis of transgenic mice expressing ODAG in the photoreceptor cells.

Watanabe, I., Miyazaki K., Honda, H., Miyagawa K. (Department of Pathology), Mishima, H. (Department of Ophthalmology and Visual Science), Furukawa, T. (Osaka Biomedical Institute)

ODAG (ocular development-associated gene) was cloned from a retinal cDNA library using a microarray method but its biological function remains unclear. To address this issue, we generated transgenic mice that express ODAG under the control of a photoreceptor-specific promoter. The transgenic mice exhibit proptosis of eyeball, elevated ocular pressure, and atrophy of ocular nerves, suggesting that the transgenic mice to be a model for buphtahlmos.

5．Functional analysis of an adaptor molecule, p130Cas (Cas), in adult tissues using conditional knockout mice

Tazaki T, Miyazaki, K., Honda, H., Sakai, R. (Growth Factor Division, National Cancer Center Research Institute)

p130Cas (Cas, Crk-associated substrate) was identified as an adaptor molecule which becomes tyrosine-phosphorylated in fibroblasts transformed by an oncoprotein, Crk or Src. We generated Cas-deficient mice and found that the biological function of Cas is bundling of actin fibers. However, because Cas-deficient mice are embryonic lethal, the role(s) that Cas plays in adult tissues has not been understood. To address this issue, we generated conditional knockout mice of Cas and crossmated them with transgenic mice expressing Cre in various tissues. Tissue-specific deletion of Cas would provide new insights of Cas function in various biological processes, such as cell proliferation, cell differentiation, cell homing, and cell migration.

6．Isolation of intestinal stem cells from mouse embryonic stem cells using a knockin method

Nakagawa, K. and Honda, H.

Musashi-1 was originally isolated by Dr. Okano in Keio University, and is regarded as a marker for intestinal (and also neuronal) stem cells. As a step for basic investigation toward regenerative medicine of radiation-sensitive organs, we knocked-in green fluorescent protein (GFP) gene into the Musashi-1 locus in mouse embryonic stem (ES) cells. Several independent clones were obtained and we are generating embryoid bodies by removing LIF and feeder cells and isolating GFP-positive cells by flowcytometry. We will characterize the in vitro function of the GFP-positive cells and will analyze its in vivo regenerative potential by transplanting these cells into intestine-irradiated mice.

7．Androgen-dependent regeneration of the prostate gland

Fujimoto, N.

In the prostate gland, castration results in involution and administration of androgen leads to complete regeneration of the normal epithelium from the stem cells. A series of androgen dependent genes, potentially involved in this regeneration process, were identified. Quantitative expression analysis revealed that several growth factors including stem cell growth factor may play a role in the regeneration process.

8．Developmental regulation of estrogen receptor expression

Fujimoto, N.

Estrogen plays a crucial role in development and regulation of the reproductive system. The promoter mechanism of one of the receptor, estrogen receptor (ER) beta, has not been identified. The 5'-flanking region of mouse ER-beta gene was cloned and proved to be a functional promoter in vitro. However, in vivo experiment revealed that the region is only able to direct testis specific expression.

A. Original papers

1．Kono H.[*1]., Kyogoku C. .[*1], Suzuki T. .[*1], Tsuchiya N. .[*1], Honda H., Yamamoto K. .[*1], Tokunaga K. .[*1], Honda Z-i. .[*1] ([*1]Department of Allergy and Rheumatology, Faculty of Medicine, Graduate School of Medicine, University of Tokyo). FcgRIIB Ile232Thr transmembrane polymorphism associated with human systemic lupus erythematosus decreases affinity to lipid rafts and attenuates inhibitory effects on B cell receptor signaling. Hum. Mol. Genet. 14: 2881-2892, 2005 (A, G, I)

2．Iwata, T[*1*2], Kawamoto, T[*1], Sasabe, E[*1], Miyazaki, K, Fujimoto, K[*1], Noshiro, M[*1], Kurihara, H[*2] and Kato, Y[*1] ([1]Dept. Dent. and Med. Biochem., [1]Dept. Dent. and Med. Biochem., [2]Dept. of Periodontal Medicine, Grad. School of Biomed. Sciences, Hiroshima Univ.) : Effects of overexpression of basic helix-loop-helix transcription factor Dec1 on osteogenic and adipogenic differentiation of mesenchymal stem cells. Eur. J. Cell Biol., in press, 2006. (I)

3．Kohno Y.[*1], Kitamura S.[*1], Sanoh S.[*1], Sugihara K.[*1], Fujimoto N., Ohta S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Metabolism of the alpha,beta-unsaturated ketones, chalcone and trans-4-phenyl-3-buten-2-one, by rat liver microsomes and estrogenic activity of the metabolites. Drug Metab Dispos. 33: 1115-1123, 2005. (I)

4．Kitamura S.[*1], Sugihara K.[*1], Fujimoto N. ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima

University School of Medicine): Endocrine Disruption by organophosphate and Carbamate pesticides In: R. C. Gupta (ed.) Toxicology of organophosphate and carbamate compounds, Elsevier Academic Press, London, 2006, pp481-494. Kitamura, S.[*1], Jinno, N.[*1], Suzuki, T.[*1], Sugihara, K.[*1], Ohta, S.[*1], Kuroki, H.[*1], Fujimoto, N. ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Thyroid hormone-like and estrogenic activity of hydroxylated PCBs in cell culture. Toxicology, 208, 377-387, 2005. (R, A, G, I)

B. Meeting presentations

1. Miyazaki, K., Miyazaki, M.[*1] Oda, Hideaki[*2], Kanno, M.[*1], Lemischka I. R.[*3], Honda H. ([1]Dept. of Immunology, [2]2nd Department of Pathology, Tokyo Women's Medical University, [3]Dept. of Molecular Biology, Princeton University, Princeton, U.S.A.) : Analysis of the zinc finger domain protein Hemp-deficient mice. The 28th Annual Meeting of the Mol. Biol. Soc. Japan, Fukuoka, 2005.12. (R, G)

2. Fujimoto, N., Suzuki, T.[*1], Akimoto, Y.[*2], Kitamura, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine, [*2]Dept. Mol. Radiobiology): Identification of mouse prostatic proteins and their hormonal regulation, Gordon Research Conferences, South Hadley, U.S.A. 2005.7. (R, A, G)

3. Araki, M.[*1], Sugihara, K.[*1], Kitamura, S.[*1], Fujimoto, N., Ohta, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Endocrine disrupting chemicals in hospital waste, Environment and Toxicology Forum 2005, Tokushima, 2005.10. (G)

4. Fujimoto, N., Asano,K.[*1], Usui, T.[*1] ([*1]Dept. Urology, Hiroshima University School of Medicine): In vivo functional analysis of the mouse estrogen receptor beta promoter, 14th Annual Meeting of Japan Steroid Hormone Society, Nagoya, 2005.11. (A, G)

5. Suzuki, T.[*1], Fujimoto, N., Ohta, S.[*1], Kitamura, S.[*1], ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Identification of mouse prostatic proteins and their hormonal regulation, 14th Annual Meeting of Japan Steroid Hormone Society, Nagoya, 2005.11. (A,G)

6. Shinohara, S.[*1], Sugihara, K.[*1], Kitamura, S.[*1], Fujimoto, N., Ohta, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): AhR ligand activity of enviromental chemicals and effect of their substituents on the activity, 126th Annual Meeting of The Pharmaceutical Society of Japan, Sendai, 2006. 3. (R, G)

7. Tange, T.[*1], Sugihara, K.[*1], Kitamura, S.[*1], Fujimoto, N., Ohta, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Metabolism of carbamate pesticides and its effect on their endocrine disrupting actions, 126th Annual Meeting of The Pharmaceutical Society of Japan, Sendai, 2006.3. (G)