Proc. Hiroshima Univ. RIRBM, 46, 2005

Division of Radiation and Regeneration Control Department of Cellular Biology

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Hiromitsu WATANABE, the former professor, retired in Mar. 2002, and Satoshi TASHIRO started his study in Dec. 2004. Osamu KATO, Associate Professor, resigned in Apr. 2004. Jiying SUN joined to the department from Mar. 2005.

The final goal of our department is to establish a system to regenerate or facilitate regeneration of damaged cells by various environmental stresses, especially radiation. For this purpose, we are studying the mechanism for dynamic organization of nuclear functions in stress response especially DNA double strand breaks (DSBs). We have already established the UVA laser microirradiation system to induce DSBs in a restricted nuclear region. We are now studying the dynamics of DNA repair proteins and chromatin in response to DNA damage using the live imaging system and the multicolor immunofluorescence technique. Furthermore, we are establishing a system to analyze the *in vivo* biochemical interaction of DNA repair proteins and damaged chromatin.

Dr. Shoji is studying the induction of neurocristopathy syndrome by environmental factors, especially the development of cardiovascular anomalies such as conotruncal anomalies, and creating an effective model that closely simulates the human one. Also, he is collaborating with the School of Medicine of the University of Tokushima (Professor Fukui) to investigate the developmental mechanism of human syndrome induced by an environmental chemical factor. He attended the Symposium A1: The Japanese Teratology Society Forty-forth Annual Meeting, Saga, Japan on July 15, 2004, and presented a paper entitled "Radiation and teratogenesis." He also attended Workshop 1: Radiation Effects; A-Bomb and other sources. 47th Annual Meeting of the Japan Radiation Research Society, Nagasaki, on Nov. 25, 2004 and presented a paper entitled "Radiation and neurocristopathy syndrome." He has also been serving as part time lecturer to teach graduate students at Graduate School of Medical Sciences, University of Tokushima.

1. Dynamic organization of nuclear domains in DNA damage response.

Tashiro S.

To examine the dynamics of chromatin and non-chromatin nuclear domains in the regulation of DNA repair, we have developed a laser UV microirradiation system to induce DNA double strand breaks (DSBs) at the restricted areas in cell nuclei. Using this system, we are studying the dynamics of DNA repair proteins and apoptosis related proteins after induction of DSBs. We have already obtained preliminary results suggesting that the dynamics of non-chromatin nuclear domains in the process of DNA repair are regulated according to the function of each nuclear domain. For the further characterization of the dynamic organization of higher order nuclear architecture, we have combined the Fluorescence Recovery after Photobleaching (FRAP) technique with laser-UV-microirradiation. This new method allows us to examine the dynamics of specific proteins in living cells after induction of DSBs.

2．Study of the mechanism to regulate dynamics and localization of chromatin and non-chromatin nuclear domains.

Tashiro, S.

In this study, we are going to analyze the basic higher order nuclear architecture regulating the formation and dynamics of non-chromatin nuclear domains. For this purpose, we will analyze the localization and dynamics of known nuclear domains using newly established imaging techniques including visualization of nuclear function and living cell imaging system. Furthermore, biochemical approach is applied to identify a key molecule for the formation of non-chromatin nuclear domains as a scaffold.

3．Molecule mechanism analysis of heme regulating heme oxygenase-1 expression

Sun, J., Tashiro, S., Igarashi, K.[1)] ([1)] Tohoku Univ)

Purpose: Heme is known participate in gene regulation as a ligand for transcription factor Bach1. Little is known regarded factor exchange at the enhances of ho-1 upon transcriptional activation or the alterations in chromatin structure that accompany the switch between repression and activation.

Methods and results: Chromatin immunoprecipitation (ChIP) revealed that Bach1 occupied the enhancer of ho-1 gene in NIH3T3 cells under normal conditions. After hemin treatment to increase the intracellular level of heme, Bach1 was displaced from the ho-1 enhances, which was followed by Nrf2 binding to these elements. These results indicate that the transcriptional regulation of ho-1 involves a direct sensing of heme levels by Bach1. In addition, under normal conditions, the chromatin structure of ho-1 is in a preactivation state (both histone H3 and H4 of ho-1 enhancers and promoters were hyperacetylated) but transcription is repressed by Bach1. Thus, heme functions as a signaling molecule induces switching of Maf dimers, resulting in ho-1 expression.

4．Function analysis of Bach1 in regulation of beta-globin expression

Sun, J., Tashiro, S., Igarashi, K.[1)] ([1)] Tohoku Univ)

Purpose: Though the transcription factors such as NF-E2, GATA-1 are known as regulators of the globin gene, the mechanism of globin gene expression remains explanation.

Methods and results: We performed quantitative RT-PCR analysis using bone marrow cells from wild- type and bach1 deficient mice. The expression of beta-globin mRNA was similar in wild type and mutant mice. In contrast, beta-globin mRNA in bach1-/- mutant was decreased. We also determined the expression of hematopoietic specific transcription factors such as NF-E2 and GATA-1. Their mRNA showed no significant change in the mutant mice.

5．Animal model of abnormal vasculogenesis and conotruncal anomalies in Neurocristopathy syndrome induced by environmental factors.

Shoji, S.

This study examines through autopsy the genetic effects of numerous anomalies found at various gestational ages in aborted and stillbirth fetuses and in intrauterine death from both the atomic bomb survivors and those who did not experience the bomb. It then investigates, from the developmental biology stand point, the morphogenesis of these anomalies found in various organs, especially in the cardiovascular system, which are experimentally induced using a variety of environmental agents. The results are used in creation of animal model of teratogenic neurocristopathy syndrome that are resembled in human DiGeorge and Velocardiofacial (CATCH 22) syndromes, more specifically the teratogenic anomalies of the cardiovascular system including conotruncal anomalies, in order to clarify their pathogenesis.

6. Radiation or environment agent-induced teratogenesis in the offspring following exposures.

Shoji, S.

The present study was conducted to determine whether following DNA damage induced by ionizing radiation or environmental agents leads to lethality and teratogenesis among the progeny and to compare them to those reported in humans.

In order to understand the mechanism of embryonic lethality and teratogenesis in the F1 offspring caused by environmental agents, we continue to study such occurrences among the offspring of parental mice that have been exposed to environmental agents such as radiation and chemical substances.

Even a single abnormal development during fertilization and embryogenesis is known to result in lethality and/or teratogenesis, with DNA damage and individual genetic background having strong influences in these processes. It is considered that these genetic factors are inherited by the next generation, and the study investigates such processes for lethality and teratogenesis.

A. Original Papers

1. Miyazaki M[*1], Kawamoto H[*2], Kato Y[*1], Itoi M[*3], Miyazaki K[*4], Masuda K[*5], Tashiro S, Ishihara H[*1], Igarashi K[*6], Amagai T[*3], Kanno R[*1], Kanno M[*1]. ([*1]Dept Immunol., [*2]RIKEN, [*3]Meiji Univ., [*4]Dept. Develop. Biol., [*5]Kyoto Univ., [*6]Dept Biomed. Chem.): Polycomb group gene mel-18 regulates early T progenitor expansion by maintaining the expression of Hes-1, a target of the Notch Pathway. J Immunol. 174: 2507-2516, 2005. (I)

2. Yamasaki C[*1], Tashiro S, Nishito Y[*1], Sueda T[*2], Igarashi K[*1]. ([*1]Dept. Biomed. Chem, [*2]Dept. Surgery): Dynamic cytoplasmic anchoring of the transcription factor Bach1 by intracellular hyaluronic acid binding protein IHABP. J Biochem (Tokyo). 137(3): 287-296, 2005. (I)

3. Suzuki H[*1], Tashiro S, Hira S[*2], Sun J, Yamazaki C[*1], Zenke Y[*1], Ikeda-Saito M[*2], Yoshida M[*3], Igarashi K[*1]. ([*1]Dept. Biomed. Chem, [*2]Tohoku Univ., [*3]RIKEN): Heme regulates gene expression by triggering Crm1-dependent nuclear export of Bach1. EMBO J. 23(13): 2544-2553, 2004. (I)

4. Tashiro S, Muto A[*1], Tanimoto K[*2], Tsuchiya H[*1], Suzuki H[*1], Hoshino H[*3], Yoshida M[*4], Walter J[*1], Igarashi K[*1]. ([*1]Dept. Biomed. Chem., [*2]Dept. Trans. Cancer Res., [*3]Univ. Tokyo, [*4]RIKEN): Repression of PML nuclear body-associated transcription by oxidative stress-activated Bach2. Mol Cell Biol. 24(8): 3473-84, 2004. (I)

5. Muto A[*1], Tashiro S, Nakajima O[*2], Hoshino H[*3], Takahashi S[*3], Sakoda E[*1], Ikebe D[*1], Yamamoto M[*3], Igarashi K[*1]. ([*1]Dept. Biomed. Chem., [*2]Yamagata Univ., [*3]Univ. Tsukuba): The transcriptional programme of antibody class switching involves the repressor Bach2. Nature. 429(6991): 566-71, 2004. (I)

6. Yoshihara T[*1], Ishida M[*1], Kinomura A[*1], Katsura M[*1], Tsuruga T[*1], Tashiro S, Asahara T[*2], Miyagawa K[*1]. ([*1]Dept. Human Genet., [*2]Dept. Surgery): XRCC3 deficiency results in a defect in recombination and increased endoreduplication in human cells. EMBO J. 23(3): 670-80, 2004. (I)

7. Tahara T[*1], Sun J, Igarashi K[*2], Taketani S[*1]. ([*1]Kyoto Inst. Thec., [*2]Dept. Biomed. Chem.): Heme-dependent up-regulation of the alpha-globin gene expression by transcriptional repressor Bach1 in erythroid cells. Biochem Biophys Res Commun. 324(1): 77-85, 2004. (I)

8. Munakata H[*], Sun JY, Yoshida K[*], Nakatani T[*], Honda E[*], Hayakawa S[*], Furuyama K[*], Hayashi N[*]. ([*]Kinki

Univ.): Role of the heme regulatory motif in the heme-mediated inhibition of mitochondrial import of 5-aminolevulinate synthase. J Biochem (Tokyo). 136(2): 233-8, 2004. (I)

9. Satoshi TASHIRO, Kazuhiko IGARASH*(*Dept. Biomed. Chem.): Repression of PML nuclear body-associated transcription by Bach2 in oxidative stress. Jikken Igaku. 22(15). 2164-2166, 2004.

B. Meetings

1. Shoji, S., Shoji, I.*1 (*1College of Medicine and Public Health, The Ohio State University) Abnormal cardiovasculogenesis and embryonic development induced by excess Fertilysin exposure. The Teratology Society Forty-forth Annual Meeting, Hyatt Regency Vancouver, Vancouver, British Columbia, Canada, 2004. (Birth defects research Part A: Clinical and Molecular Teratology 70 (5), 360, 2004) (A, R)

2. Shoji, S., Shoji, I.*1 (*1医, College of Medicine and Public Health, The Ohio State University) Cardiovascular anomalies and embryonic development following maternal gamma rays exposure. The Teratology Society Forty-forth Annual Meeting, Hyatt Regency Vancouver, Vancouver, British Columbia, Canada, 2004. (Birth defects research Part A: Clinical and Molecular Teratology 70 (5), 360, 2004) (A, R)

3. Shoji, S., Shoji, I.*1 (*1College of Medicine and Public Health, The Ohio State University) Symposium A1: SA1-5 Radiation and teratogenesis. The Japanese Teratology Society Forty-forth Annual Meeting, Saga, 2004. (Cong. Anom. 68 (5), 360, 2004) (A, R)

4. Shoji, S., Shoji, I.*1 (*1College of Medicine and Public Health, The Ohio State University) Workshop 1: Radiation Effects; A-Bomb and other sources. W1-6 Radiation and neurocristopathy syndrome. 47th Annual Meeting of the Japan Radiation Research Society, Nagasaki, 2004. (English Abstracts pp. 50; Japanese Abstracts pp.57) (J. Radiat. Res. 45, 430, 2004). (A, R)

5. Satoshi TASHIRO, Kiyoshi MIYAGAWA, Kazuhiko IGARASHI : DNA repair, apoptosis and nuclear domains. 63rd Annual meeting of the Japanese Cancer Association, Fukuoka. 2004. 9. 29-10. 01. (Program, p127)

6. Tsuyoshi IKURA, Kenji KAMIYA, Satoshi TASHIRO: Dynamics of the TIP60 complex and histone H2AX in DNA damage response. 63rd Annual meeting of the Japanese Cancer Association, Fukuoka. 2004. 9. 29-10. 01. (Program, p127)

7. Satoshi TASHIRO: Dynamics of higher order nuclear architecture after induction of DNA damage. 47th Annual Meeting of Japan Radiation Research Society, Nagasaki. 2004. 11. 25-27 (J. Radiat. Res. 45, 430, 2004)

8. Satoshi TASHIRO, Yumi HARANO, Kazuki KONO, Atsushi ONO, Akihiko MUTO, Kazuhiko IGARASHI: Regulation of dynamics and function of Bach2, a transcription repressor, by SUMOylation. 27th Annual Meeting of the Molecular Biology Society of Japan, Kobe, 2004. 12. 8-11. (Abstract, p329)

9. Tsuyoshi IKURA, Satoshi TASHIRO, Isao KURAOKA, Kenji KAMIYA: Dynamics of the TIP60 complex and histone H2AX in DNA damage response. The 27th Annual Meeting of the Molecular Biology Society of Japan, Kobe, 2004. 12. 8-11. (Abstract, p366)

10. Junya KOBAYASHI, Hiroshi TAUCHI, Satoshi TASHIRO, Shuichi SAKAMOTO, Kenichi MORISHIMA, Shinya MATSUURA, Kenshi KOMATSU: NBS1 recognizes DNA double-strand breaks by two kinds of mechanisms. The 27th Annual Meeting of the Molecular Biology Society of Japan, Kobe, 2004. 12. 8-11. (Abstract, p648)

11. Yukari ZENKE, Satoshi TASHIRO, Jiying SUN, Kazuhiro IWAI, Kazuhiko IGARASHI: Heme-binding transcription factor Bach1 is degradated by ubiquitin-proteasome system. The 27th Annual Meeting of the Molecular Biology Society of Japan, Kobe, 2004. 12. 8-11. (Abstract, p441)

12. Akihiko MUTO, Satoshi TASHIRO, Osamu NAKAJIMA, Hideto HOSHINO, Satoru TAKAHASHI, Eiichirou SAKODA, Masayuki YAMAMOTO, Kazuhiko IGARASHI: The transcriptional programme of antibody class switching involves the repressor Bach2. The 27th Annual Meeting of the Molecular Biology Society of Japan, Kobe, 2004. 12. 8-11. (Abstract, p442)

13. Kyoko OCHIAI, Akihiko MUTO, Satoshi TASHIRO, Kazuhiko IGARASHI: Repression of Blimp-1 gene by B cell-specific transcription factors Bach2 and Bcl-6. The 27th Annual Meeting of the Molecular Biology Society of Japan, Kobe, 2004. 12. 8-11. (Abstract, p442)

14. Yoshihiro DOHY, Shinji OMURA, Jiying SUN, Tsutomu OHTA, Kazuhiko IGARASHI: Cross-talk of Bach1 and p53 in the regulation of apoptosis effector Perp. The 27th Annual Meeting of the Molecular Biology Society of Japan, Kobe, 2004. 12. 8-11. (Abstract, p442)

15. Kazuhiko Igarashi, Jiying Sun, Hiroshi Suzuki, Yukari Zenke, Satoshi Tashiro: In situ switching of heterodimeric transcription factor complexes induced by heme. The 77th Annual Meeting of the Japanese Biochemical Society, Yokohama 2004. 10. 13-16. (Abstract, p741)

16. Satoshi Tashiro, Akihiko Muto, Minoru Yoshida, Hideto Hoshino, Kazuhiko Igarashi: Repression of PML nuclear body-associated transcription by oxidative stress-activated Bach2. The 77th Annual Meeting of the Japanese Biochemical Society, Yokohama 2004. 10. 13-16. (Abstract, p953)

17. Akihiko Muto, Satoshi Tashiro, Osamu Nakajima, Hideto Hoshino, Satoru Takahashi, Eiichirou Sakoda, Dai Ikebe, Masayuki Yamamoto, Kazuhiko Igarashi: The transcriptional programme of antibody class switching involves the repressor Bach1. The 77th Annual Meeting of the Japanese Biochemical Society, Yokohama 2004. 10. 13-16. (Abstract, p957)

C. others

1. Satoshi Tashiro[1], Akihiko Muto1, Keiji Tanimoto1[5], Haruka Tsuchiya1, Hiroshi Suzuki1, Hideto Hoshino[2,4,6], Minoru Yoshida[3,4], Joachim Walter[1,7], and Kazuhiko Igarashi: Repression of PML nuclear body-associated transcription by oxidative stress-activated Bach2. 46th SYMPOSIUM OF THE SOCIETY FOR HISTOCHEMISTRY. Praque (2004. 9. 22-25)

2. Satoshi TASHIRO: Dynamics and SUMOylation of a transcription factor Bach2. The 2nd SUMO meeting. Okazaki 2004. 7. 9-10.

3. Satoshi Tashiro: Dynamic organization of nuclear domains in DNA damage response. The 21st Radiation Biology

Center International Symposium on Chromatin and Epigenetic Memory in Damage Response. Kyoto 2004. 11. 27-29.

4. Satoshi TASHIRO, Atsushi ONO, Dai IKEBE, Kazuhiko IGARASHI: Subnuclear localization of BACH2 gene in chronic myelocytic leukemia cells after imatinib treatment. JBS Bio-symposium 2005 & The 4th transcription meeting. Kusatsu 2005. 1. 11-12.

5. Satoshi TASHIRO, Tsuyoshi IKURA, Kiyoshi MIYAGAWA, Kazuhiko IGARASHI, Kenji KAMIYA: DNA repair and dynamics of higher order nuclear architecture. The 22nd Chromosome workshop. Sendai 2005. 1. 27-29.