5. Research Center for Radiation Protection

Kazuo Sakai, Ph.D.
Director, Research Center for Radiation Protection

Outline of Research Career:
In 1982, Dr. Sakai obtained a Ph. D. degree majoring in biochemistry from the University of Tokyo. He worked as a Research Associate in the Department of Radiation Biophysics, Faculty of Medicine, University of Tokyo (1982-1989), and then as a Lecturer in the Department of Radiation Oncology, Graduate School of Medicine, University of Tokyo (1989-1999). The main subject of his research was radiation-induced DNA damage and its repair, and the mechanism of radiation-induced cell death. From 1983 to 1985 he worked as a research fellow in the Genetics Division, Children's Hospital, Harvard Medical School. The research subjects there were gene amplification and cloning of genes responsible for radiosensitivity. He moved to the Central Research Institute of Electric Power Industry in 1999 to research biological effects of low dose radiation. He joined NIRS in 2006.  
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Objectives:

The Research Center for Radiation Protection was newly established in 2006. The aim of the Center is to provide a scientific basis for radiation protection and safety. Toward this goal, radiation exposure from various sources is measured, the dose-effect relationships for various endpoints are examined, and the mechanisms underlying the effects are investigated. The Research Center disseminates its research results to promote public understanding of radiation effects and to encourage the enactment of more reasonable regulations concerning the use of radiation. The scope of its activity is not limited to Japan. It has been appointed a collaborating centre by the International Atomic Energy Agency.

Overview:

The Research Center consists of 4 Research Groups (Regulatory Sciences Research Group, Experimental Radiobiology for Children's Health Research Group, Radiation Effect Mechanisms Research Group, and Environmental Radiation Effects Research Group), the Nakaminato Laboratory for Radioecology, and the Department of Advanced Technologies for Radiation Protection Research.

The activities of the Research Groups and the Nakaminato Laboratory are described in the respective section of this Report.

The Department of Advanced Technologies for Radiation Protection Research consists of 4 sections.

In the Advanced Analytical Technology Section, cooperative work with other research groups from inside and outside of this institute were carried out to measure trace elements and naturally occurring radionuclides in environmental and biological samples. Also, newly developed analytical techniques to determine trace elements have been compared with conventional ones to show the accuracy of these developed methods.

The Animal Pathology Section conducted technical and diagnostic histopathological support for NIRS intramural research.

The Advanced Animal Research Section supports integrated research of molecular and genetic studies with physiological studies in whole animals. Although remarkable progress of radiation biology has been made at genetic, molecular and cellular levels, physiological analysis of whole animal models is inevitable for extrapolation to human health. The group supports radiobiological research by application of assisted reproductive technologies (ARTs) in genetically modified laboratory mice, including in vivo fertilization, embryo transfer, micromanipulation of embryos and cryopreservation. Such technologies have also become essential to efficiently conduct large-scale animal experiments by providing a large number of animals synchronously. The Animal Research Section also has supported research using Medaka fish by providing tumor-bearing fish, generating transgenic fish, and the quality control of frozen oocytes of qualified strains of Medaka fish.

The Environmental Radioactivity Survey Section initiated three collaborative studies with three universities in Japan. They involve the "Construction of Natural Radiation Exposure Study Network" from the Special Coordination Funds for the Foundation for the Promotion Science and Technology of Ministry of Education, Culture, Sports, Science and Technology. In addition, several collaborative studies were conducted with domestic and foreign institutions. This section also carried out several commissioned work utilizing its technologies and facilities.

The Research Center was designated by the International Atomic Energy Agency as a Collaborating Centre for the 2nd term (2009-2013) for Low Dose Radiation Biology.

In the Research Center 47 permanent and 57 temporary members actively conducted research during the fiscal year 2009. They produced 90 original papers and 72 reviews and proceedings. The Center held the second "KIDS Workshop: Radiation Protection for Children" in conjunction with IAEA/NIRS Workshop on "Low Dose and Medical Exposure" and WHO Global Initiative Meeting on "Towards a Safer use of Radiation in Paediatrics".

Dr. Kazuo Sakai continued to be the Director of the Research Center; Dr. Hidenori Yonehara, the Director of the Regulatory Sciences Research Group; Dr. Yoshiya Shimada, the Director of the Experimental Radiobiology for Children's Health Research Group; Dr. Mitsuru Neno, the Director of the Radiation Effect Mechanisms Research Group; and Dr. Satoshi Yoshida, the Director of the Environmental Radiation Effects Research Group. As of July 2008, Dr. Kiyomi Eguchi-Kasai was named as the Head of the Planning and Coordination Section of the Research Center to promote its activity further.
5.1. Regulatory Sciences Research for Radiation Safety and Protection

Hidenori Yonehara, Ph.D.
Director, Regulatory Sciences Research Group

Outline of Research Career
Dr. Yonehara received a Ph.D. from Shiga University of Medical Science in 1995 for his study on the issue of risk from exposure to residential radon. He joined NIRS in 1996 and began working on studies related to dose evaluation from environmental radiation. From 2003 to 2006 he worked on development of radiation safety standards as the Director for Radiation Protection Policy in the Ministry of Education, Culture, Sports, Science and Technology (MEXT). Since his return to NIRS, he has studied dose evaluation from natural radiation sources as well as issues of radiation safety regulation. Since March 2007, he has been working as Director of the Regulatory Sciences Research Group.

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Objectives

The objectives of regulatory sciences research for radiation safety and protection are to summarize scientifically based information for radiation safety regulation and to exchange this information among different stakeholders to bridge the gap between science and society. The research programs focus on the following four points.

1) Summarizing information on radiation protection issues

The group aims to summarize achievements of research projects on radiation protection provided by NIRS, as well as other research institutes to contribute to activities of relevant international organizations such as UNSCEAR and ICRP. The group also constructs a research information network on radiation protection for sharing information with scientific organizations, regulatory authorities and the public.

2) Radiation risk assessment and construction of information databases

The group constructs information databases on risk assessment for people who are exposed to low dose radiation and controllable natural radiation sources. Scientific information on radiological archives of experimental research, on the exposures and health effects of radiation among different human populations, and on effects of environmental radiation from epidemiological studies are collected for the databases.

The group carries out epidemiological studies on health effects of the exposure to natural radiation sources for the purpose of risk assessment.

3) Development of mathematical models

Using the results of basic research related to the effects of radiation on human health and the environment, the group develops mathematical models to estimate the risk from exposure to natural radiation sources, medical exposure, and the models for analysis of radiological effects on the environment.

4) Development of a method for risk communication

The group collects examples in which risk information on radiation safety would be communicated to the public, and the group analyzes methods of risk communication with sociological consideration.

Progress of Research

1) Construction of information databases for radiation risk assessment

An original database for information on exposure due to industrial use of naturally occurring radioactive materials (NORMs) has been developed previously and published on the web. The database provides a search system by which users of the materials can investigate the level of activity concentration in more than 1000 types of materials and estimate a dose when handling the materials.

The archive system for long-term animal experiment data/material was also constructed and is on a trial basis. The accumulated information associated with researchers, radiation sources, biological results, macro/microscopic observations, etc. are going to be registered in the "STORE", the international long-term animal experiment archive which is operated within the framework of EURATOM FP7.

2) Development of mathematical models

The group aims to develop two types of mathematical models for regulatory science. The first type of model is simulation modeling of carcinogenesis for the main purpose of evaluating the risk of radiation at a low dose of exposure. The second type is to evaluate the effects of ionizing radiation on environmental biota and ecosystems.

Recently, international concerns about framework for protection of non-human biota have been increasing and European and North American countries have respectively developed assessment frameworks and tools to evaluate the radiological impact on non-human biota. The last year, we applied the assessment tools developed by Europe and the U.S.A. to the environment of Japan. It was found that the assessment framework can work, although default parameters which are used in both tools were different from the Japanese environment, so that we collected Japanese concentration ratio data from the literature and compared the collected data with default values of the ERICA tool (European assessment model).

For the purpose of comparison, we focused on the transfer factor of vegetables because of the amount of data available. From a comparison of the data for transfer factor value of grass and herbs in ERICA, we found that almost all TF values in ERICA were greater than those from Japanese data (i.e. ERICA has adopted a conservative value), except for Sb and Zr. The Japanese transfer factor of Sb was within the same order of ERICA TF; however, Japanese TF of Zr was less than that in ERICA. Therefore, assessment with the ERICA default value of Zr will be an underestimation in the Japanese environment.

3) Epidemiological study

The possible effects of exposure to controllable natural radiation and medical radiation are our main research interests. We continued a case-control study of residential radon and thoron and lung cancer among cave-dwelling residents in Gansu Province, China, in cooperation with researchers inside and outside NIRS. A total of 103 cases and 200 controls have been entered in the study so far, and 1-year measurements for radon, thoron and their decay products will be soon completed for all subjects. Preliminary analysis of the interview
data from questionnaires showed an increased risk of lung cancer in relation to smoking which should be adjusted in the main analysis. Data on the detailed measurements were also analyzed, showing temporal variations according to dwelling type which should be taken into account for better assessment of exposure to radon and its decay products in epidemiological studies of residential radon.

We also continued a meta-analysis of second cancer risk among childhood cancer survivors treated with radiotherapy to quantitatively evaluate the possible effects of medical exposure. In the meta-analysis, we have developed a methodology to estimate an excess relative risk from individual studies in which only category-specific risk estimates were available. The number of eligible studies has increased more than two-fold, and detailed analysis is ongoing.

4) Investigation into justification of medical radiological procedures.

The surveys of national and international circumstances for judgement on whether radiation diagnostic procedures would be justifiable were investigated. The guidelines to determine the most appropriate diagnostic imaging examinations and to reduce unnecessary exposure of patients to radiation based on the available evidence have been well equipped in the UK and the USA. Few programs of undergraduate medical education have been achieved in order to choose the most appropriate imaging investigation or intervention for their patients in each Japanese medical college so far. Also few practical tools have been equipped for risk communication in hospitals regarding each radiodiagnostic examination and radiation exposure among medical doctors, radiological technicians and patients.

Results of survey on risk perception done in FY 2007 were analyzed using risk ranking techniques. The survey had been conducted in all parts of Japan using web-based questionnaires and 658 responses were obtained. Subjects were asked to rank 30 items of various types of technologies and human activities according to their subjective judgments on the order of perceived magnitude of risk. Irrespective of sex, age, occupation and academic major, all groups examined perceived handguns, nuclear power and cigarettes as having the highest risk, while X-ray exposure was perceived as a moderate risk. Respondents tended to believe the information from TV more than that from public organizations. We also interviewed researchers within the NIRS. The NIRS researchers perceived nuclear power as less risky and bicycles and motor vehicles as more risky compared with the perception of the general public.

5) Dialogue seminars for risk communications among stakeholders

A series of meetings called "Dialog Seminars" on themes of optimization of radiation in medicine for children of radiodiagnostics exposure and radioactive waste was held to communicate information on risk among scientists, persons in regulatory authorities, those in relevant companies, mass communicators and the public. In the seminars regarding optimization of radiodiagnostic exposure, international trends, the present circumstances and experimental and epidemiological data of risk assessments, the present regulatory circumstances and problems in clinical fields issues related to protection of medical exposure were discussed among medical doctors, radiological technicians, experts for radioprotection and regulators. In the seminar regarding radioactive waste, fundamental information on high-level waste and radiation health effects was provided by experts to the public, and issues related to radiation waste were discussed among stakeholders. To clarify the factors on risk perception and acceptance of nuclear fuel cycle, the record of the discussion has been analyzed in terms of message analysis.

Major publications
2. Francois BRECHIGNAC, Masahiro Doi: Challenging the current strategy of radiological protection of the environment: arguments for an ecosystem approach, Journal of Environmental Radioactivity, 100 (12), 1125-1134, 2009
Yoshiya Shimada, Ph.D.
Director, Experimental Radiobiology for Children's Health Research Group

Outline of Research Career
Dr. Shimada received a Ph.D. in 1985 from the University of Tokyo. In the Mizuno Biohoronics Project of JST (1985-1987) and at the Tokyo Metropolitan Institute of Gerontology (1987-1989), he worked on innate immunity in carcinogenesis and aging, respectively. Since 1989 at NIRS, he has focused on molecular and cellular mechanisms of radiation carcinogenesis from the viewpoint of a combined effect of environmental carcinogens and the age-at-exposure effect.

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Photo: Shimada (front right)
Objectives

With the advent of an era of low birthrate and prolonged longevity, concern about the safety of fetuses and children has been growing. Programs to protect the health of fetuses and children and the safety of the environment are being instituted, particularly in the USA and Europe. These regulations are mainly directed at foodstuffs and chemicals. Recently, a progressive increase in medical uses of radiation for children has forced ICRP, IAEA and WHO to draft global initiatives on radiation protection of children. This group carries out studies to provide information on the risk of carcinogenesis due to radiation exposure during fetal and childhood periods, for which there are at present insufficient data. Using animal models, we study the effects of radiation exposure on cancer induction and lifespan shortening. Final goals of this research group are to propose age-weighting factors and relative biological effectiveness (RBE) of neutrons and heavy ions for fetuses and children for radiation protection.

Progress of Research
1) Age dependence of lifespan shortening by irradiation in B6C3Fl mice

Fifty female and male B6C3Fl mice per group, which have been used in a wide variety of toxicological studies such as the National Toxicology Program (NTP) in the USA, were exposed to gamma rays (60Co), carbon ions (energy, 290 MeV/n; LET, 13 keV/μm) and neutrons (energy, 2 MeV) at various ages during fetal to mature adulthood periods. The ages examined were pre-implantation (3 days post-conception (dpc)), major organogenesis (13 dpc), late fetal (17 dpc), neonatal (1 week after birth), prepubertal (3 weeks), postpubertal (7 weeks) and mature adulthood stages (15 weeks).

The doses ranged between 0.2 and 4 Gy for gamma rays, 0.2 and 2 Gy for carbon ions, and 0.05 and 1 Gy for neutrons. These mice are now being kept under observation other wise autopsied at moribundity or soon after death. The result of the experiment for gamma-ray exposure at the adult stage indicated that female mice appeared more susceptible to radiation-induced lifespan shortening than male mice. Male mice at the neonatal stage came to be more sensitive than those at the adult stage. Surprisingly, irradiation at the late fetal stage had little influence on lifespan shortening for both genders. Irradiation with carbon ions at the adult stage shortened the lifespan to a similar extent as that with gamma-rays. Carbon ions were more potent, however, in reducing lifespan than gamma rays when fetal and neonatal mice were exposed. These results suggest a larger relative biological effectiveness (RBE) of carbon ions for fetus and infants.

2) Age dependence of cancer risks in mammary gland, lung, bone marrow, liver, kidney, brain and intestine

Radiation risks are dependent upon both tissue types and the age at exposure. Breast is one of the most susceptible organs to radiation-associated cancer. We have been using the Sprague-Dawley (SD) rat mammary cancer model to investigate the age effect of Cs-137 gamma rays or carbon ions (13 keV/μm) on breast cancer risk. In FY2009, pathological diagnosis for 250 rats and autopsy of 380 rats were performed. Tentative data suggest that gamma irradiation of prepubertal rats at 1 Gy resulted in smaller diminishment of the ovarian follicular pool and greater effectiveness on mammary cancer induction than irradiation at 2 Gy. A neutron irradiation experiment was also commenced, where the dose range was determined to be 0.05-0.5 Gy based on past literature. Genomic DNA analysis of gamma-ray-induced mammary cancers of SD × COP hybrid rats indicated that copy number aberrations were high on chromosomes 2, 3 and 5 at a frequent of more than 30%.

The lung is one of the important organs for radiological protection of workers and the public because of its high radiation-associated cancer risk. The incidence of radiation-induced lung tumors was compared in 1, 5 and 15 week-old female Wistar rats (total 760 animals in total) following thoracic X-ray irradiations (0, 1, 3 and 5 Gy). Lung tumor induction increased in a dose-dependent manner, but the dose-effect relationship did not change much depending on the age at irradiation.

The effect of age on tumor development of kidney, brain (medulloblastoma), intestine, liver and lymphoid organ (thymus) was also examined using mutant and genetically engineered animals as well as B6C3Fl mice. Perinatal and infant stages were the most sensitive to the development of kidney and brain tumors in Eker rats and Ptk1+/− mice, respectively. Brain tumors developed in a dose-dependent fashion, showing considerable effects even at a low dose of 0.1 Gy. Late embryonic stages were also sensitive to radiation-induced brain tumorogenesis. We confirmed that radiation-induced brain tumors in Ptk1+/− mice had wild-type Ptk1 loss caused by interstitial chromosomal deletions, which was characteristic of radiation-induced tumors. This enabled us to distinguish radiation-induced and spontaneous tumors and consequently led to the finding of brain tumor induction even at a low dose, as low as 50 mGy. We also found that irradiation at the infantile stage induced more intestinal tumors in Apet1+/− mice than at the adult stage, and the second hit event was, again, intra-chromosomal deletions in tumors of mice irradiated. Crypt cells in infant colon were more resistant to apoptosis than those of adult.
intestines, which may account for the age difference in the susceptibility to tumorigenesis. The incidence of T-cell lymphomas in B6C3F1 and Mlh1−/− mice exposed at 17 dpc, 2 or 10 week of age was examined. Infant mice were the most susceptible to radiation, but 17-dpc mice were unexpectedly resistant. In the tumors from Mlh1−/− mice, frequent frameshift mutations at mononucleotide repeat sequences in Ihh mouse gene were observed, which resulted in the loss of protein.

3) Combined effect of radiation and chemical carcinogens on lung and intestinal tumorigenesis

The age effect of combined exposure to radiation and chemicals has been investigated on pulmonary and intestinal carcinogenesis. For lung tumors, the thoracic region of female Wistar rats was irradiated with X rays (3 Gy) at neonatal (1 week of age), pubertal (5 weeks) or adult (22 weeks) stages, and then N-nitrosober (2-hydroxyspropyl) amine (BHP) was intraperitoneally injected one week after irradiation. Synergistic effects of the X rays and BHP were found in rats exposed at pubertal and adult stages, and the synergism was more effective at the pubertal stage. In Mlh1−/− mice, intestinal tumors were induced by combined exposure to X rays (2 Gy) and dextran sodium sulfate. In male but not female mice, the incidence of tumors increased in a supra-additive fashion. There was no significant age difference in the susceptibility to tumor induction.

4) Detrimental effect of uranium on the developing kidney

The health effects on children in depleted uranium-polluted areas and uranium mining areas are of recent concern. Uranium and its compounds have the potential to cause nephrotoxicity. We found that the dynamics of uranium deposit and adenocarcinoma in kidney differed between 1 week and 10 weeks of age in rats: uranium concentration was lower, and elimination was delayed in neonates than in adults. This was because the volume of S3 segments of the proximal tubules, which are the selective site of uranium accumulation and induction of apoptosis, was quite small in neonates. Moreover, rapidly growing S3 segments during the infant period re-accumulated uranium, which was released from somewhere in the body, thereby resulting in persistent apoptotic figures observed up to 3 weeks of age. Experimental groups for the late effect of uranium are being set up.

5) Mutation induction in the Aprt locus

In order to determine the age-dependence of mutation induction, Aprt−/− mice at 1 or 7 weeks of age were exposed to a single dose at 1 or 4 Gy, or four fractions at 1 Gy of X rays, as well as to a single dose of 0.25 or 1 Gy, or four fractions at 0.25 Gy of neutrons. Observations suggest that exposure at a younger age with the higher dose resulted in more Aprt−/− mutations in cultured kidney cells derived from the exposed mice. Fractionated exposure of X rays did not show any mutation inductions; however, fractionated exposure of neutrons enhanced the effect of irradiation.

Major publications

5.3. Studies on Radiation Effect Mechanisms

Mitsuru Nenoi, Ph.D.
Director, Radiation Effect Mechanisms Research Group

Outline of Research Career
Dr. Nenoi received a Ph.D from Kyoto University in 1992 for his study on induced accumulation of polyubiquitin gene transcripts after UV-irradiation and TPA-treatment. His research interest is mechanisms of gene transcription after exposure to DNA damaging agents.
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Objectives

Estimation of the low-dose radiation risk has been made using the high-dose data from atomic bomb survivors at Hiroshima and Nagasaki under the assumption that the risk is proportional to the radiation dose without a threshold. However, we do not have the scientific evidence to necessarily support this assumption. We do not have sufficient scientific data on the effects of low-dose radiation on developmental and differentiation anomalies either. Because it is now considered to be difficult to assess the risk of low-dose radiation from animal experiments or in epidemiological data, this research group conducts studies on the mechanism of radiation effects caused by low-dose radiation. The purpose of this research group is to derive findings useful in the risk assessment of low-dose radiation that can be used as a basis for the development of an appropriate regulatory framework. The following study items are investigated respectively by the four teams.

1) Radiation Carcinogenesis Research Team: Evaluation of indirect effects of low-dose radiation on carcinogenesis (carcinogenesis due to changes in the microenvironment caused by irradiation) and examination of the involvement of DNA repair mechanisms in low-dose radiation-induced carcinogenesis.

2) DNA Repair-Gene Research Team: Clarification of low-dose radiation risk-modifying factors in nonhomologous end-joining DNA-repair and its molecular mechanism.

3) Developmental and Differentiation Anomaly Research Team: Verification of the validity of radiation regulations relating to developmental and differentiation anomaly by evaluating the effects of low-dose radiation on abnormalities in neural crest cell differentiation.

4) Radiobiological Response Research Team: Determination of risk-modifying factors specific to low-dose radiation by identifying genes associated with biological responses to low-dose radiation, including radiobiological responses and signal transduction.

Progress of Research

1) Radiation Carcinogenesis Research Team

Radiation risk of cancer induction has been evaluated based on direct effects of radiation on irradiated cells. It is known that radiation causes cancer through two types of damage: DNA damage directly induced in target cells and radiation-induced change of a microenvironment. The contribution of the latter untargeted carcinogenesis to radiation-induced cancer risk has not been evaluated. To elucidate its contribution to radiation risk, we have established a thymus transplantation system for assessment of untargeted effects of radiation on carcinogenesis. When thymuses of unirradiated new-born wild type mice were transplanted in thymectomized, irradiated scid mice, T-cell lymphomas of transplanted thymus origin were induced at 0.1 or 0.2 Gy, depending on the transplantation sites (subcutaneous or under the kidney capsule). The results indicate that low doses of γ-rays induce untargeted lymphomagenesis in a Prkdc-deficient condition. Bone marrow transplantation prevented this untargeted carcinogenesis by supplying progenitor T cells into transplanted atrophic thymuses and relieving them from radiation-induced thymic hypoplasia, which demonstrated a relationship between induction of untargeted lymphomagenesis and thymic hypoplasia. We also determined whether Notch1, one of the major oncogenes related to lymphomagenesis, was rearranged in the 5' region of the locus in untargeted lymphomas. Notch1 was interstitially deleted in untargeted lymphomas at a frequency similar to that in lymphomas induced by whole-body irradiation. Furthermore, Notch1 was deleted in untargeted lymphomas through mechanisms similar to those in lymphomas induced by whole-body irradiation. These results suggest that the development of radiation-induced untargeted lymphomas share the same mechanisms with those in lymphomas induced by whole-body irradiation.

2) DNA Repair-Gene Research Team

DNA double strand breaks (DSBs) are highly cytotoxic lesions that are generated by ionizing radiation (IR), various DNA-damaging chemicals and DNA replication itself. Failure to repair DSBs, or their misrepair, may result in cell death or chromosomal rearrangements, including deletions and translocations. This chromosomal instability can promote carcinogenesis and accelerate aging. The repair of DSBs is indispensable for genomic integrity. Cells, therefore, have invested in at least two pathways to repair DSBs, namely homologous recombination repair (HRR) and non-homologous end-joining (NHEJ). In higher organisms, NHEJ can function in all phases of the cell cycle and is the predominant repair pathway. Our chief aim is, in this context, to clarify the induction-mechanism of mutation by radiation. In particular, identification of the modulatory factor (s) for a low-dose radiation-risk in NHEJ and the elucidation of the molecular mechanism (s) involved with those factor (s) are the focus of our interest. Up to the present, we have established three cell lines having \(\text{ATRC4}, \text{Artemis and MDC1} \) (mediator of DNA damage checkpoint) disrupted, respectively, by a gene targeting technique in a human colon tumor cell line HCT116 to define the biological roles of NHEJ-related...
genes on DNA damage induced by IR. We then demonstrated higher sensitivities of these three knockout cell lines to IR and various chemical reagents that induce different types of DNA damages by a survival assay in comparison with parental HCT116 cells. Frequencies of chromosomal aberration induced by IR were also significantly higher in all deficient cell lines than that in the parental cells. In addition, we showed that MDC1 closely correlates with regulation of the phosphorylation, at least, of ATM and DNA-PKcs after IR.

In the current study, we determined that frequency of the HPRT gene mutation induced by X-rays (0.5-2 Gy) was significantly increased in a dose-dependent manner in MDC1−/− cells, whereas the induction of mutation beyond the basal level was not observed in parental cells up to 2 Gy of X-rays. This radiation-induced mutagenic phenotype of MDC1−/− cells is consistent with previous findings in survival rate and chromosomal aberration assays. Subsequently, these findings suggest that MDC1 plays an important role in DNA damage signaling/repair machinery in human cell lines.

Meanwhile, we analyzed gene expression by use of a DNA micro-array technique to find genes influenced by low-dose radiation in MDC1−/− cells as well as in parental HCT116. Enhancement of expression levels of genes coding for factors related to DNA replication, cell cycle and DNA repair was exhibited in MDC1−/− cells compared with parental HCT116 cells under normal culture conditions, while the expression levels of genes related to translation and protein folding were suppressed. Interestingly, we found that MDC1 is associated with the expression of genes coding for factors which function in pathways of aging and circadian rhythms. In any event, MDC1 may regulate many aspects of DNA damage response pathways, and may be associated with stabilizing the interactions and retention of NHEJ components at the site of DSDs. We are currently working on validation of the expression profiles of the genes mentioned above, and investigating the influence of X-ray irradiation on gene expression profiles in MDC1−/− cells.

3) Developmental Anomalies Research Team

To elucidate the mechanism of the effects of low dose radiations on the development of mice as well as neural crest-derived cells, melanocytes at the cellular level, pregnant females of C57BL/10J mice at 9 days of gestation were whole-body irradiated with a single acute dose of γ-rays (0.1, 0.25, 0.5, and 0.75 Gy). The effect was studied by scoring changes in the postnatal and prenatal development of mice as well as cutaneous costs 22 days after birth and in the melanocyte development in the prenatal epidermis and hair follicles. The percentage of live birth and body weight at day 22 were not affected by the irradiation, whereas the survival to day 22 was significantly decreased in mice irradiated with 0.75 Gy γ-rays. The frequency and size of white spots (white hair skin devoid of melanoblasts and melanocytes) in the midventrum increased in irradiated mice in a dose-dependent manner. In 18-day-old embryos, the frequency of abnormalities in tails and eyes as well as of hemorrhage increased as dose increased. In contrast, the number and body weight of embryos were not affected by the irradiation (0.1 to 0.75 Gy). The numbers of melanoblasts and melanocytes in the epidermis and hair follicles also decreased in a dose-dependent manner. The numbers decreased significantly even in mice irradiated with 0.1 Gy γ-rays. These results suggest that γ-rays seem to have a great effect on post- and prenatal development of mice as well as on melanocyte development.

4) Radioadaptive response research team

Exposure of low doses of ionizing radiation can induce protective mechanisms against a subsequent higher dose of irradiation. This phenomenon, called radiation-induced adaptive response (AR), has been described in a wide range of biological models. We previously demonstrated the existence of AR in mice during late organogenesis. In the present study, induction of AR by priming X-rays in combination with challenging irradiations from high LET accelerated heavy ions (HI) in C57BL/6J mice was examined, using 30-day survival after challenging irradiations as an index. Three kinds of accelerated HI from mono beams of carbon, silicon and iron with LET values of about 15, 55, and 200 keV/m, respectively were examined. The priming low dose of X-rays at 0.50 Gy significantly reduced mortality from the high challenging dose of carbon or silicon particles, but not from iron particles. These results indicate that AR could be induced by priming low LET X-rays in combination with subsequent challenging high LET irradiations from certain kinds of accelerated heavy ions, and successful induction of AR would be a possible event relating to the LET value or the HI particle of the challenging irradiations. We here demonstrated the existence of AR induced by low LET X-rays against high LET irradiations at the whole body level in a mouse model for the first time. These findings would provide new insight into studies on radiation-induced AR in vivo.

Major Publications

1. Hirobe, T., Shinpo, T., Higuchi, K. and Sano, T. Life cycle of human melanocytes is regulated by endothelin-1 and stem cell factor in synergy with

2. Hirobe, T. Ferrous Ferric Chloride Stimulates the Proliferation of Human Skin Keratinocytes, Melanocytes, and Fibroblasts in Culture, Journal of Health Science (Tokyo, Japan), 55, 447-455, 2009


5.4. Studies on Environmental Radiation Effects

Satoshi Yoshida, Ph.D.
Director, Environmental Radiation Effects Research Group

Outline of Research Career
Dr. Yoshida received a BE in safety engineering from Yokohama National University in 1983 and a ME and Ph.D. in environmental chemistry in 1985 and 1989, respectively, from Tokyo Institute of Technology. He joined NIRS in 1989. His main research interests are radioecology, environmental chemistry, and ecotoxicology.

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Photo: Yoshida (right)
**Objectives**

The recent rapid changes in energy production systems and life styles of people worldwide have made environmental radiation research even more important. In order to satisfy the needs for radiation safety and regulations, this research group aims to investigate three subjects related to environmental radiation and radioactivity: 1) effects of radiation on organisms and ecosystems; 2) exposure of the public to natural radiation; and 3) marine dynamics of important radionuclides. The group consists of five research teams: Terrestrial Radiation Ecotoxicology Research Team, Aquatic Radiation Ecotoxicology Research Team, Natural Radiation Exposure Research Team, Cosmic Radiation Exposure Research Team, and Marine Radioecology Research Team. The following describes the progress of each of these teams during FY 2009.

**Progress of Research**

1) **Effects on organisms and ecosystems**

While the importance of radiological protection of the environment based on scientific principles is increasingly recognized internationally as environmental issues garner more attention, the relevant scientific data are extremely limited. This group conducts studies to evaluate the effects of radiation on representative terrestrial and aquatic organisms as well as studies to estimate radiation doses on those environmental organisms. In addition, the group develops methods to evaluate the ecological effects of radiation using experimental model ecosystems containing various species.

**Terrestrial Radiation Ecotoxicology Research Team**

To understand the impact of radiation on terrestrial ecosystems, plants, fungi, earthworms and springtails were selected, and the dose-effect relationships for radiation have been studied. In order to detect radiation responsive genes, a novel technology, high-coverage expression profiling (HiCEP), has been applied. Among many transcript-derived fragments (TDFs) up-regulated by irradiation, poly (ADP-ribose) polymerase gene was identified as a sensitive radiation responsive gene in several animals and plants, i.e., springtail (Folsomia candida), a model plant (Arabidopsis thaliana) and an earthworm (Enchytraeus japonicus).

Since the biological effects of long-term irradiation have more relevance to studies in radiation ecotoxicology, the team continued a study on chronic exposure. The model plant, *A. thaliana*, exposed to gamma rays for 2 weeks at a dose rate of 20 Gy/day was assessed for metabolic analyses. Among 125 compounds identified in metabolic profiling of the plant, 30 showed significant elevation in the levels following irradiation. The elevations were not only observed in primary metabolites in such processes as the TCA cycle, amino acid metabolism and sugar metabolism, but also notably in intermediates of secondary metabolism. This suggested that the metabolic balance had changed by irradiation, which probably caused successive growth reduction in the plant.

The effects of high LET radiation must be also considered because of the presence of alpha and beta emitters in the environment as well as gamma emitters. Based on the idea that an exposure study of environmental organisms to heavy ions at NIRS-HIMAC might provide valuable information to judge whether or not the radiation weighting factors defined in human radiation protection could be applied to the other environmental organisms, we have studied the effects of heavy ions at NIRS-HIMAC on the growth of *E. japonicus*. Earthworms were exposed to C, Ne, Si, Ar or Fe ion with energy of 290, 400, 490, 500 and 500 MeV, respectively. Heavy ions clearly showed stronger effects than gamma rays with respect to the growth inhibition of the earthworm. As LET was increased, heavy ions appeared to inhibit growth more effectively; however, the effects of Si, Ar and Fe ion were not significantly different.

**Aquatic Radiation Ecotoxicology Research Team**

Radiation effects on aquatic ecosystems at various endpoints were investigated in some selected organisms and experimental model ecosystems.

We have been trying to generate medaka (*Oryzias latipes*) strains that have mutations in some DNA repair genes by the targeting induced local lesions in genome (TILLING) system, which includes random mutagenesis, followed by screening for induced mutations in target genes. We could obtain a homo mutant that has a point mutation in a conserved region of gadd45a, which is involved in cell cycle arrest and DNA double strand break repair. Radiosensitivity of this mutant will be examined.

Genome-wide gene expression was examined in acutely γ-irradiated green alga *Pseudokirchneriella subcapitata*, which is one of the species most commonly used for ecotoxicity evaluation of chemicals but for which genomic sequence information is lacking. Approximately 7,000 expressed genes were detected by HiCEP, which is based on an amplified fragment length polymorphism (AFLP) and thus requires no sequence information for analysis. Expression levels of approximately 800-900 genes were affected at 100 to 300 Gy. Nucleotide sequences of 41 up-regulated genes were determined. The quantitative reverse transcription polymerase chain reaction (qRT-PCR) validated the up-regulation. Two genes had homology to some DNA repair genes. One resembled DEAD/DEAH box helicase genes, and the other resembled
SNF2/RAD54 family genes and rad26. Further characterization of the affected genes will contribute to finding biomarkers for detection of radiation effects and elucidating molecular mechanisms of radiation responses.

In our previous studies, bacterial community structure in the flooded paddy soil microcosm had been affected by chronic γ-irradiation at a dose rate of 1.2 Gy/day for 5 days. This year, the bacterial species composition of the pre-irradiated soils was comprehensively clarified by 16S rDNA clone library analysis and bacterial species affected by irradiation were isolated from the microcosm. Effects of chronic γ-irradiation on fungus communities were also examined in this microcosm by denaturant gradient gel electrophoresis (DGGE) of 18S rDNA. Differences were not observed in DGGE band profiles between control and irradiated microcosms, suggesting that fungus communities are less radiosensitive than bacterial communities.

2) Exposure to natural radiation

Since natural radioactive substances and cosmic radiation at high altitudes contribute greatly to the radiation dose received by the general public, it is necessary to quantify the actual level of exposure and to document its features. The group therefore investigates the concentration and exposure doses of radon (222Rn), thoron (220Rn), and related radionuclides, mainly in areas with high natural radiation, and analyzes the results together with epidemiological data. The group also aims to collect scientific information on dose and effects of cosmic radiation in aircraft and to provide them in an intelligible and easy to access way for the general public such as on the Internet.

Natural Radiation Exposure Research Team

Recent epidemiological studies indicated that lung cancer risk significantly increases due to exposure to relatively low-level residential radon (100 Bq/m³). We are conducting an epidemiological study in China, cooperating with the Radiation Epidemiology Team of the Regulatory Sciences Research Group. Passive radon detectors developed by NIRS are used for this study. Measurements with passive detectors are conducted as follows: (1) a large number of passive detectors are assembled at NIRS, (2) these detectors are sent to China by post, (3) Chinese collaborators place them in dwellings selected in studied areas, (4) they are retrieved after six months of exposure, (5) the exposed detectors are sent back to NIRS, and (6) they are processed at NIRS and radon concentration for each dwelling is estimated. This series of work was conducted twice in 2009 and the estimated radon concentrations will be used for an epidemiological study.

We are also investigating potential exposure due to natural radionuclides contained in building materials. Building material samples were collected for this purpose and concentrations of natural radionuclides such as radium and uranium were estimated using a high purity germanium detector (HPGe) and inductively coupled plasma - mass spectrometry (ICP-MS). Exposure due to these radionuclides was estimated assuming typical environmental parameters.

Cosmic Radiation Exposure Research Team

More than 16 million Japanese people go abroad every year using aircraft and about 20 thousand persons are working as crew on aircraft of Japanese airline companies. At aviation altitudes, they are exposed to enhanced cosmic radiation of which the annual personal dose generally exceeds 1 mSv. However, the situation and health effects of cosmic radiation exposure are still uncertain. The team therefore makes efforts to collect scientific information on dose and effects of cosmic radiation and also to provide them in an easy-to-understand way by the general public. Major tasks are (1) calculation of aviation route doses (effective doses received in aircraft) using the most up-to-date method, (2) development of new detectors to verify calculations in aircraft, and (3) improvement of a comprehensive system for radiation protection dosimetry of aircraft crew. Some research outputs of the team are open to the public as a web program entitled "Japanese Internet System for Calculation of Aviation Route Doses (JISCARD) " on the NIRS home page. In FY 2009, we summarized the results of in-flight measurements that were performed for verification of the simulation code developed for aviation dose calculation. In the measurements, we employed advanced instruments such as an extended energy rem meter and a Bonner-ball neutron detector (BBND); the BBND was borrowed from the Japan Aerospace Exploration Agency (JAXA). Satisfactory precision was found in the results. A real-time monitoring system of cosmic-ray neutrons in the upper atmosphere has been constructed at the summit of Mt. Fuji. We also continue to cooperate with airline companies in Japan, regarding management of radiation exposure for aircraft crew.

3) Marine dynamics of important radionuclides

Because many Japanese nuclear facilities are located in coastal areas facing the Pacific Ocean and the Japan Sea, it is very important to predict the environmental behavior, and thus the fate of radionuclides in marine ecosystems. The group focuses on the development of highly sensitive analytical methods for important radionuclides (e.g., plutonium, americium, iodine, etc.) for which data are scarce, and provides data on
their activities and isotopic ratios to understand their environmental behavior in marine ecosystems.

**Marine Radiocology Research Team**

The chemical form is one of the most important factors controlling iodine environmental behavior in the ocean. The actual mechanisms responsible for iodate reduction and iodide oxidation, however, have yet to be fully elucidated due largely to the lack of sensitive iodine speciation analytical methods. In addition, the knowledge of biogeochemical cycling of stable iodine can be useful for the safety assessment of radioactive iodine which is released from nuclear facilities. We have developed a sensitive hyphenation technique, HPLC-ICP-MS for the speciation of stable iodine in seawater. The vertical distributions of total iodine, iodate and iodide in coastal seawater off Aomori, Japan were investigated using the developed method. The concentration of total iodine increased with depth down to 700 m. On the contrary, iodide decreased with depth from 12 ng/ml in the surface seawater to 1.2 ng/ml in the bottom layer at 700 m. The highest concentration of iodide was found in the surface water, suggesting the reduction of iodate due to high biological productivity in the surface water.

To deal with the problem of global warming, a rapid growth in nuclear power generation is expected in East Asia. Prior to this expected growth, it is important to study the behavior of plutonium (Pu) isotopes in coastal seas of East Asia. Seawater samples were collected in the East China Sea and Yellow Sea, and their $^{238}$Pu/$^{239}$Pu atom ratios were determined by sector-field ICP-MS. The atom ratios of $^{239}$Pu/$^{239}$Pu for surface and bottom water on the East China Sea continental shelf had no significant difference, ranging from 0.222±0.011 to 0.246±0.019. The atom ratios in the Yellow Sea were 0.199±0.013 for surface water and 0.211±0.017 for bottom water and were slightly lower than those in the East China Sea. The atom ratios in Chinese coastal seawaters were significantly higher than the mean global fallout ratio of 0.18. We proposed that Pu isotopes were transported from the open ocean to the East China Sea by oceanic currents and removed to the sediment column by enhanced scavenging from the water column by high particle fluxes in the Changjiang Estuary. Data on $^{238}$Pu/$^{239}$Pu atom ratios will provide useful keys for presenting the background data of $^{239}$Pu/$^{239}$Pu atom ratio before the expected expansion of nuclear power capacity in East and South Asian countries and for distinguishing potential sources of Pu in the future.

**Major publications**

5.5. Office of Biospheric Assessment for Waste Disposal

Shigeo Uchida, Ph.D.
Head, Biospheric Assessment for Waste Disposal

Outline of Research Career
Dr. S. Uchida received his doctor degree from Kyoto University. He has about 30 years' experience in the fields of radioecology and environmental radiochemistry, and, especially, his interest is the behaviors of long-lived radionuclides in the environment, e.g., $^{60}$Ni, $^{75}$Se, $^{89}$Sr, $^{99}$Tc, $^{129}$I, $^{137}$Cs, Th, U, etc. He has improved models and parameters for radionuclides in soil-to-crop systems. He has been proceeding a project to collect and estimate environmental transfer parameters of radionuclides in relation to radioactive waste management. 

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Photo: Uchida (front right)
Objectives

The biospheric assessment of radiation dose to human beings related to the releases of long-lived radionuclides from underground nuclear waste disposal sites is very important for the peaceful use of atomic energy. For the assessment, radiocological transfer models and transfer parameters are needed. Environmental conditions, such as climate, vegetation and soil, affect these parameters. Additionally, agricultural products and food customs in Japan differ from those in Europe and North America. Therefore, we should have our own practical data in Japan using data from European and North American countries as references.

In this office, environmental transfer parameters, such as soil-to-plant transfer factors (TFs) and soil-soil solution distribution coefficients (Kds), have been collected from agricultural fields throughout Japan. Recently, we have also been measured parameters to clarify radon emission mechanisms from soil, as well as to understand the fate of elements in coastal areas in Japan. Analyses of stable isotopes and some natural radioisotopes in soil and the edible part of crop sets, and coastal water and seafood sets have been carried out in order to obtain TFs / concentration factors under equilibrium conditions, while radionuclide experiments have been applied for Kds in various soils. For the case of ¹⁴C transfer parameters, radionuclide experiments were carried out to obtain TFs and ¹⁴C distribution in soil. In addition, transfer models for predicting the behavior of radionuclides in atmosphere-paddy soil-nice plant systems have been developed.

Progress of Research

1) Radionuclide behavior in Japanese estuarine areas

For estuarine systems, we made a report on sediment-water distribution coefficients (Kds) observed in four estuarine areas, that is, Mabuchi River (off Aomori), Mogami River (off Yamagata), Yura River (off Toyoto), and Kuma River (off Kumamoto). The total concentrations of stable elements and naturally occurring radionuclides (i.e., Na, Mg, K, Ca, V, Mn, Fe, Co, Ni, Cu, Rh, Sr, Y, Mo, Cd, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu, Pb, and U) in the estuarine water at each sampling point and in the corresponding sediment sample were measured since stable elements can be used as analogues of radionuclides. The results showed that the Kds of most of the elements varied within one order of magnitude regarding their differences between minimum and maximum values of each element at all the stations. However, a wide variation of Kds of Mn, Fe, Co, Cu, Rh, and La was observed. In addition, geometric means (GMs) of observed Kds were compared with the recommended values in the IAEA Technical Report Series 422 (TRS-422). The results showed that GMs of Kds for most of the elements agreed well with the recommended values, but GMs of Kds for Mn, Fe, and Cd were more than 10 times lower than the recommended values. The obtained Kd values could be important to investigate the behavior, transport, and fate of artificial radionuclides and to assess the radiological doses in estuarine areas. In order to provide more generic Kd data, we have been collecting samples from other estuarine areas.

2) Carbon-14 mobility in agricultural soil systems

Among the truranic (TRU) waste-related radionuclides, ¹⁴C in organic forms is important for dose assessment. Because there is little information regarding reliable migration and realistic transport models for such organic ¹⁴C, the possible migration of organic ¹⁴C from a TRU repository sited to the biosphere through groundwater presents some concern, especially in soil systems. This year, therefore, the partitioning ratios of ¹⁴C in solid, liquid, and gas phases were determined by batch sorption tests using 97 healthy soil samples. Each of the soil samples was suspended in deionized water containing ¹⁴C sodium acetate, one of the possible organic ¹⁴C forms from TRU waste, and shake-incubated for 7 days. More than 65% of the spiked ¹⁴C was released into the air, approximately 30% was partitioned into the solid phase, and the ¹⁴C remaining in the liquid phase was only a few percent. These results suggested that if the ¹⁴C incorporated into acetate migrated from a TRU repository site to paddy fields, most of the ¹⁴C would be released into the air and the rest would be partitioned into the soil phase. It is likely that microorganisms in the soils are responsible for these partitioning ratios because about 97% of the spiked ¹⁴C remained in the liquid phase in the microorganism-depleted sample.

3) Estimation methods for environmental parameters

Estimation models for soil-to-plant transfer factors (TFs) of some elements and radionuclides were developed using several crop and soil characteristics; one of them is the TF of ²²²Rn (TF-Ra). The radionuclide should be assessed to determine the safety of geological disposal of high-level radioactive and TRU wastes. However, reported TF data for ²²²Rn are still limited due to the low concentration of ²²²Rn in plants in the natural environment. Thus, we collected TF-Ra for crops and then applied a statistical approach to estimate TF-Ra instead of directly measuring the radionuclide. Since TF is defined as the plant/soil concentration ratio, concentrations of ²²²Rn in soil and crops were estimated separately. Among the various soil characteristics, that
is, water content, pH (H₂O), and 58 elemental concentrations, we found the highest correlation between concentrations of log (²²⁶Ra) and log (U) in soils with a high correlation factor, R=0.82 (p<0.001), possibly because ²²⁶Ra is a progeny in the ²³⁸U series. We also found a high correlation between concentrations of log(²²⁶Ra) and log(Ba) in plants with R=0.89 (p<0.001) because they could be chemically similar in plant uptake. Using concentrations of U in soil and Ba in plant, we could estimate TF-Ra with good accuracy as shown in Fig. 5-1. The difference between estimated and measured TF-Ra values was a factor of 1.2 on average for crops. The method could estimate TF-Ra for the soil-to-plant systems; however, ²²⁶Ra concentration in plants may increase linearly with increasing ²²⁶Ra concentration in soil. Thus, the soil-plant systems should be considered as under normal Ba and Ra concentration range conditions in soil to use an equation to estimate ²²⁶Ra concentration in plants. As for the normal ²²⁶Ra and Ba ranges, the following values can be used for a rough estimation: 85-95 Bq/kg-dry for ²²⁶Ra (or that for U in soil is 0.8-7.1 mg/kg-dry), and 84-960 mg/kg-dry for Ba for these values were within the range of reported values for non-contaminated soils.

**Major publications**


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**Fig. 5-1.** Comparison of measured TF-Ra values with estimated TF-Ra values obtained by using Ba in plant and U in soil concentrations to estimate Ra concentrations in plant and soil, respectively.