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Division of Genome Biology
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The purpose of our research group is to elucidate the molecular mechanisms of chromosome instability by using human cell lines from the patients with cancer-prone genetic disorder. We are studying the molecular basis of the radiation-hypersensitive genetic disorders, including NBS, AT, and LIG4 syndrome, and also studying the molecular mechanism of chromosome segregation, using the patients' cells of premature chromatid separation (PCS) syndrome. These studies will be useful to uncover the regulation of the cell-cycle checkpoints and DNA double strand break repair. Following research projects were carried out during the fiscal year of 2005.

1. Genetic and functional analysis of cancer-prone genetic disorder, PCS syndrome.
2. Isolation of the gene that regulates the inhibition of centrosome re-duplication.
3. Analysis of radiation-induced TopBP1 focus formation.
4. Identification of the underlying gene for the radiation hypersensitive disorder.
5. Mutation screening of the Werner syndrome variant.
6. Genetic analysis of Smith-Lemli-Opitz syndrome in Japan.

1. Genetic and functional analysis of cancer-prone genetic disorder, PCS syndrome.

Matsumoto, Y., Ikeuchi, T. (Tokyo Med. Dent. Univ.), Kajii, T. (Hachioji), Matsuura, S.

A chromosomal instability syndrome of premature chromatid separation (PCS syndrome) is a mitotic-spindle checkpoint disorder, characterized by "mosaic variegated aneuploidy (MVA)" and "premature chromatid separation (PCS)". We found that the expression level of BubR1 protein was remarkably decreased in PCS cells, and only faint BubR1 signals were detected on kinetochores by immunofluorescence analysis. Each patient had one heterozygous mutation (flameshift mutation, nonsense mutation, and missense mutation), and there was no detectable mutation in another allele of the BubR1 gene of the six patients in spite of full sequencing. However, haplotype analysis and Western blot analysis both provided strong evidence suggesting the presence of a common cryptic mutation that result in a reduced transcript. Therefore, PCS patients were compound heterozygote for a null mutation and a cryptic hypomorphic mutation, and had reduced level of BubR1 protein, less than 50% of normal control. This is the first demonstration to confirm functionally that BubR1

insufficiency is the cause of abnormal mitotic checkpoint in PCS syndrome. We are trying to identify the BubR1 mutation that results in reduced protein expression.

2. Isolation of the gene that regulates the inhibition of centrosome re-duplication.

Izumi H., Matsumoto, Y., Matsuura S.

Centrosome is a small nonmembranous organelle, consisting of a pair of centrioles and a number of different proteins surrounding the centriole pair, which localizes at nearby nucleus in animal cells. In interphase cell, centrosome functions as microtubule organizing center (MTOC) that stabilizes cell polarity. In mitotic phase, centrosome plays a key role in establishing bipolar spindle, which is essential for balanced segregation of chromosomes into two daughter cells. Centrosome duplication occurs at G1/S transition and must do only once in a single cell cycle. Abrogation of such control mechanism results in abnormal amplification of centrosomes. The presence of amplified centrosomes results in high frequencies of aberrant mitotic spindle formation, which in turn increases the unequal segregation of chromosomes or chromosome instability. It is reported that normal cells dominantly inhibit centrosome re-duplication. However, the centrosome re-duplication mechanism is still enigma. We studied the centrosome re-duplication in mouse A9 hybrid cells, and found that a transfer of some human chromosomes suppressed centrosome re-duplication in mouse A9 cells. Now, we are narrowing down the candidate chromosomal regions by a technique of radiation hybrid mapping.

3. Analysis of radiation-induced TopBP1 focus formation.

Morishima, K., Kobayashi, J. (Kyoto Univ.), Komatsu, K. (Kyoto Univ.), Matsuura, S

Human TopBP1 shares sequence homology with Cut5/Rad4 identified in fission yeast, suggesting that TopBP1 is involved in damaged DNA repair and DNA replication. Recently, we found that formation of TopBP1 foci was significantly inhibited in AT cells or the cells with an NBS1 phosphorylation mutant. On the other hand, NBS1 focus formation was not affected in the TopBP1 siRNA cells. We also found that the TopBP1 siRNA cells showed a high frequency of premature chromatin condensation (PCC), which is a hallmark of ATR deficient cells. These results suggested that TopBP1 localizes to damaged DNA in a manner of ATM/NBS1 dependence and regulates ATR activity.

4. Identification of the underlying gene for the radiation hypersensitive disorder.

Suda T., Izumi H., Fujimoto H. (Kyoto Univ.), Komatsu K. (Kyoto Univ.), Matsuura S.

We previously reported a Japanese girl with the novel chromosomal instability syndrome. The clinical symptoms included severe microcephaly, short stature, combined immunodeficiency, and development of malignant lymphoma. The primary skin fibroblast from the patient showed radiation hypersensitivity. The clinical and cellular phenotypes were similar to those of the patients with Nijmegen breakage syndrome (NBS). However, no mutation was detected in the NBS1 gene, and western blot revealed normal expression of NBS1, Mre11, and Rad50 proteins. To identify the underlying gene for the patient, we utilized the technique of microcell-mediated chromosome transfer (MMCT) to introduce a human chromosome into the patients' fibroblast cell line, and functional complementation assays were carried out. We found that a chromosome 13 complemented the radiation hypersensitivity of the patient's cells. Since the chromosome 13 contains the DNA ligase IV (LIG4) gene, which is involved in non-homologous end joining (NHEJ) pathway of DNA DSB repair, mutation screening was performed in the LIG4 gene of the patient, and biallelic mutations were detected in the LIG4 gene. These results demonstrated that this is the first Japanese patient with the LIG4 syndrome.

5. Mutation screening of the Werner syndrome variant.

Matsumoto, H., Matsumoto, Y., Matsuura, S.

We recently encountered a patient with Werner syndrome variant. The 13-year old boy showed severe short stature (-8SD), but did not show senescence and immunodeficiency. Chromosome analysis of the peripheral lymphocytes showed high frequency of variegated translocation mosaicism (VTM), and a 2.5 times increase of SCE frequency. These results suggested that the disorder might be the novel genetic disorder, with clinical symptoms similar to those of Bloom syndrome and chromosomal findings similar to those of Werner syndrome. Mutation screening is being carried out for candidate genes, and functional analysis using the patient's cells is in progress.

6. Genetic analysis of Smith-Lemli-Opitz syndrome in Japan.

Matsumoto, Y., Tsukahara, M. (Yamaguchi Univ.), Matsuura, S.

Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive malformation syndrome characterized by microcephaly, syndactyly of toes, ambiguous genitalia, and mental retardation. The underlying DHCR7 gene has been isolated and a wide variety of distinct mutations were reported in USA and European SLOS patients. A significant difference has been suggested in the frequency of SLOS among different ethnic populations. We have analyzed DHCR7 genes of seven Japanese SLOS patients, and identified distinct mutations. R352Q mutation appeared to be most prevalent among Japanese SLOS patients, suggesting that this mutation might be a predominant founder mutation in Japan.

A. Original Papers

1. Matsuura, S., Matsumoto, Y., Morishima, K., Izumi, H., Matsumoto, H., Ito, E., Tsutsui, K., Kobayashi, J.[*1], Tauchi, H.[*2], Kajiwara, Y.[*3], Hama, S.[*3], Kurisu, K.[*3], Tahara, H.[*4], Oshimura, M.[*5], Komatsu, K.[*1], Ikeuchi, T.[*6], Kajii, T[*7]. ([*1]Radiat. Biol. Center, Kyoto Univ., [*2]Faculty of Sci., Ibaraki Univ., [*3]Dept. Neurosurgery, Hiroshima Univ., [*4]Dept. Cell. Mol. Biol., Hiroshima Univ., [*5]Dept. Mol. Cell. Biol., Tottori Univ., [*6]Div. Genet., M. R. I., Tokyo Med. Dent. Univ., [*7]Hachioji): Monoallelic BUB1B mutations and defective mitotic-spindle checkpoint in seven families with premature chromatid separation (PCS) syndrome. Am. J. Med. Genet. 104A: 358-367, 2006. (R)(G)(I)

2. Matsumoto, Y., Morishima, K., Honda, A.[*1], Watabe, S.[*2], Yamamoto, M.[*2], Hara, M.[*3], Hasui, M.[*4], Saito, C.[*5], Takayanagi, T.[*6], Yamanaka, T.[*7], Saito, N.[*8], Kudo, H.[*9], Okamoto, N.[*10], Tsukahara, M.[*2], Matsuura, S. ([*1]Dept. Gastroentrol. Univ. of Tsukuba, [*2]Faculty Health Sci., Yamaguchi Univ., [*3]General Isotope Cent., Tokyo Med. Dent. Univ., [*4]Hasui Pediat. Clinic, [*5]Dept. Psychiatr. Nat. Sanatorium Hokuriku Hosp., [*6]Dept. Pediatr., Nat. Saga Hosp., [*7]Dept. Hum. Welfare, Okazaki Women's Junior College, [*8]Shin-Koga Hosp., [*9]Asahigawasou Ryoiku Cent. Ryoikuen, [*10]Osaka Med. Cent. Res. Inst. Maternal Child Health): R352Q mutation of the DHCR7 gene is common among Japanese Smith-Lemli-Opitz syndrome patients. J. Hum. Genet. 50: 353-356, 2005. (G)(I)

3. Suda T.[*1], Katoh M.[*1], Hiratsuka M.[*1], Takiguchi M.[*2], Kazuki Y.[*2], Inoue T.[*1], Oshimura M.[*1,2]. ([*1]Dept. Hum. Genome Sci., Tottori Univ., [*2]Dept. Biomed. Sci., Instit. of Regenerative Med. Biofunction, Tottori Univ.): Heat-regulated production and secretion of insulin from a human artificial chromosome vector. Biochem. Biophys. Res. Commun., 340: 1053-1061, 2006. (I)

B. Meeting Presentations

1. Matsuura, S., Matsumoto, Y., Morishima, K., Izumi, H., Tauchi, H.[*1], Kobayashi, J.[*2], Komatsu, K.[*2], Ikeuchi,

T.*3, Kajii, T.*4 (*1Faculty Sci., Ibaraki Univ., *2RBC, Kyoto Univ., *3MRI, Tokyo Med. Dent. Univ., *4Hachioji): monoallelic BUB1B mutations in seven patients with PCS syndrome. 49th Annual Meeting of the Japan Society of Human Genetics. Kurashiki, September 19-22, 2005. (G)

2. Ikeuchi, T.*1, Yoshida, M.*2, Oda, S.*3, Mukai, Y.*4, Yokomori, K.*5, Numabe, H.*6, Matsuura, S., Kajii, T.*7 (*1MRI, Tokyo Med. Dent. Univ., *2Nat. Inst. Radiol. Sci., *3Nat. Kyushu Cancer Center, *4Jpn. Red Cross Med. Center, *5Tokyo Med. Univ., *6Hchioji): Chromosomal instability and cancer susceptibility in PCS syndrome. 49th Annual Meeting of the Japan Society of Human Genetics. Kurashiki, September 19-22, 2005.

3. Morishima, K., Kobayashi, J.*1, Tauchi, H.*2, Komatsu, K.*1, Matsuura, S. (*1RBC, Kyoto Univ., *2Faculty Sci., Ibaraki Univ.): Functional analysis of TopBP1 in DNA repair. 64th Annual Meeting of Japanese Cancer Association. Sapporo, September 14-16, 2005. (R)(G)

4. Sakamoto, S.*1, Tauchi, H.*2, Kobayashi, J.*1, Teshigawara, K.*3, Matsuura, S., Komatsu, K.*1 (*1RBC, Kyoto Univ., *2Faculty Sci., Ibaraki Univ., *3Lympyocyote Bank, Co., Ltd): NBS1 function in homologous recombination repair. 64th Annual Meeting of Japanese Cancer Association. Sapporo, September 14-16, 2005.

5. Matsuura, S., Morishima, K., Izumi, H., Kobayashi, J.*1, Tauchi, H.*2, Komatsu, K.*1, Ikeuchi, T.*3, Kajii, T.*4 (*1RBC, Kyoto Univ., *2Faculty Sci., Ibaraki Univ., *3MRI, Tokyo Med. Dent. Univ., *4Hachioji): Cancer-prone PCS syndrome: mutations and their function of BUB1B. 64th Annual Meeting of Japanese Cancer Association. Sapporo, September 14-16, 2005. (G)

6. Tauchi, H.*1, Kobayashi, J.*2, Sakamoto, S.*2, Matsuura, S., Komatu, K.*2 (*1Faculty Sci., Ibaraki Univ., *2RBC, Kyoto Univ.): NBS1 regulates DNA damage checkpoint and apoptosis. 64th Annual Meeting of Japanese Cancer Association. Sapporo, September 14-16, 2005.

7. Kobayashi, J.*1, Tauchi, H.*2, Sakamoto, S.*1, Morishima, K., Tashiro, S.*3, Matsuura, S., Komatsu, K.*1 (*1RBC, Kyoto Univ., *2Faculty Sci., Ibaraki Univ., *3RIRBM, Hiroshima Univ.): Modulation effect of gamma-H2AX on ATM-dependent DNA damage response. 64th Annual Meeting of Japanese Cancer Association. Sapporo, September 14-16, 2005.

8. Komatsu, K.*1, Antoccia, A.*2, Sakamoto, S.*1, Nakamura, K.*1, Teshigawara, K.*3, Kobayashi, J.*1, Matsuura, S., Tauchi, H.*4 (*1RBC, Kyoto Univ., *2Univ. Roma Tre, *3Lymphocyte Bank Co., Ltd, *4Faculty Sci., Ibaraki Univ.): ATM associates with NBS1 for a cell cycle checkpoint regulation. 48th Annual Meeting of the Japan Radiation Research Society, Hiroshima, November 15-17, 2005.

9. Matsumoto, Y., Izumi, H., Morishima, K., Kobayashi, J.*1, Tauchi, H.*2, Komatsu, K.*1, Ikeuchi, T.*3, Kajii, T.*4, Matsuura, S. (*1RBC, Kyoto Univ., *2Faculty Sci., Ibaraki Univ., *3MRI, Tokyo Med. Dent. Univ., *4Hachioji): Mutation analysis of BUB1B gene in seven patients with PCS syndrome. 48th Annual Meeting of the Japan Radiation Research Society, Hiroshima, November 15-17, 2005. (G)

10. Nakamura, K.*1, Samamoto, S.*1, Kobayashi, J.*1, Tauchi, H.*2, Teshigawara, K.*3, Matsuura, S., Komatsu, K.*1 (*1RBC, Kyoto Univ., *2Faculty Sci., Ibaraki Univ., *3Lymphocyote Bank Co., Ltd): Distinctive homologous recombination pathways between NBS1 and BRCA1/BRCA2. 48th Annual Meeting of the Japan Radiation Research Society, Hiroshima, November 15-17, 2005.

11. Morishima, K., Sakamoto, S.[*1], Kobayashi, J.[*1], Tauchi, H.[*2], Komatsu, K.[*1], Matsuura, S. ([*1]RBC, Kyoto Univ., [*2]Faculty Sci., Ibaraki Univ.): Response of TopBP1 in NBS cells after DNA damage. 48th Annual Meeting of the Japan Radiation Research Society, Hiroshima, November 15-17, 2005. (R)(G)

12. Ohishi, S.[*1], Iijima, K.[*1], Sakamoto, S.[*2], Kobayashi, J.[*2], Matsuura, S., Komatsu, K.[*2] ([*1]Faculty Sci., Ibaraki Univ., [*2]RBC, Kyoto Univ.): Functional domain of NBS1 for DNA double strand break repair. 48th Annual Meeting of the Japan Radiation Research Society, Hiroshima, November 15-17, 2005.

13. Kobayashi, J.[*1], Tauchi, H.[*2], Morishima, K., Matsuura, S., Komatsu, K.[*1] ([*1]RBC, Kyoto Univ., [*2]Faculty Sci., Ibaraki Univ.): Modulation effect of gamma-H2AX on ATM-dependent DNA damage response. 48th Annual Meeting of the Japan Radiation Research Society, Hiroshima, November 15-17, 2005.

14. Suda, T., Izumi, H., Fujimoto, H.[*1], Morishima, K., Yamada, M.[*2], Kobayashi, K.[*2], Kobayashi, J.[*1], Tauchi, H.[*3], Komatsu, K.[*1], Matsuura, S. ([*1]RBC, Kyoto Univ., [*2]Dept. Pediatr., Hokkaido Univ., [*3]Faculty Sci., Ibaraki Univ.): Functional complementation assays and identification of the underlying gene for the radiation hypersensitive disorder. 48th Annual Meeting of the Japan Radiation Research Society, Hiroshima, November 15-17, 2005. (R)(G)

15. Kobayashi, J.[*1], Tauchi, H.[*2], Morishima, K., Matsuura, S., Komatsu, K.[*1] ([*1]RBC, Kyoto Univ., [*2]Faculty Sci., Ibaraki Univ.): Functional role of gamma-H2AX on ATM-dependent DNA damage response. 28th Annual Meeting of the Molecular Biology Society of Japan, Fukuoka, December 7-10, 2005.

16. Iijima, K.[*1], Kobayashi, J.[*2], Muranaka, C.[*1], Sakamoto, S.[*2], Mochizuki, D.[*1], Ishizaki, K.[*3], Matsuura, S., Komatsu, K.[*2] ([*1]Faculty Sci., Ibaraki Univ., [*2]RBC, Kyoto Univ., [*3]Aichi Cancer Inst.): Regulation of DNA damage-induced apoptosis by NBS1. 28th Annual Meeting of the Molecular Biology Society of Japan, Fukuoka, December 7-10, 2005.

17. Suda T.[*1], Hiratsuka M.[*1], Katoh M.[*1], Takiguchi M.[*2], Kazuki Y.[*2], Inoue T.[*1], Oshimura M.[*1,2]. ([*1]Department of Human Genome Science, Tottori Univ., [*2]Department of Biomedical Science, Institute of Regenerative Medicine and Biofunction, Tottori Univ.): Heat-regulated production and secretion of insulin from a human artificial chromosome vector. 28th Annual Meeting of the Molecular Biology Society of Japan, Fukuoka, December 7-10, 2005.

18. Matsumoto, Y., Morishima, K., Izumi, H., Tsutsui, K., Kobayashi, J.[*1], Tauchi, H.[*2], Komatsu, K.[*1], Ikeuchi, T.[*3], Kajii, T.[*4], Matsuura, S. ([*1]RBC, Kyoto Univ., [*2]Faculty Sci., Ibaraki Univ., [*3]MRI, Tokyo Med. Dent. Univ., [*4]Hachioji): Genetic analysis of seven Japanese patients with PCS syndrome. 46th Annual meeting of Research Society for Delayed Effects of Atomic Bomb Detonation, Hiroshima, June 6, 2005. (G)

19. Suda T., Izumi H., Fujimoto H.[*1], Morishima K., Yamada M.[*2], Kobayashi K.[*2], Kobayashi J.[*1], Tauchi H.[*3], Komatsu K.[*1], Matsuura S.. ([*1]Rad. Biol. Center, Kyoto Univ., [*2]Department of Pediatrics, Hokkaido Univ., [*3]Faculty of Sci., Ibaraki Univ.): Identification of novel DNA ligase IV mutations in a Japanese patient with radiohypersensitivity. The Third International Symposium DNA Damage Response and Cancer, Hiroshima, February 1-2, 2006. (R)(G)

20. Morishima, K., Sakamoto, S.[*1], Kobayashi, J.[*1], Tauchi, H.[*2], Komatsu, K.[*1], Matsuura, S. ([*1]Rad. Biol. Center, Kyoto Univ., [*2]Faculty of Sci., Ibaraki Univ.): Involvement of TopBP1 in homologous recombination repair. The

Third International Symposium DNA Damage Response and Cancer, Hiroshima, February 1-2, 2006. (R)(G)

C. **Others**

1. Izumi, H.: Centrosome duplication and chromosome aneuploidy. In the textbook of the 12th Clinical Cytogenetics Seminar, pp89-100, 2005.

(R), (A), (G) and (C) are reports on the study using Radiation Experiments, Animal Experiments, Gene Technology Facilities and Studies established at the International Radiation Information Center, respectively. (I) indicates reports printed in the scientific journals listed in Current Contents.