

7. Frontier Research Center RadGenomics Project



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Dr. Murata obtained his Doctor of Medical Science from the Hokkaido University School of Medicine in 1973. After he worked as a clinical fellow in the Hokkaido University Hospital, he was promoted to Head of the Division of Nuclear Medicine & Radiology, Tokyo Metropolitan Geriatric Hospital in 1974. From 1983 to 1997, he worked as the Director of the Division of Radiology, Toranomon Hospital. Dr. Murata was invited to join NIRS as the Director of the Research Center of Charged Particle Therapy in 1997 and served in that position until 2003. Since 2001 he has been concurrently the Supervisory Director of the Frontier Research Center. His research themes have been the patho-physiological analysis of myocardial disorders with nuclear cardiology and developing a new strategy for cancer treatment by radiotherapy using heavy ion beams.

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Director

Dr. Imai received a Ph.D. from the University of Tsukuba in 1986. Following a fellowship from the Japan Society for the Promotion of Science for Japanese Junior Scientists at the Institute of Applied Biochemistry, University of Tsukuba, he joined the Tsukuba Life Science Center, Institute of Physical and Chemical Research (RIKEN). From 1988 to 1989, he worked in the Department of Genetics, Washington University Medical School (St. Louis, Missouri, USA) as a visiting research associate. Here Professor Maynard Olson sparked his interest in the human genome project. After joining The Cancer Institute, (Japanese Foundation for Cancer Research) in 1991, Dr. Imai worked on cancer and population genomics. Since 1994 he has been a senior researcher at NIRS. In 2001 he was named project leader of the RadGenomics Project.

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Objectives:

Cancer patients vary considerably in normal tissue reactions after radiotherapy. Several observations have indicated that certain genetic factors play important roles in this variability. It has been hypothesized that the clinical radiosensitivity of normal tissues should be regarded as a so-called complex trait dependent on the cumulative effect of many minor genetic determinants. Thus single nucleotide polymorphisms (SNPs) on certain genes may somehow associate with the severity of normal tissue reactions after radiotherapy. It is important to uncover a molecular basis underlying radiation sensitivity of normal tissues for further investigation of the more complex character of cancer cells. In this study we have searched for polymorphisms that are associated with normal tissue radiation sensitivity of various cancer patients. We believe the

results will open a way for achieving individual-oriented radiotherapy with high-therapeutic ratio.

The outcome of this research will allow us to identify any correlations between an individual DNA sequence and radiation susceptibility (treatment efficiency and adverse effects). If a correlation is found, the DNA sequence in blood cells will enable the prediction of an individual's radiation susceptibility. Therefore, it will be possible to provide information to determine treatment protocols, such as the irradiation method and the avoidance of adverse effects, leading to personalized radiotherapy.

The project will also contribute to future research on the molecular mechanisms of radiation sensitivity in humans.

Progress of Research:***Patients***

The 1,834 patients who were registered between 2001 and 2006 included 699 breast cancer patients, 251 cervical cancer patients, 324 prostate cancer patients, and 268 head and neck cancer patients. Normal tissue reactions until the 3rd month after completion of the treatment were graded according to the National Cancer Institute-Common Toxicity Criteria (NCI/CTC). Late effects on normal tissues were graded according to the Radiation Therapy Oncology Group/ the European Organization for Research and Treatment of Cancer (RTOG/EORTC) scoring system and the Late Effects of Normal Tissues-Subjective, Objective, Management and Analytic (LENT-SOMA) scoring system. Patients were divided into two groups (radiosensitive and radioresistant) according to the grades determined by the above scoring systems.

Optical detection system for allele-specific extension of oligonucleotides immobilized on a plastic chip

Single nucleotide polymorphisms (SNPs) are useful as genetic association markers for various human diseases as well as for prediction of individual responses to therapeutic treatment such as drugs and ionizing radiation. For routine molecular biology research and bedside clinical diagnosis, readily available technologies are required to genotype limited numbers of SNPs that are selected in advance in large scale association studies.

A novel optical detection system for on-chip allele-specific primer extension was developed to conveniently genotype multiple SNPs. This method used selective incorporation of biotin-modified deoxynucleotide by allele-specific extension reaction with oligonucleotides immobilized on a plastic chip and provided simplification of experimental procedures and reduction of reaction time to as short as 10 minutes at a constant temperature of 65 °C. The methodology is easily carried out at reasonable running cost with regular laboratory instruments.

Inter-strain variance in late phase of erythematous reaction or leg contracture after local irradiation among three strains of mice

To gain insights into inter-strain differences in radiosensitivity, mice of inbred strains, A/J, C57BL/6J, and C3H/HeMs, were irradiated at graded doses ranging from 20-60 Gy. Skin reaction and leg contraction were observed for a period of 230 days and between 175-350 days, respectively. Gene expressions in leg skin tissue were quantified by quantitative RT-PCR assay at 1, 12 and 72 hour

after 30 Gy irradiation. The three strains showed various degrees of susceptibility to irradiation as evaluated by skin scores. Large inter-strain differences were also detected in the lengths of contraction. Expressions of several genes such as Per3 and Rad51ap1 displayed inter-strain differences. We concluded that the continuum model of tissue injury revealed that genetic factor, which varies among strains, is one of the causes of variances in severity of damage after irradiation.

Strain-dependent differences in locomotor activity after local brain irradiation with 30 GyE of carbon ions

This study investigated strain differences in brain damage among male A/J, C57BL/6JNrs and C3H/HeNrs mice after local brain irradiation. Whole brains were irradiated with a single dose of 30 GyE carbon ion beams and then locomotor activity was determined as body heat of each animal. The daily locomotor activities of untreated mice differed among strains. Non-irradiated C57BL/6JNrs mice were more active than A/J mice. This variance became more obvious immediately after irradiation, when the activity of A/J and C3H/HeNrs mice diminished, whereas that of C57BL/6JNrs mice increased at the beginning of the active phase and remained elevated for three days after irradiation. The altered activities of all three strains of irradiated mice gradually recovered to normal within three to four days.

DNA repair capacity measured by high throughput alkaline comet assays in EBV-transformed cell lines and peripheral blood cells from cancer patients and healthy volunteers

We collected peripheral blood (PB) from 556 patients with various types of cancer who had undergone radiotherapy and from 81 healthy volunteers. We exposed whole PB and Epstein-Barr virus-transformed lymphoblastoid cell lines (EBLs) derived from the PB mononucleocytes to X-ray irradiation (5 Gy). Using the alkaline comet assay, we measured the immediate DNA damage and, at 15 min, the % residual damage. In PB, the immediate damage was similar in patients and healthy volunteers while the % residual damage (mean +/- S.D.) was significantly higher in patients with breast (54.3 +/- 23.9), cervical (54.7 +/- 23.9), head/neck (56.8 +/- 24.4), lung (60.1 +/- 23.5), or esophageal cancers (59.5 +/- 33.7) than in healthy donors (42.9 +/- 19.6) ($P < 0.05$). We did not observe such differences in the EBV-transformed cell lines. Thus, radiation sensitivity of fresh PB cells measured by the alkaline comet assay was related to cancer status.

Gene expression profile changes correlating with radioresistance in human cell lines

To identify gene expression profiles specific to radioresistance of human cells, global gene expression profiles of a total of 15 tumor and normal fibroblast cell lines were analyzed using DNA microarrays and statistical clustering methods. Initially, six of the cell lines were categorized into radioresistant (RG) or nonradioresistant (NRG) groups according to the radiation dose required to reduce their survival to 10% (D_{10}). Genes for which expression was specific to each group at 1 or 3 h after irradiation were identified using statistical procedures including analysis of variance and a two-dimensional hierarchical clustering method. The remaining nine cell lines were subjected to the k-nearest neighbor pattern classification. The nine test cell lines were successfully classified by their D_{10} value using 46 and 44 genes for which transcription levels had significantly changed at 1 and 3 h after irradiation, respectively. Of these genes, 25 showed altered expression at both time points in the NRG or RG, but independently it was not possible to classify the test cell lines. These results suggested that radioresistant cell lines showed certain radiation-induced changes in gene expression profiles that are different from the profile changes of the more-sensitive cell lines.

Potential in a single cancer cell to produce heterogeneous morphology, radiosensitivity and gene expression

Morphologically heterogeneous colonies were formed from a cultured cell line (KYSE70) established from one human esophageal carcinoma tissue. Two subclones were separated from a single clone (clone13) of KYSE70 cells. One subclone (clone13-3G) formed mainly mounding colonies and the other (clone 13-6G) formed flat, diffusive colonies. X-ray irradiation stimulated the cells to dedifferentiate from the mounding state to the flat, diffusive state. Clone 13-6G cells were more radiosensitive than the other 3 cell lines. Clustering analysis for gene expression level by oligonucleotide microarray demonstrated that in the radiosensitive clone13-6G cells, expression of genes involved in cell adhesion was upregulated, but genes involved in the response to DNA damage stimulus were downregulated. The data demonstrated that a single cancer cell had the potential to produce progeny heterogeneous in terms of morphology, radiation sensitivity and gene expression, and irradiation enhanced the dedifferentiation of cancer cells.

Radiation sensitivities of 31 human esophageal squamous cell carcinoma cell lines

The purpose of this study was to determine the radiosensitivities of 31 human oesophageal squamous cell carcinoma cell lines with a colony-formation assay. A large variation in radiosensitivity existed among 31 cell lines. Such a large variation may partly explain the poor results of radiotherapy for this cancer. One cell line (KYSE190) demonstrated an unusual radiosensitivity. Ataxia-telangiectasia-mutated (ATM) gene in these cells had five missense mutations, and ATM protein was truncated or degraded. Inability to phosphorylate Chk2 in the irradiated KYSE190 cells suggests that the ATM protein in these cells had lost its function. The dysfunctional ATM protein may be a main cause of unusual radiosensitivity of KYSE190 cells. Because the donor of these cells was not diagnosed with ataxia telangiectasia, mutations in ATM gene might have occurred during the initiation and progression of cancer. Radiosensitive cancer developed in non-hereditary diseased patients must be a good target for radiotherapy.

A fast, simple method for screening radiation susceptibility genes by RNA interference

Radiotherapy can cause unacceptable levels of damage to normal tissues in some cancer patients. To understand the molecular mechanisms underlying radiation-induced physiological responses, and to be able to predict the radiation susceptibility of normal tissues in individual patients, it is important to identify a comprehensive set of genes responsible for radiation susceptibility. We have developed a simple and rapid 96-well screening protocol using cell proliferation assays and RNA interference to identify genes associated with radiation susceptibility. We evaluated the performance of alamarBlue-, BrdU-, and sulforhodamine B-based cell proliferation assays using the 96-well format. Each proliferation assay detected the known radiation susceptibility gene, PRKDC. In a trial screen using 28 shRNA vectors, another known gene, CDKN1A, and one new radiation susceptibility gene, ATP5G3, were identified. Our results indicate that this method may be useful for large-scale screens designed to identify novel radiation susceptibility genes.

Major publications:

- 1) Shuhei Noda, Mayumi Iwakawa, Toshie Oota, Masaru Iwata, Minfu Yang, Miyako Gotou, Hiroko Tanaka, Yoshinobu Harada, Takashi Imai: Inter-strain variance in late phase of erythematous reaction or leg contracture after local irradiation among three strains of mice, *Cancer Detection and Prevention*, **29(4)**:376-82, 2005.
- 2) Mayumi Iwakawa, Miyako Gotou, Shuhei Noda, Masashi Sagara, Shigeru Yamada, Naohito Yamamoto, Yoshihiro Kawakami, Yoshifumi Matsui, Yukimasa Miyazawa, Hideya Yamazaki, Hiroshi Tsuji, Tatsuya Ohno, Junetsu Mizoe, Hirohiko Tsujii, Takashi Imai: DNA repair capacity measured by high throughput alkaline comet assays in EBV-transformed cell lines and peripheral blood cells from cancer patients and healthy volunteers. *Genetic Toxicology and Environmental Mutagenesis : A Section of Mutation Research*, **588(1)**:1-6, 2005.
- 3) Ken-ichi Ishikawa, Kumiko Saegusa, Yoshimi Otsuka, Atsuko Ishikawa, Seiko Kawai, Kaori Yasuda, Tomo Suga, Yuichi Michikawa, Masao Suzuki, Mayumi Iwakawa, Takashi Imai: Gene expression profile changes correlating with radioresistance in human cell lines. *Int. J. Radiat. Oncol. Biol. Phys.*, **65(1)**:234-245, 2006.
- 4) Sadayuki Ban, Yuichi Michikawa, Ken-ichi Ishikawa, Masashi Sagara, Koji Watanabe, Yutaka Shimada, Jouji Inazawa, Takashi Imai: Radiation sensitivities of 31 human esophageal squamous cell carcinoma cell lines. *International Journal of Experimental Pathology*, **86**: 231-240, 2005.
- 5) Atsushi Tsuji, Hitomi Sudou, Aya Sugyo, Marika Ohtuki, Makoto Miyagishi, Kazunari Taira, Takashi Imai, Yoshinobu Harada: A Fast, Simple Method for Screening Radiation Susceptibility Genes by RNA Interference. *Biochem. Biophys. Res. Commun.*, **333**: 1370-1377, 2005.