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.
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. **Bio-dosimetry in human body.**

Participants: Masaaki Tatsuka, Hidehiko Kawai, Shiho Suto, Fumio Suzuki, Takahide Ota, Takashi Takata, Jun Hihara, Kouichi Wadasaki, Daikichi Nalkamura, Genichiro Tsuji, Fumio Shimamoto (Division of Molecular Oncology and Virology, Medical Research Institute, Kanazawa Medical University, Programs for Biomedical Research Oral Maxillofacial Pathobiology, Hiroshima University Graduate School of Biomedical Sciences, Division of Clinical and Experimental Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University, Department of Radiology, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima University Bio-Venture Program, Tow Cells Co. Ltd., Department of Pathology, Faculty of Human Culture and Science, Prefecture University of Hiroshima)

**Purpose:** A new method for bio-dosimetry in human is to be established.

**Methods and Results:** Dental panoramic radiography patients and cancerous radiotherapy treatment patients were tested for the new method utilizing the truncation of LyGDI. We were able to detect truncated LyGDI proteins in these patients.

2. **Transmission of ionizing radiation effects into germ-line.**

Participants: Hidehiko Kawai, Akifumi Kanda, Shiho Suto, Fumio Suzuki, Takahide Ota, Masao Inoue, Otsura Niwa, and Masaaki Tatsuka (Kanazawa Medical University, 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:** After fertilization of sperm from irradiated male mouse, phosphorylated H2AX foci were found in nuclei of unirradiated egg. In addition, phosphorylated H2AX foci were found in polar body of fertilized egg.

3. **Overexpression of Aurora-A in oral carcinogenesis.**

Participants: Masaaki Tatsuka, Sunao Sato, Shoujiro Kitajima, Shiho Suto, Hidehiko Kawai, Mutsumi Miyauchi, Ikuko Ogawa, Masayo Maeda, Takahide Ota, and Takashi Takata (Programs for Biomedical Research Oral Maxillofacial Pathobiology, Hiroshima University Graduate School of Biomedical Sciences, Oral Anatomy and Morphological Basic Dentistry, Pathological Examination, Hiroshima University Hospital, Division of Molecular Oncology and Virology, Medical Research Institute, Kanazawa Medical University)

**Purpose:** Aurora kinases are known to play a key role in maintaining mitotic fidelity, and overexpression of aurora kinases has been noted in various tumors. Overexpression of aurora kinase activity is thought to promote cancer development through a loss of centrosome or chromosome number integrity. In this program, the role of Aurora-A overexpression in oral carcinogenesis is elucidated.

**Methods and Results:** We observed augmentation of G12V-mutated HRAS-induced neoplastic transformation in BALB/c 3T3 A31-1-1 cells transfected with Aurora-A. Aurora-A-short hairpin RNA (shRNA) experiments showed that the expression level of Aurora-A determines susceptibility to transformation. Aurora-A gene amplification was noted in human patients with tongue or gingival squamous carcinoma (4/11). Amplification was observed even in pathologically normal epithelial tissue taken at sites distant from the tumors in two patients with tongue cancer. However, overexpression of Aurora-A mRNA was observed only within the tumors of all patients examined (11/11). Our data indicate that Aurora-A gene amplification and overexpression play a role in human carcinogenesis, largely due to the effect of Aurora-A on oncogenic cell growth, rather than a loss of maintenance of centrosomal or chromosomal integrity.
4. Role of Aurora-B/AIM-1 kinase activity in cell transformation.

Participants: Akifumi Kanda, Hidehiko Kawai, Shiho Suto, Shoujiro Kitajima*, Sunao Sato*, Takashi Takata*, Masaaki Tatsuka (Programs for Biomedical Research Oral Maxillofacial Pathobiology, Hiroshima University Graduate School of Biomedical Sciences, *Oral Anatomy and Morphological Basic Dentistry, Pathological Examination, Hiroshima University Hospital)

Purpose: Aurora-B, previously known as AIM-1, is a conserved eukaryotic mitotic protein kinase. In mammals, this kinase plays an essential role in chromosomal segregation processes, including chromosome condensation, alignment, control of spindle checkpoints, chromosome segregation, and cytokinesis. Aurora-B is overexpressed in various cancer cells, suggesting that the kinase activity perturbs chromosomal segregation processes. Its forced overexpression induces chromosomal number instability and progressive tumorigenicity in rodent cells in vitro and in vivo. Nevertheless, based on focus formation in BALB/c 3T3 A31-1-1 cells, Aurora-B is not oncogenic. We elucidate the role of Aurora-B in cell transformation.

Methods and Results: We show that Aurora-B kinase activity augments Ras-mediated cell transformation. RNA interference with short hairpin RNA inhibits transformation by Ras and its upstream oncogene Src, but not by the downstream oncogene Raf. In addition, the inner centromere protein, which is a passenger protein associated with Aurora-B, has a similar ability to potentiate the activity of oncogenic Ras. These data indicate that elevated Aurora-B activity promotes transformation by oncogenic Ras by enhancing oncogenic signaling and by converting chromosome number-stable cells to aneuploid cells.

5. Multiple mixed lineage leukemia (MLL) fusion proteins suppress p53-mediated response to DNA damage.

Participants: Hidehiko Kawai, Dmitri Wiederschain*, Yuan Zhi-Min* (*The Harvard School of Public Health)

Purpose: Chromosomal translocations involving the mixed lineage leukemia (MLL) gene are often observed in acute leukemia. As a result of these chromosomal translocations, the N terminus of MLL is consistently fused in-frame to a number of partner proteins. Expression of MLL fusion proteins is known to induce malignant transformation of normal blood progenitors. Although more than 30 genes have been identified as fused to MLL in human leukemias, AF4, AF9, ENL, AF10, and ELL account for the absolute majority of recurrent MLL partners. The precise molecular mechanisms underlying the oncogenic function of MLL fusions are still poorly understood. It has been shown that transient expression of MLL-ELL results in a potent and specific inhibition of p53, a critical tumor suppressor protein. In this study, we analyze the effect of additional frequently detected MLL fusion proteins on p53 activity.

Methods and Results: We investigate the effect of several frequently detected MLL fusion proteins on p53 transcriptional activity. Our data show that MLL-AF9, MLL-AF10, MLL-ENL, and MLL-ELL substantially down-regulate p53-mediated induction of p21, MDM2, and Bax in response to DNA damage. Furthermore, we identify the reduction in p53 acetylation by p300 as a major mechanism of the inhibitory effect of MLL leukemic fusions. Our data suggest that abrogation of p53 functional activity can be a common feature of MLL fusion-mediated leukemogenesis.


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 within 2 h of incubation after irradiation, 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, H-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 X-rays or UV irradiation using two dimensional gel electrophoresis (2DE).

**Methods and Results:** In this study, Jurkat cells were irradiated with 15 Gy of X-rays or 20 J/m² 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. Samples were applied to immobilized pH gradient strips (pH range of 5.5 - 6.7) and isoelectric focusing was carried out. Then following SDS-PAGE was performed and these gels were stained with silver nitrate. These protein spot positions and intensities were compared between patterns of irradiated and un-irradiated cells, and between patterns of X-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. Proteins responding to both radiations were analyzed after tryptic digestion by peptide mass fingerprinting using a MALDI-TOF/TOF mass spectrometry. As a result, six most prominent protein spots were identified as delta3, 5-delta2, 4-dienoyl-CoA isomerase mitochondrial precursor, stathmin, RuvB-like, Cap G, acidic ribosomal protein P0, and Rho GDP dissociation inhibitor beta. In order to know whether these proteins contribute to apoptosis signaling pathway or not, the analyses of time dependent manner of changes in the protein spot patterns are in progress.

**List of contributions**

**A. Original Papers**


**B. Meeting Presentations**


C. Others


(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.