Research Center for Radiation Casualty Medicine
Department of Radiation Medicine

Professor Yoshio HOSOI, M.D., Ph.D. (~Dec. 31, 2012)
Assistant Professor Keiji TANIMOTO, D.D.S., Ph.D.
Assistant Professor Akinori MORITA, Ph.D.
Postgraduate Student Andika Chandra PUTRA, M.D. (~Mar. 31, 2013)
Postgraduate Student Ippei TAKAHASHI M.D. (Apr. 1, 2012~)

The area of interest of this department is to understand and control biological radiation response for purpose of improving radiation therapy and providing important insight into radiation protection and casualty medicine.

Our current research activities are focused on biological effects of low-dose radiation exposure in terms of radiation protection and nuclear safety, development of a novel kind of radioprotectors that inhibit radiation-induced cell death, and molecular mechanisms of hypoxic response relating to therapy resistance in cancer cells.

The research projects have been carried out and/or are being planned from various aspects during the academic year of 2012 as follows:

1. **Mechanisms of reactive oxygen species (ROS)-induced activation of ATM**
   Searching for the subcellular localization of ATM activation by oxidative stress

2. **Development of novel kind of radioprotectors that suppress radiation-induced cell death**
   Development of novel kind of radioprotectors that target p53

3. **Molecular mechanisms of hypoxic responses in solid tumors as potential molecular targets of anti-tumor therapy**
   1) Transgenic mice of the human hypoxia-inducible factor-1α gene
   2) Molecular mechanisms of genome damage response under hypoxic conditions

These researches were supported by several grants as follows: The Science Promotion Fund of the Ministry of Education, Culture, Science, Sports and Technology of Japan, 1) Challenging Exploratory Research (23659588) : A novel radiosensitization that targets radioresistant G0 phase cancer cells and cancer stem cells by inhibiting ATM protein kinase (delegate: Y. Hosoi), 2) Grant-in-Aid for Scientific Research (C) (23592766) : Significance of HIF-DEC pathway in DNA-damage response in oral cancer cells (delegate: K. Tanimoto), 3) Grant-in-Aid for Young Scientists (A) (24689050) : Development of novel kind of radioprotectors that relieve the damage of normal tissues in radiation injury (delegate: A. Morita); The Science Promotion Fund of the Ministry of Health, Labour and Welfare of Japan (e-Rad ID: 11103425) : Development of a novel anticancer drug that exerts multiple anticancer potencies (collaborator: A. Morita)

We had a total of 13 presentations at symposium, invited, educational, and special lectures in this year. Prof. Y. Hosoi was appointed as members of “Council of University, the Ministry of Education, Culture, Sports, Science & Technology in Japan”, “Council of Low Dose Ionizing Radiation, the Institute for Environmental Sciences”, “Council of Environmental Evaluation around Atomic Power Plant, Niigata Prefecture”, “Council of Japanese Radiological Society”, “Council of Japanese Society for Therapeutic Radiology and Oncology”, “Council of Radiation Biology, Japanese Society for Therapeutic Radiobiology and Oncology” and “Council of
Japan Radiation Research Society. Assist. Prof. K. Tanimoto was appointed as members of “Board of Secretary of the Japanese Association for Cancer and Hypoxia Research”, “Part-time lecturer of Saitama Medical University”, and “Part-time lecturer of Shimane University”.

1. Mechanisms of reactive oxygen species (ROS) -induced activation of ATM

Hosoi, Y., Morita, A.

**Purpose:** ATM is known to be activated by oxidative stress; however, the subcellular localization of its activation is still unclear. In this study, we attempted to identify the subcellular localization of its activation using some subcellular fractionation approaches.

**Methods and Results:** To avoid the influence of nuclear ATM responses, we initially determined the concentration of hydrogen peroxide that gave extranuclear-limited activation of ATM, and then subcellular fractionated the cells under this condition. A subcellular fractionation showed that a certain membrane fraction only contained ROS-activated ATM, while the fraction was not composed of single organelle, but contained mitochondria and peroxisomes. It is currently under investigation whether the activation occurs at mitochondria or peroxisomes.

2. Development of a novel kind of radioprotectors that inhibit radiation-induced cell death

Morita, A., Takahashi I., Hosoi, Y.

**Purpose:** Radiation therapy for cancer often has severe side effects that limit its efficacy. Because these side effects are in part determined by p53-mediated apoptosis, temporary suppression of p53 has been suggested as a therapeutic strategy to relieve the damage of normal tissues during treatment of p53-deficient tumors. On the other hand, it is known that dissociation of a zinc ion, which is coordinated to metal ion binding site of p53, could induce p53 denaturation, hence we evaluated some zinc chelators as radioprotective p53 inhibitor.

**Methods and Results:** We found 5-chloro-8-quinolinol (KH-3) as a radioprotector that can protect mice from a sublethal dose of 7.5 Gy total-body irradiation. Some cellular analyses revealed that KH-3 modulated p53 target-gene expression without affecting p53 itself expression. In particular, p21, which has anti-apoptotic activity, was markedly up-regulated by KH-3. Furthermore, KH-3 was ineffective against the radiation-induced apoptosis of p53-knockdown transformant, but suppressed that of p53 revertant. Results indicated that suppression of radiation-induced apoptosis by KH-3 was specifically mediated through p53 signaling pathways.

3. Molecular mechanisms of hypoxic response in solid tumors as potential molecular targets of anti-tumor therapy

Tanimoto, K.

**Purpose:** Since homeostasis of oxygen supply is the most important for life, hypoxic responses are maintained strictly at the molecular levels. Recently, it is getting clearer that these hypoxic response systems are related to the development and progression of some diseases including solid tumors. Especially, hypoxia-inducible factor-1 (HIF-1), which is a transcription factor activated under hypoxia, is thought to be a key regulator of those responses. In our projects, we are trying to clarify the role of HIF-1-mediated signaling pathway in solid tumors to develop the molecular targeted therapies.

1) Transgenic mice of the human hypoxia-inducible factor-1α gene

Tanimoto, K.

**Purpose:** To evaluate a role of HIF1A in carcinogenesis and tumor progression, we generated transgenic (tg) mice of human HIF1A gene.

**Methods and Results:** We have generated HIF1A tg mice which had FLAG tagged human HIF1A gene, and several lines were confirmed by PCR. We have also confirmed their expression of transgene and HIF-1 target genes by real-time RT-PCR and found that significant expression of human HIF1A in brain, heart, lung, stomach, small intestine, kidney, spleen, uterus, and skin of tg mice. Interestingly, multiple tumor formations were observed in tg mice older than 3 months, and tumors in lung, mammary gland, uterus, and submandibular gland were observed in almost 1 year old or older mice, and significantly shorter survival of tg mice was observed. Molecular analyses are ongoing now.

2) Molecular mechanisms of genomic response under hypoxic conditions

Tanimoto, K.

**Purpose:** Although it is well known that oxygen sensitizes cells to radiation and oxygen depletion (hypoxia) confer resistant phenotypes, molecular mechanisms remain unclear. Recently, it was reported that
expression levels of several DNA repair related genes were decreased in hypoxic cells. We also reported that hypoxia can regulate the transcription of hMLH1 via HIF-1-DEC pathway in cancer cell lines. Here, to understand the dynamic change of DNA damage and repair system under hypoxic conditions, we tried to clarify the regulation mechanisms of genomic response including DNA repair enzymes.

**Methods and Results:** We first performed comprehensive gene expression analysis using CodeLink™ Expression Bioarray System, UniSet Human 20K I Bioarray (19881 probes), and demonstrated that 24-hour hypoxic treatment up-regulated 2638 genes and down-regulated 2881 genes in HSC2. We found that they included both already known genes and a lot of novel genes. Notably, 22 of DNA repair enzyme genes were down-regulated under hypoxic conditions. Among them, promoters of MLH1, MSH2, MBD4, MRE11A, BRCA1, or RAD51 were subcloned into pGL3, luciferase reporter plasmid, and subjected to transient transfection experiments. Reporter assays and knock-down experiments demonstrated that these promoters were regulated by HIF-1-DEC pathway at the multiple mechanisms. Further investigation of their regulation mechanisms and significance in radiation therapy to cancers are on-going now.

**List of contributions**

A. **Original Papers**


5. Mohammed AES 1, Eguchi H 1, Wada S 1, Koyama N 1, Shimizu M 1, Otani K, Ohtaki M, Tanimoto K, Hiyama K 2, Gaber MS 1, Nishiya M 1 (*1Saitama Med Univ Int Med Cntr, *2Fukuhara Clinic). TMEM158 and FBLP1 as Novel Marker Genes of Cisplatin Sensitivity in Non-small Cell Lung Cancer Cells. Experimental Lung Research 2012, 38: 463-74. (G) (I)


B. **Meeting Presentations**


16. Junko Kitagawa*1, Yoji Yoshimi*1, Mari Mochizuki*1, Akinori Morita, Fumio Sugawara*1, Masahiko Ikekita*1 (*1Tokyo Univ. of Sci.): Characterization of a candidate anticancer drug isolated from Fuscoporia

18. Chisa Uchida*1, Mari Mochizuki*1, Yoji Yoshimi*1, Akinori Morita, Yoshimune Hasome*1, Noriko Ida*1, Osamu Funatsu*1, Fumio Sugawara*1, Masahiko Ikekita*1 (*1Tokyo Univ. of Sci.): Genetic analysis related to DDTCT-induced cell growth inhibition. The 85th Annual Meeting of the Jpn. Biochem. Soc., Fukuoka, Dec. 15, 2012. (in Jpn)


(R) and (G) are reports on the study using Radiation Experiments and Gene Technology Facilities, respectively. (I) indicates reports printed in the scientific journals listed in Current Contents.