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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.

Nariaki Fujimoto is investigating the metabolism-activating and functional mechanisms of hormone- and endocrine-disrupting chemicals in collaboration with Dr. Ohta (Dept. of Xenobiotic Metabolism and Molecular Toxicology).

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 (a collaboration with Dr. Furukawa in Osaka Bioscience Institute and Dr. Miyagawa in Dept. of Human Genetics).

Toshiyuki Mizuno, a graduate student from Dept. of Orthopaedic Surgery, is analyzing transgenic mice expressing a dominant negative form of PPAR γ under the control of a chondrocyte-specific promoter (a collaboration with Dr. Tsumaki in Osaka).

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

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., Nakagawa, K.

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 which exhibit CML-like myeloproliferative disorder reproducibly. Using this transgenic system as a tool and by combining it with random genomic mutagenesis by retroviral insertion, we are planning to identify candidate genes whose overexpression or inactivation might contribute to BC of CML. BHX2, a mouse

strain which transmits retroviruses through milk, is used for this study. We have back-crossed p210BCR/ABL transgenic mice with BXH2 mice more than 3 generations to obtain BHX2 genetic background and are monitoring peripheral blood counts of the transgenic mice. We already found that several p210BCR/ABL transgenic mice developed acute leukemia and are now identifying genes where retroviruses were integrated by using virus-specific primers.

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

Miyazaki, K., Honda, H., Inaba, T., 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 have already created knockin mice for BCR/ABL, AML1/ETO, AML1/EVI1, E2A/HLF. These mice will be useful not only for investigating molecular mechanism(s) of how these chimeric proteins cause leukemia in vivo but also for developing new therapies for chromosomal translocation-associated leukemias.

3. Functional analysis of a polycomb gene, Hemp, isolated from a hematopoietic stem cell library

Miyazaki, K., Honda, H., Lemischka, I.R. (Department of Molecular Biology, Princeton University, USA)

Hemp is a polycomb gene isolated from a highly purified hematopoietic stem cell library. To investigate its biological function, we generated Hemp/GFP knockin mice, in which MBT domain of Hemp was replaced by GFP. Intercrossing heterozygotes resulted in no alive homozygotes, strongly suggesting that Hemp/GFP homozygotes are embryonic lethal. We are currently analyzing when and how the homozygous mice become lethal.

4. 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.

5. Developmental changes in 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. The in vivo function was also characterized in the promoter-lacZ-transgenic mice.

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

Watanabe, I., Honda, H., 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 an interesting phenotype in the eyeball and in the retina and we are analyzing the molecular mechanism underlying the phenotype.

8. Functional analysis of a dominant negative form of PPARγ dominant negative form in chondrocytes using transgenic mice

Mizuno, T., Honda, H., Ochi, M. (Dept. of Orthopaedic Surgery), Tsumaki, N. (Dept. of Orthopedics, Osaka University)

PPARγ is a nuclear receptor protein which modulates intracellular signaling networks. To investigate its role in chondrocytes, we generated transgenic mice expressing a dominant negative form of PPARγ under the control of a chondrocyte-specific promoter. We obtained several independent mouse lines and are analyzing their phenotype.

9. 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.

A. Original papers

1. Niki, M.[*1*2], Di Cristofano A.[*1], Zhao M.[*3], Honda H., Hirai, H.[*4], Aelst, L.V.[*3], Cordon-Cardo, C.[*2], and Pandolfi, P.P[*1*2] (1 Cancer Biology and Genetics Program, Memorial Sloan-Kettering Cancer Center, New York, [*2]Department of Pathology, Memorial Sloan-Kettering Cancer Center, New York, [*3]Cold Spring Harbor Laboratory, Cold Spring Harbor, [*4]Department of Hematology & Oncology, Graduate School of Medicine, University of Tokyo): Critical role of Dok-1 and Dok-2 in leukemia suppression. J. Exp. Med. 20: 1689-95, 2004. (A, G, I)

2. Fujimoto, N., Asano, K.[*1], Usui, T.[*1], Honda, H., Kitamura, S.[*2] ([*1]Dept. Urology, [*2]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Cloning and characterization of the 5-flanking region of the rat estrogen receptor β gene J. Steroid Biochem. Mol. Biol. 93, 151-158, 2005. (R, A, G, I)

3. Fujimoto, N., Suzuki, T.[*1], Honda, H., Kitamura, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Estrogen enhancement of androgen responsive gene expression in hormone induced hyperplasia in the ventral prostate of F344 Rats. Cancer Sci. 95, 711-715, 2004. (R, A, G, I)

4. Fujimoto, N., Igarashi, K.[*1], Kanno, J.[*1], Honda, H., Inoue, T.[*1] ([*1]Div. Toxicology, NIHS) Identification of estrogen responsive genes in the GH3 cell line by cDNA microarray analysis, J. Steroid Biochem. Mol. Biol. 91, 121-129, 2004.(I)

5. Fujimoto, N., Jinno, N.[*1], Kitamura, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Activation of ERE responses by thyroid hormone with increase in estrogen receptor levels in a rat pituitary cell line, GH3. J. Endo. 181, 77-83, 2004.

6. Kitamura, S.[*1], Kato, T.[*1], Iida, M.[*1], Jinno, N.[*1], Suzuki, T[*1], Ohta, S.[*1], Fujimoto, N., Hanada, H.[*1], Kashiwagi, K.[*1], Kashiwagi, A.[*1]. ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Anti-thyroid hormonal activity of tetrabromobisphenol A, a flame retardant, and related compounds: affinity to the mammalian thyroid hormone receptor, and effect on tadpole. Life Sci. 76, 1589-1601, 2005. (R, A, G, I)

7. 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)

8. Suzuki, T.[*1], Kitamura, S.[*1], Khota, R.[*1], Sugihara, K.[*1], Fujimoto, N.[*1], Ohta, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Estrogenic and antiandrogenic activities of 17 benzophenone derivatives used as UV stabilizers and sunscreens. Toxicol. Appl. Pharmacol., 203, 9-17, 2005. (A, G, I)

9. Kitamura, S.[*1], Suzuki, T.[*1], Sanoh, S.[*1], Kohta, R.[*1], Jinno, N.[*1], Sugihara, K.[*1], Yoshihara, S.[*1], Fujimoto, N., Watanabe, H., Ohta, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Comparative study of the endocrine-disrupting activity of bisphenol A and 19 related compounds. Toxicol. Sci., 84, 1-12, 2005. (A, G, I)

10. Miyazaki M.[*1], Kawamoto, H.[*2], Kato, Y.[*1], Itoi, M.[*3], Miyazaki, K., Masuda, K.[*4], Tashiro, S.[*5], Ishihara, H.[*1], Igarashi, K.[*5], Amagai, T.[*3], Kanno, R.[*1] and Kanno M.[*1] ([*1]Dept. of Immunology and [*5]Biomed. Chemistry, Grad. School of Biomed. Sciences, Hiroshima Univ., [*2]Lab. for Lymphocyte Development, RIKEN Res. Center for Allergy and Immunology, [*3]Dept. of Immunology and Microbiology, Meiji Univ. of Oriental Medicine, [*4]Dept. of Immunology and Cell Biology, Grad. School of Biostudies, Kyoto Univ.): Polycomb group gene mel-18 regulates early T progenitors expansion by maintaining Hes-1 expression. J. Immunology, 174, 2507-2516, 2004 (I)

11. Matsubara, T.[*1], Suardita K.[*2], Ishii, M.[*2], Igarashi, A.[*2], Oda, R.[*3], Nishimura, M.[*4], Saito, M.[*5], Nakagawa, K., Yamanaka, K.[*2], Miyazaki, K., Shimizu, M.[*2], Tsuji, K.[*1], Nakamura, K.[*6], Kato,Y[*1*2]. ([*1]JST, [*2]Dept. Dent. and Med. Biochem., Grad. School of Biomed. Sciences, Hiroshima Univ., [*3]Dept. of Operative dent., [*4]Dept. of Prosthetic dent., Grad. School of Biomed. Sciences, Hiroshima Univ., [*5]Dept. Operative dent. and Endodontics Kanagawa Dental College, [*6]Dept. of Orthopedic Surgery, Grad. School of Medicine, Univ. of Tokyo): Alveolar bone marrow as a cell source for regenerative medicine: differences between alveolar and iliac bone marrow stromal cells. J. Bone. Miner. Res., 313(3): 503-508, 2004 (I)

12. Higashi Y, Kimura M, Hara K, Noma K, Jitsuiki D, Nakagawa K, Oshima T, Chayama K, Sueda T, Goto C, Matsubara H, Murohara T, Yoshizumi M. Autologous bone-marrow mononuclear cell implantation improves endothelium-dependent vasodilation in patients with limb ischemia. Circulation. 2004 Mar 16;109(10):1215-1218. (I)

13. Jitsuiki D, Higashi Y, Goto C, Kimura M, Noma K, Hara K, Nakagawa K, Oshima T, Chayama K, Yoshizumi M. Effect of edaravone, a novel free radical scavenger, on endothelium-dependent vasodilation in smokers. Am J Cardiol. 2004 Oct

15;94(8):1070-1073. (I)

B. Meeting presentations

1. Honda, H. "The front-line research of hematopoietic neoplasms: Analysis of molecular mechanisms underlying development of hematopoietic neoplasms using model mice" Symposium in the 63th Annual Meeting of Japanese Cancer Association, Fukuoka, 2004 9. (R,A,G)

2. 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-disruputing activity in hospital sewage. International Symposium of the Environmental Risk of Endocrine Disrupter, Kyoto, 2005 (R, A, G)

3. Asano,K.[*1], Fujimoto, N., Usui, T.[*1] ([*1]Dept. Urology, Hiroshima University School of Medicine): Cloning and characterization of the 5¤-flanking region of the rat estrogen receptor β gene (Proceedings 80,402). 12th Annual Meeting of Japan Steroid Hormone Society, Osaka, 2003 (R, A, G)

4. Suzuki, T.[*1], Fujimoto, N., Honda, H., Ohta, S.[*1], Kitamura, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Androgen responsive gene expression in rat prostatic lobes (Proceedings 80,415). 12th Annual Meeting of Japan Steroid Hormone Society, Osaka, 2003 (R, A, G)

5. Fujimoto, N., Honda, H., Igarashi, K.[*1], Kanno, J.[*1], , Inoue, T.[*1] ([*1]Div. Toxicology, NIHS): Estrogen enhancement of androgen responsive gene expression in hormone induced hyperplasia in the ventral prostate of F344 Rats (Abstract 318). 63rd Annual Meeting of Japan Cancer Association, Nagoya, 2003 Fukuoka, 2004 (R, A, G)

6. Fujimoto, N., Suzuki, T.[*1], Honda, H., Kitamura, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Synergisic effects of estrogen on androgen responsive gene exrepssino in rat prostate (Proceddings 80, 140). 77th Annual Meeting of the Japanese Endocrine Society, Kyoto, 2004 (R, A, G)

7. Asano,K.[*1], Fujimoto, N., Usui, T.[*1] ([*1]Dept. Urology, Hiroshima University School of Medicine): Cloning and characterization of the 5¤-flanking region of the rat estrogen receptor β gene. (Proceedings 80, 202). 77th Annual Meeting of the Japanese Endocrine Society, Kyoto, 2004 (R, A, G)

8. Fujimoto, N., Honda, H. and Kitamura, S.[*1] ([*1]Dept. Xenob. Metab. Mol. Toxicol., Inst. Pharm. Sci., Hiroshima University School of Medicine): Cloning and characterization of the 5¤flanking region of rat estrogen receptor beta gene. 16th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology, Seefeld, Austria, 2004 (R, A, G)

9. Miyazaki M.[*1], Kawamoto H.[*2], Kato Y.[*1], Itoi M.[*3], Miyazaki K., Masuda K.[*4], Tashiro S.[*5], Ishihara H.[*1], Igarashi K.[*5], Amagai T.[*3], Kanno R.[*1] and Kanno M.[*1] ([*1]Dept. of Immunology and 5Biomed. Chemistry, Grad. School of Biomed. Sciences, Hiroshima Univ., [*2]Lab. For Lymphocyte Development, RIKEN Res. Center for Allergy and Immunology, [*3]Dept. of Immunology and Microbiology, Meiji Univ. of Oriental Medicine, [*4]Dept. of Immunology and Cell Biology, Grad. School of Biostudies, Kyoto Univ.): Polycomb group gene mel-18 controls Notch signaling in early T progenitor expansion. The 12th International Congress of Immunology, Montreal, 2004.7 (R,G)

10. Miyazaki K., Miyazaki M.[*1], Kanno M.[*1], Miyatake S.[*2], Kato Y.[*3], Honda H. ([*1]Dept. of Immunology, [*3]Dept. of Dent. and Med. Biochem., Grad. School of Biomed. Sciences, Hiroshima Univ., [*2]Dept. of Immunology, TMIMS) : Analysis of roles of DEC1 and DEC2 in T cell differentiation. The 27th Annual Meeting of the Mol. Biol. Soc. Japan, Kobe, 2004.12. (R, G)