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Major biological effects of ionizing radiation have been considered to arise from errors in the repair of DNA damage produced by irradiation. In fact, the induction of cell killing, mutation, and transformation in mammalian cells is known to increase with increasing doses of radiation, and also reported to strongly depend on their repair ability of DNA damage. However, recent reports have demonstrated that the irradiated cells show a transient expression of specific genes in response to ionizing radiation and that the dependence of gene expression on dose is not simply linear. Because the mammalian cells respond to various external stress such as heat treatment, UV radiation and synthetic chemicals, the cellular response to radiation may be derived from more general defense systems that have been developed in the living organisms during long evolutionary process.

Arrest of the cell cycle and induction of apoptosis are thought to be major cellular response to external stimuli. In the case of ionizing radiation, DNA damage like strand breaks and oxidative base lesions trigger to cell cycle arrest through the generation of signals that slow down the cell cycle progression and induce the expression of repair genes. Apoptosis plays a central role in maintaining multicellular organs to control cell number and is often used as a defense system to remove damaged or mutated cells. The research aim of this department is to analyze the molecular mechanisms of signaling pathways between radiation-induced DNA damage and cellular consequences such as cell cycle arrest and apoptotic cell death. Analysis of the genes regulating multistep processes of neoplastic cell transformation and its final step, cancer metastasis is also a major research project.

The research projects, which were carried out in 2004 and are being planned for the following years, are summarized as follows.

1. Elucidation of the molecular mechanisms of radiation-induced cellular response.
2. Analysis of the genes controlling mitotic checkpoints.
3. Studies of radiation-induced apoptosis using various cultured mammalian cells.

4. Analysis of the cellular signaling regulators associated with apoptotic cell death
5. Molecular analysis of radiation effects on meiotic cycle and embryonic development.
6. Research on molecular mechanisms of neoplastic cell transformation and cancer metastasis.

1. Role of Rho family regulator LyGDI during thymic apotosis induced by ionizing irradiation.

Participant: Xin-weng Zhou, Hidehiko Kawai, Shiho Suto, Masaaki Tatsuka, Masayo Maeda*, Takahide Ota*, Fumio Suzuki (*Kanazawa Medical University)

Purpose: LyGDI, which is abundant in haematopoietic cells, is proteolysed by the activated caspase-3 during thymic apotosis induced by ionizing irradiation. The cleaved LyGDI is translocated into cellular nucleus. Biological roles of the cleaved LyGDI are to be elucidated for our understanding about thymic apotosis induced by ionizing irradiation.

Methods and Results: A truncated form LyGDI, which is produced by activated caspase-3, was forcedly expressed in mouse thymic cells. The nuclear translocated LyGDI induced JNK (c-Jun N-terminal kinase) activation. On the other hand, cytoplasmic LyGDI was found to be translocated into mitochondria, and subsequently cytochrome c was released from mitochondria to activate caspase-3. These results indicate that LyGDI is an important molecule to amplify thymic apoptotic signals.

2. Effects of ionizing radiation on segregation processes of germ-line chromosomes.

Participant: Participants: Masaaki Tatsuka, Akifumi Kanda, Shiho Suto, Hidehiko Kawai, Takahide Ota*1, Masao Inoue*1 Otsura Niwa*2, and Fumio Suzuki (*1 Kanazawa Medical University, *2 Kyoto University)

Purpose: Cellular processes of the production of germ-line transmitted mutations should be elucidated for understanding the radiation effects caused through the generation.

Methods and Results: Methods and Results: The in vitro culture system of testiculuses isolated from male mice was established. Using this system, the sperms capable to fertilize were collected. On the other hand, unfertilized and fertilized eggs were obtained from female mice. Both sperms and eggs were used for visualization of mitotic apparatuses which were required by segregation of meiotic or mitotic chromosomes.

3. Analysis of the X-irradiation-induced G2/M checkpoint signaling via Aurora kinases.

Participants: Kawai, H., Suto, S., Kanda, A., Suzuki, F., Ota*, T., Inoue*, M., and Tatsuka (*Kanazawa Medical University)

Purpose: Aurora kinases belong to an A-kinase subfamily, and at least three members of this subfamily are present in mammals although yeast has only one kinase, Ipl1. Aurora-A regulates Cdk1 activity via Ajuba suggesting the implication of this kinase in the G2 checkpoint signal pathway. Aurora-B kinase is responsible for mitotic phosphorylation of histone H3, similar to yeast Ipl1, but its implication in the G2 checkpoint signal pathway and the functional relationship between two kinases in DNA damaged cells have not been elucidated.

Methods and Results: We now provide functional insight into the production mechanism of an increased chromosomal instability after exposure to IR in cells defective in the regulation of G2 checkpoint. In TP53-/- and ATM-/- cells, IR repressed Aurora-B kinase activity during mitotic progression, preserving Aurora-A kinase activity. Using enhanced green fluorescence protein (EGFP)-tagged Aurora-B (EGFP-AIM-1)-expressing cells, we found that IR induces an impaired mitosis having no alignment/segregation of chromosomes without cytokinesis in G2 checkpoint-defective cells. The tight regulation of the balance of two Aurora kinase activities regulates chromosomal segregation fidelity during mitosis, and its impairment, an unbalanced regulation, causes chromosome number instability.

4. Screening of functional genes responsible to ionizing radiation-induced cell cycle checkpoint regulation.

Participants: Hidehiko Kawai, Akifumi Kanda, Shiho Suto, Masaaki Tatsuka, Takahide Ota[*1], Yoshitaka Nobukuni[*2], Kiyoshi Miyagawa[*2], and Fumio Suzuki ([*1]Kanazawa Medical University, [*2]Depaetment of Human Genetics)

Purpose: Functional genes responsible to ionizing radiation-induced cell cycle checkpoint regulation were screened by using a mammalian cell system having saturation mutagenesis.

Methods and Results: The system used by us had been established from Chinese hamster ovary cells utilizing virus-derived fragment-induced insertion saturation mutagenesis. About 1,000,000 cells were screened for identifying functional genes responsible to ionizing radiation-induced cell cycle checkpoint regulation. Candidate genes inserted in the cells obtained from the library would code important regulator for radiation-induced checkpoint regulation.

5. Proteome analysis of γ and UV-irradiated Jurkat cells using two dimensional gel electrophoresis.

Participant: Akimoto, Y., Fukuda, E., and Suzuki, F.

Purpose: Since UV generally causes a rapid cell death and ionizing radiation induces a delayed form of apoptosis, the activation of signal transduction by radiation would be different between γ-rays and UV. In fact, exposure of Jurkat cells to UV resulted in a large amount of cytochrome *c* being released into the cytosol, and a clear laddering pattern of DNA fragments and activation of caspase-9 and its downstream caspases were observed within 3 h of incubation after irradiation. However, γ-irradiated cells showed extensive release of cytochrome c and caspase-9 and -3 activation at 24 h or longer incubation periods. In order to identify the signaling regulators associated with apoptotic cell death, we analyzed cellular proteins responding to γ-rays or UV irradiation using two dimensional gel electrophoresis (2DE). In this study, Jurkat cells were irradiated with 15 Gy of γ-rays or 20 J/㎡ of UV, both of which show a similar effect on cell killing, incubated for 48 or 6 h, respectively, and then treated with digitonin for preparing their cytosolic extracts.

Methods and Results: Samples were applied to immobilized pH gradient strips and isoelectric focusing was carried out on an Ettan IPGphor (Amersham Biosciences). Then following SDS-PAGE was performed on an Ettan DALTsix Electrophoresis System (Amersham Biosciences). Protein spot position and intensities were compared between patterns of irradiated and un-irradiated cells, and between patterns of γ-rays and UV irradiated samples. Comparison of the high resolution 2DE protein patterns of the extracts from irradiated and un-irradiated cells showed differences in many spots including protein modifications. We found that protein spots changed quantitatively or qualitatively in the irradiated cells were classified into three groups; proteins specifically responding to either γ-rays or UV and proteins responding to both radiations. Some of these spots were analyzed after tryptic digestion by peptide mass fingerprinting using a MALDI-TOF/TOF mass spectrometry, and three most prominent differing protein spots were identified as chloride intracellular channel protein 1 (CLIC1), Rho-GDP-dissociation inhibitor and acidic ribosomal protein P2. Further 2DE analyses of proteins responding to γ-rays or UV irradiation with different high resolution conditions are in progress.

6. Dephosphorylation of acidic ribosomal protein P2 in UV-irradiated Jurkat cells.

Participant: Fukuda, E., Akimoto, Y., and Suzuki, F.

Purpose: Most hematopoietic cells, such as thymocytes and lymphocytes, are known to be hypersensitive to radiation and exhibit interphase cell death with a typical morphological characteristic of apoptosis. Recently, it has reported that many human tumor cell lines which generally undergo necrotic cell death show a delayed apoptosis following exposure to UV irradiation. However, the mechanism of cell-type-dependence of apoptosis has not been well analyzed, and it is still not certain whether there is a key regulator in signaling pathways leading to either rapid or delayed apoptosis that is activated by UV-induced DNA damage. In this study, we analyzed cellular proteins responding to UV irradiation using two dimensional gel electrophoresis

(2DE) using two kinds of cell lines, Jurkat cells which undergo rapid apoptosis, and HeLa S3 cells which are known to be characterized by delayed apoptosis.

Methods and Results: Cells were irradiated with 20 J/㎡ of UV, incubated for various time intervals, and then treated with digitonin for preparing their cytosolic extracts. Comparison of the high resolution 2DE protein patterns of the extracts from irradiated and un-irradiated cells showed differences in many spots including protein modifications. In addition, the 2DE patterns of cytosolic fraction from apoptotic and nonapoptotic cells in Jurkat cells were totally different from those in HeLa S3 cells, although more than 2000 protein spots were detected in both cell lines. Intensive protein spots appeared in UV-irradiated Jurkat cells were analyzed after tryptic digestion by peptide mass fingerprinting using a MALDI-TOF/TOF mass spectrometry. Interestingly, one of 12 proteins identified, acidic ribosomal proteinP2 (P2) which is known to be mainly located within the 60s ribosomal subunit, was detected in three different p*I* spots, indicating the presence of two phosphorylation residues in cytoplasmic P2. Since only the phophorylated P2 was found in un-irradiated Jurkat cells and the amount of unphophorylated forms of P2 increased with increasing time of incubation after UV irradiation, the dephosphorylation of P2 may be associated with the activation of signaling pathways leading to apoptosis in UV-irradiated Jurkat cells.

List of contributions

A. Original Papers

1. Suzuki, F.: Cell Analysis -II (1.7 Measurement of cell growth activity), *Methods of Cell Biology III (Ohkuma, K., ed..)*, pp.22-36, Hirokawa Pub. Co., Tokyo, 2004.

2. Ota, T[*1]., Kawai, H., Kanda, A., Suto, S., Tatsuka, M., Suzuki, F., Takegami, Y[*1]., Maeda, M[*2]., Inoue, M[*1]. ([*1]Med. Res. Inst., Kanazawa Med. Univ., [*2]Dept. Pathol., Kanazawa Med. Univ.): Localization, dynamics, and function of RhoGDI-2 revealed by expression of functional RhoGDI-2-DsRed fusion proteins in the irradiated apoptotic living cells. *J. Med. Res. Inst. Kanazawa Med. Univ.,* 13 (2): 68-72, 2004. (R) (G)

3. Tatsuka, M.: Cell cycle regulation and Aurora kinases. *Seminor Cell Cycle Cancer.*22 (3): 42-63, 2004. (R) (G)

4. Tatsuka, M., Ota, T[*]. ([*]Med. Res. Inst., Kanazawa Med. Univ.): RhoGDI: key molecules of Rho signaling. *J. Med. Res. Inst. Kanazawa Med. Univ.,* 13 (5): 16-21, 2004. (R) (G)

5. Maeda, M[*1]., Kawai, H., Kanda, A., Suto, S., Tatsuka, M., Suzuki, F., Murakami, S[*2]., Inoue, M[*], Ota, T[*2]. ([*1]Dept. Pathol., Kanazawa Med. Univ., [*2]Med. Res. Inst., Kanazawa Med. Univ.): Involvement of RhoGDI-2 in radiation-induced mammalian G2-chekpoint regulation. *J. Cell Cycle Res.* 35 (6): 112-120, 2004. (R) (G)

6. Ota, T[*]., Tatsuka, M., Inoue, M[*], Kawai, H., Kanda, A., Suto, S., Suzuki, F., Maeda, M[*]., Odashima, S[*]. ([*]Med. Res. Inst., Kanazawa Med. Univ.): RhoGDI-2: A major regulator of cell behavior after irradiation in hematopoietic cells. *Cell Dynamics Res.* 3 (4): 56-63. 2004. (R) (G)

7. Zhou X., Suto, S., Ota, T[*]., and Tatsuka, M. ([*]Med. Res. Inst., Kanazawa Med. Univ.): Nuclear translocation of cleaved LyGDI dissociated from Rho and Rac during Trp53-dependent ionizing radiation-induced apoptosis of thymus cells *in vitro*. *Radiat. Res.*, 162 (3): 287-295, 2004. (R) (G) (I)

8. Tatsuka, M., Inoue, M[*], Ota, T[*]. ([*]Med. Res. Inst., Kanazawa Med. Univ.): Meiosis and Aurora kinases. *Seminor Cell Cycle Cancer.* 22 (4): 8-13, 2004. (R) (G)

9. Tatsuka, M., Ota, T*. (*Med. Res. Inst., Kanazawa Med. Univ.): RhoGDI- 2 /LyGDI: a critical factor to produce metasitatic cancer cells during carscinogenesis. *Seminor Cell Cycle Cancer.*22 (4): 23-31, 2004. (R) (G)

10. Fukuda E., Akimoto, Y., Yajima, H., Izumi, S*., Hirata, T*., and Suzuki F. (*Dept. Math. Life Sci., Grad. Sch. Sci.): Proteome analysis of cellular responses to radiation in Jurkat cells. *Nagasaki Medical J.,* 79 (Special ed.), 63-266, 2004. (R) (G)

11. Sasai, K*1., Katayama, H*1., Stenoien, D. L*2., Fujii, S*1., Kimura, M*3., Okano, Y*3., Tatsuka, M., Suzuki, F., Earnshaw, W. C*4., Brinkley, B. R*2., and Sen, S*1. (*1Dept. Mol. Pathol., Univ. Texas M.D. Anderson Cancer Center, *2Dept. Mol. Cell. Biol., Baylor College Med., *3Dept. Mol. Pathol., Gifu Univ. Sch. Med., *4Inst. Cell Mol. Biol., Edinburgh Univ.): Aurora-C is a novel chromosomal passenger protein that can complement Aurora-B function in mitotic cells. *Cell Motility & Cytoskeleton,* (in press) (G) (I)

B. Meeting Presentations

1. Tatsuka, M.: RhoGDI-2/LyGDI and cancer metastasis . 15th Annual Meeting of the Japan Society for Cancer Metastasis and Invasion, Fukushima, 2004. 5. (Abstract, p.13, 2004) (R) (G)

2. Fukuda E., Akimoto, Y., Yajima, H., Izumi, S*., Hirata, T*., and Suzuki F. (*Dept. Math. Life Sci., Grad. Sch. Sci.): Extensive analysis of cellular proteins responding to radiation using a human leukemia cell line, Jurkat. 45th Annual Meeting on Late Effects of Atomic Bomb, Nagasaki, 2004. 6. (Abstract, p.34, 2004) (R) (G)

3. Suzuki, F., Akimoto, Y., Sasai, K., and Yajima, H.: Sensitivity to radiation and activation of apoptosis signal-transduction pathways in human cancer cells. 34th Annual Meeting of the Japan Radiological Society (Bioloby), Kyoto, 2004. 6. (Abstract, p.68, 2004) (R) (G)

4. Zhou, X., Suto, S., Kanda, A., Kawai, H., Suzuki, F., Ota, T*., and Tatsuka, M. (*Med. Res. Inst., Kanazawa Med. Univ.): Appearance of cleaved LyGDI during X-irradiated apoptotic thymic cells. 29th Annual Meeting of the Chugoku Radiation Society, Hiroshima, 2004. 7. (*Rad. Biol. Res. Commun.,* 39 (4), 432, 2004) (R) (G)

5. Fukuda E., Akimoto, Y., Yajima, H., Izumi, S*., Hirata, T*., and Suzuki F. (*Dept. Math. Life Sci., Grad. Sch. Sci.): Proteome analysis of cellular proteins responding to UV with a MALDI-TOF/TOF mass spectrometry. 29th Annual Meeting of the Chugoku Radiation Society, Hiroshima, 2004. 7. (*Rad. Biol. Res. Commun.,* 39 (4), 432-433, 2004) (R) (G))

6. Kawai, H., Suto, S., Kanda, A., Suzuki, F., Ota, T*., Inoue, M., and Tatsuka, M. (*Med. Res. Inst., Kanazawa Med. Univ.): Analysis of the X-irradiation-induced G2/M checkpoint signaling via Aurora kinases. 63th Annual Meeting of the Japanese Cancer Association, Fukuoka, 2004. 9. (Abstract, p.151-152, 2004, *Cancer Sci.,* 95 (Suppl.), 151-152, 2004) (R) (G)

7. Suzuki, F., Fukuda E., Akimoto, Y., Yajima, H., Izumi, S*., and Hirata, T*., (*Dept. Math. Life Sci., Grad. Sch. Sci.): Analysis of cellular proteins associated with radiation-induced apoptosis using two-dimentional electrophoresis. 63th Annual Meeting of the Japanese Cancer Association, Fukuoka, 2004. 9. (Abstract, p.169, 2004, *Cancer Sci.,* 95 (Suppl.), 169, 2004) (R) (G)

8. Maetda, M*1., Murakami, M*2., Takegami, T*2., Tatsuka, M., and Ota, T*2. (*1Dept. Pathol., Kanazawa Med. Univ., *2Med. Res. Inst., Kanazawa Med. Univ.): Expression and localization of ERM proteins, E-cadherin, and beta-catenin in human colon cancer cell lines. 63th Annual Meeting of the Japanese Cancer Association, Fukuoka, 2004. 9. (Abstract, p.187, 2004,

Cancer Sci., 95 (Suppl.), 187, 2004) (G)

9. Ota, T[*1]., Maeda, M[*2]., Murakami, M[*1]., Takegami, T[*1]., and Tatsuka, M. ([*1]Med. Res. Inst., Kanazawa Med. Univ., [*2]Dept. Pathol., Kanazawa Med. Univ.): A new role of LyGDI in the regulation of Rho family proteins during metastatic processes. 63th Annual Meeting of the Japanese Cancer Association, Fukuoka, 2004. 9. (Abstract, p.264-265, 2004, *Cancer Sci.*, 95 (Suppl.), 264-265, 2004) (G)

10. Tatsuka, M., and Takata, T[*]. ([*]Dent. Pathol): Aurora kinases: their important roles involving chromosome distribution errors and increased cancer susceptibility. 63th Annual Meeting of the Japanese Cancer Association, Fukuoka, 2004. 9. (Abstract, p.418, 2004, *Cancer Sci.*, 95 (Suppl.), 418, 2004) (R) (G)

11. Tastuka, M.: Meiosis and Aurora. 25th Kanazawa Med. Sci. Cho-Yo Seinor, Kanazawa, 2004. 10. (Abstract, p.10, 2004) (R) (G)

12. Akimoto, Y., Fukuda, E., Izumi, S[*]., Hirata, T[*]., and Suzuki F. ([*]Dept. Math. Life Sci., Grad. Sch. Sci.): Proteome analysis of γ and UV-irradiated Jurkat cells using two dimensional gel electrophoresis. The 47th Annual Meeting of the Japan Radiation Research Society, Nagasaki, 20034 11. (Proceedings, p.90 & p.116, 2004) (R) (G)

13. Suzuki, F., Fukuda E., Akimoto, Y., Izumi, S[*]., and Hirata, T[*]. ([*]Dept. Math. Life Sci., Grad. Sch. Sci.): Analysis of the cellular signaling regulators associated with radiation-induced apoptosis. The 27th Annual Meeting of the Molecular Biology Society of Japan. Kobe, 2004. 12. (Program and Abstract, p.959, 2004) (R) (G)

14. Akimoto, Y., Fukuda, E., Izumi, S[*]., Hirata, T[*]., and Suzuki, F. ([*]Dept. Math. Life Sci., Grad. Sch. Sci.): Changes in two dimensional gel electrophoresis patterns of cytoplasmic proteins expressed in Jurkat cells after γ or UV irradiation. The Second International Symposium on Hiroshima University 21st Century COE Program ≲ Radiation Casualty Medical Research Center -, Hiroshima, 2004. 12. (Abstracts , p.47, 2004) (R) (G)

15. Fukuda E., Akimoto, Y., Izumi, S[*]., Hirata, T[*]., and Suzuki F. ([*]Dept. Math. Life Sci., Grad. Sch. Sci.): Accumulation of dephosphorylated acidic ribosomal protein P2 in the cytosol of cultured human cells after UV irradiation. The Second International Symposium on Hiroshima University 21st Century COE Program ≲ Radiation Casualty Medical Research Center -, Hiroshima, 2004. 12. (Abstracts, p.48, 2004) (R) (G)

C. Others

1. Suzuki, F.: Lecture on —Radiation effects and utility—. Lecture for visitor to the institute. Hiroshima, 2004. 9.

2. Suzuki, F.: Basic understanding of radiation (Radiation property and practical units for the measurement). Okayama forum on —Medical care and treatment for radiation casualties—. Okayama, 2004. 10.

(R) and (G) are reports on the study using Radiation Experiments and Gene Technology Facilities, respectively. (I): Report printed in the scientific journal that has been listed in Current Contents.