Departmental Overview

The Department of Molecular Biosciences is responsible for the conduct of basic science program primarily involving (1) Studies of genetic effects and (2) Studies of carcinogenic mechanisms. We are also conducting studies examining biological mechanisms of noncancer diseases among A-bomb survivors.

In the studies of genetic effects, we aim to determine the frequency and nature of heritable mutations in members of survivor families (mother, father, and offspring). Previous studies of the survivor families did not indicate any significant genetic effects of parental exposure to radiation, and our recent animal studies indicated the rate of $1 \times 10–2$/Gy per genome of relatively large deletion/amplification mutations for effects of parental exposure. This mutation induction rate is substantially lower than those predicted from mouse maker gene studies. The reason why the effect of parental exposure is lower in humans than in mice is unknown. To address this question directly, we are planning whole genome sequencing-based genetic studies using next-generation sequencing (NGS) technology that will provide the capability to detect the entire spectrum of mutations in survivor families. We are also developing a mouse model for NGS-based measurement of spermatogonia stem cells mutations to investigate the fate of mutant germ cells during meiosis, fertilization and embryonic development.

In the studies of carcinogenic mechanisms, we aim to clarify mechanistic relationships between radiation exposure and cancer development. Previous studies of thyroid cancer tissue specimens in the LSS indicated that gene rearrangements involving *RET* or *ALK* frequently occurred in papillary thyroid cancer cases exposed at young ages to high radiation doses, and the carcinogenic potential of these rearranged genes is currently being assessed using in vivo and in vitro experiments. Based on potential involvement of liver inflammation and fibrosis in radiation-associate liver cancer, we hypothesize that chronic inflammation due to radiation exposure may contribute to the development of liver cancer through liver metabolic abnormality and fibrosis. To test this hypothesis, an animal model to examine involvement of liver steatogenesis and fibrosis in radiation-induced liver cancer is being developed. We are also examining genetic factors in breast and thyroid cancers. Previous studies indicated that lymphocyte chromosomal translocations did not dose-dependently increase in survivors exposed in utero. To test the hypothesis that chromosomally aberrant tissue stem cells were negatively selected during fetal development, cytogenetic damage of in utero-exposed mice is being evaluated for cells in various organs and systems, such as the thyroid, and the hematopoietic system.

We are also making efforts to identify and evaluate biomarkers linking radiation exposure to diseases among A-bomb survivors. Biomarkers currently being assessed involve immunological endpoints and obesity indicators potentially related to enhanced risks of chronic diseases including cancer among A-bomb survivors. In the AHS, we are developing longitudinal study designs to test the hypothesis that hematopoietic and immune-cell homeostasis perturbed following radiation exposure may affect the development of inflammation-associated diseases such as cardiovascular diseases and liver fibrosis/cancer. For better understanding of biological mechanisms of radiation-related diseases, we are also planning collaborative studies with outside experts to perform integrated analyses of multiple

molecular (omic) endpoints such as genomics, transcriptomics, metabolomics, and proteomics. The biodosimetry data for the frequency of chromosome aberrations in blood T cells as well as the intensity of electron spin resonance (ESR) signals in tooth enamel are anticipated to provide information on possible random and systematic dose uncertainties in individual doses calculated by DS02R1 and prove to be valuable for use in cancer risk estimation.

FY2017 Achievements

Radiation and Genetics Effects

- Radiation-induced deletions in mouse spermatogonia are usually large (over 200 kb) and contain little sequence similarity at the junctions (Kodaira, Radiat Res 2017; 187:722-31). *Purpose:* This study aimed to characterize the structures and sequences of radiation-induced deletions occurring in mouse spermatogonia cells which are inherited to offspring. *Methods:* We analyzed the sequences of the regions around the rejoined junctions of 33 de novo copy-number mutations (27 deletions and 6 duplications) obtained from offspring sired by male mice that were irradiated at the spermatogonia stage and from non-irradiated controls. *Results:* The deletions can be classified into three major groups. Group 1, deletion size 1kb to 1 Mb, sharing long blocks of similar sequences (200–6,000 bp) at the junctions (e.g., illegitimate recombination). Group 2, deletion size shorter than 200 kb, sharing only 0-7 bp homology (micro-homology-mediated). Group 3, deletion size longer than 200 kb, sharing 0-2 bp homology (typical NHEJ). The group 3 consisted primarily of deletions that occurred in the irradiated genomes. *Conclusion:* It was suggested that large size (>200 kb) with little sequence similarity around the rejoined sites are likely to be a hallmark of radiation-induced deletions in mice.

- Radiation-induced small-size indels (insertions and deletions) and complex mutations were identified in F1 mice born to exposed spermatogonia or mature oocytes. *Purpose:* To evaluate genetic effects of radiation exposure to spermatogonia or mature oocytes, WGS examination was conducted in a mouse system. *Methods:* We compared WGS data between F1 mice from parents before and after exposure to 4 Gy of gamma-ray. *Results:* The frequency of small-size indels increased in F1 mice born to either exposed spermatogonia or oocytes. Furthermore, multiple mutations within 10 bp appeared to be induced in these mice. The frequency of such complex mutations increased in those born after mature oocyte exposure and born after spermatogonia exposure. *Conclusion:* Radiation induced small-size indels in spermatogonia and mature oocytes that were heritable. Complex mutations were also induced following γ-ray exposure for mature oocytes and spermatogonia. These results will provide useful information for planning WGS analysis in A-bomb survivor families.

- A preliminary study to establish mouse experiments for culture, gene manipulation and cell transplantation of spermatogonia stem cells was initiated to investigate radiation

effects on germ cell mutations.

Radiation Dosimetry

- Cytogenetic reconstruction of gamma-ray doses delivered to atomic bomb survivors: dealing with wide distributions of photon energies and contributions from hematopoietic stem/progenitor cells (Nakamura, Radiat Res 2017; 187:412-8). *Purpose:* To overcome the uncertainty of retrospective estimation of A-bomb doses with cytogenetic methods, ESR measurements which exhibit less dependency on photon energy were applied. *Methods:* Both ESR and cytogenetic doses were estimated from 107 survivors of Hiroshima, in which the latter estimates were made by the use of standard ^{60}Co gamma rays. *Results:* It was shown that the two sets of kerma doses were in close agreement, indicating that perhaps no correction is necessary in estimating A-bomb gamma-ray doses. *Conclusion:* The results will make it possible to directly compare cytogenetic doses with physically estimated doses of the survivors. Nagasaki ESR data was also summarized thereafter.

- To investigate the effect of A-bomb radiation to humans, a cytogenetic dosimetry study was conducted for A-bomb survivors in the AHS cohort. A total of 1,869 survivors (1,179 in Hiroshima and 690 in Nagasaki) were examined using 2-color-FISH for detection of translocations involving chromosomes 1, 2, and 4. The preliminary results are summarized as follows: (1) clear nonlinear dose responses were observed in both Hiroshima and Nagasaki; (2) a wide scatter of individual translocation frequencies against physical dose was observed in both cities as seen in the previous Giemsa staining study; (3) The city difference seen in previous studies using Giemsa staining became much smaller now with FISH; (4) Nagasaki factory workers had significantly lower dose responses than people who were exposed in Japanese houses; (5) the reduced intercity difference suggests that the previous city difference by the solid Giemsa method was mainly due to different aberration detection rates between Hiroshima and Nagasaki laboratories.

- Radiation-induced unrepairable DSBs: Their role in the late effects of radiation and possible applications to biodosimetry (Noda, J. Radiat Res, 2017; 1-7). *Purpose:* This study aimed to characterize the structures and biological roles of unrepairable and persistent DSBs remaining in the past-irradiated cells and tissues. *Methods:* Irradiated normal human diploid fibroblasts (NHDFs) were used as a model system for detecting unrepairable DSBs. The persistency and molecular specificity of the damages that were

distinct from the repairable (easy-to-repair) DSBs were determined in microscopic and biochemical analysis. We also examined the detection of the damage in past-irradiated mouse tissues. *Results:* It was found that the unrepaired DSB-ends made pairs locating at distant sites in nucleus so that one DSB produced a distinct pair of broken ends. (torn-off structure). A new candidate protein that was associated with the damage was identified. *Conclusion:* Such protein-based factors may be useful as a new biomarker for detecting unrepairable DSBs as well as for retrospective dose estimation.

Radiation and Cancer

- To evaluate involvements of ALK rearrangements in lung adenocarcinoma among A-bomb survivors, we analyzed archival lung cancer tissue specimens in LSS. ALK rearrangements were identified in 2 (1 exposed and 1 unexposed) cases among 24 (12 exposed and 12 unexposed) surgically dissected cases, but in none among 50 autopsy adenocarcinoma cases. Due to the small number of cases with ALK rearrangements, any conclusion has not been drawn from the results.

- To provide insight into the mechanisms of increased cancer risk among A-bomb survivors, we conducted a preliminary study using a mouse colon cancer model (CT26.CL25) testing the hypothesis that radiation accelerates intestinal tumor progression through the PD-1/PD-L1 immune checkpoint pathway; however, any immunosuppressive effects through the immune checkpoint molecules on tumor growth was not shown when mice were irradiated with 3.5 Gy of X-rays three weeks prior to cancer cell transplantation. It is therefore difficult to investigate immunosuppressive effects of radiation in this mouse colon cancer model.

- To understand if mutation heterozygotes of a gene involved in DNA repair are at an increased risk of developing cancer, skin cancer risk among XPA founder mutation carriers was chosen as a model system. Out of 1,698 control individuals screened, 14 were found as XPA heterozygotes (0.8%) whereas the frequency was 2.5% (11/440) among skin cancer patients who developed basal cell carcinomas in areas exposed to sunlight ($p=0.01$). No increased risk was found among patients of squamous cell carcinomas. The manuscript has been submitted for publication.

- HPRT-dup-GFP mice, the first recombinant mice created at RERF, were sent to MMRRC mutant mouse repository and designated as MMRRC43688, B6J.Cg-Hprt^{tm1Rerf}. We have created p53-GFP knock-in mice in which radiation-induced tumors carrying dominant-negative mutations at p53 are supposed to become fluorescent. As a collaborative study with Prof. Ootsuyama of University of Occupational and Environmental Health, Fukuoka, a feasibility study using a typical chemical carcinogen, 3-MC (3-methylcholanthrene), has initiated to develop tumors in p53-GFP knock-in mice.

Radiation and Immunologic Effects

- Impact of early life exposure to ionizing radiation on influenza vaccine response in an elderly Japanese cohort (Hayashi, submitted). *Purpose:* This study was designed to test the hypothesis that radiation exposure early in life exacerbates age-associated decreases in immune function and thus further decreases the ability of elderly individuals to mount a protective adaptive immune response to prophylactic seasonal influenza vaccination. *Methods:* Immune response to influenza vaccination was tested with serum antibody titers that were measured prior to and three weeks after influenza vaccination in 292 AHS subjects over two influenza vaccination seasons. *Results:* Although individuals exposed to 1 Gy or higher were more likely to seroconvert to two antigens than lower dose exposure, there is no negative effect of radiation exposure on vaccine response in individuals. *Conclusion:* Early life exposure to ionizing radiation does not prevent responses of elderly A-bomb survivors to seasonal influenza vaccine. The plasma levels of inflammation-related proteins and profiles of lymphocyte subsets measured before influenza vaccination can predict influenza vaccine response.

- Fate decision between group 3 innate lymphoid and conventional natural killer cell lineages by Notch signaling in human circulating hematopoietic progenitors (Kyoizumi, J Immunol 2017;199:2777-2793). *Purpose:* The role of Notch signaling in human innate lymphoid cell (ILC) differentiation is unclear. In this study, we analyzed the functions of Notch in the differentiation of human hematopoietic progenitor cell (HPC) subpopulations circulating in peripheral blood. *Methods:* The fate of HPC subpopulations was determined by limiting dilution and clonal assays using high-throughput flow cytometry. *Results:* Notch signaling in combination with IL-7 induced differentiation of human HPCs into NKp44+ILC3 but conversely suppressed IL-15-dependent NK generation. *Conclusion:* Notch signaling plays Janus-faced roles in the fate decision between NKp44+ILC3 and cNK lineages at different stages of human HPCs.

- Radiation and age-associated changes in peripheral blood dendritic cell populations among aging atomic bomb survivors in Japan (Kajimura, Radiat Res 2018; 89:84-94). *Purpose:* Late effects of radiation on dendritic cells (DCs), key coordinators for activation and differentiation of T cells were investigated to test the hypothesis that A-bomb radiation exposure induced premature aging of DCs, resulting in reduced numbers and impaired functions of DCs. *Methods:* Numerical and functional changes related to age and radiation dose in conventional DCs (cDCs) and plasmacytoid DCs (pDCs) were analyzed among 229 A-bomb survivors. *Results:* We observed 1) a

dose-dependent decreases in the number of plasmacytoid DCs (pDCs) in females; and 2) a hierarchical cluster of two distinct types in gene expression profiles of conventional DCs (cDCs), with different median values of age and cytokine production levels. *Conclusion:* The numbers and functions of circulating DCs generally recovered to normal levels 65 years after A-bomb radiation exposure, i.e., levels typical for the unexposed group of the same generation.

- Aging-related changes in human T-cell repertoire over 20 years delineated by deep sequencing of peripheral T-cell receptors (Yoshida, Exp Gerontol. 2017; 96:29-37). *Purpose:* The human T-cell receptor (TCR) repertoire declines with age, but this decline has not been fully investigated longitudinally in individuals. *Methods:* Using a deep sequencing approach, we analyzed TCR β repertoires longitudinally over approximately 20 years, with ages ranging from 23 to 50 years at the start (23 to 65 years overall), in peripheral-blood CD4 and CD8 T-cell populations that were collected and cryopreserved 3 times at intervals of approximately 10 years from each of 6 healthy adults (3 men and 3 women). *Results:* Deep sequencing of longitudinal TCR repertoire changes revealed 1) age-dependent decrease and increase in CD8 TCR repertoire diversity (p=0.0008) and clonal populations (p=0.0015), respectively; 2) retained CD4 TCR repertoires and diversity; and 3) persisted and even expanded T-cell clones over 20 years. *Conclusion:* The study design therefore enabled us to reveal the longitudinal behavior of individual T-cell clones over 20 years in people of various ages.

- Late effects of exposure to ionizing radiation and age on human thymus morphology and function (Ito, Radiat Res. 2017;187:589-598). *Purpose:* To examine the long-term effects of radiation on thymic function, detailed histological evaluations were conducted in thymus tissue samples that collected from A-bomb survivors. *Methods:* A detailed morphometric analysis of thymus activity and architecture was conducted among autopsy thymus tissues obtained from 165 A-bomb survivors. *Results:* Hallmarks of thymic involution, i.e., decreases in thymic epithelium and immature lymphocyte areas, increased in individuals exposed to both low (5 – 200 mGy) and moderate to high (>200 mGy) doses of ionizing radiation compared to non-exposed (< 5 mGy) individuals. *Conclusion:* Even low-dose radiation exposure can accelerate thymic aging, with decreased thymopoiesis.

Radiation and Other Noncancer Conditions

- The onset time of symptoms related to stroke was significantly earlier in spontaneously

hypertensive stroke prone rat (SHRSP) irradiated with 0.1 Gy than in controls but not with 0.05 Gy. This suggested a threshold in the radiation dose effect between 0.05 and 0.1 Gy. Preliminary examinations, where SHRSP rats were chronically irradiated with an accumulated dose of 0.5 or 1 Gy (dose rate was 0.05 or 0.1 Gy/day, respectively), did not show any significant radiation effects. In addition, metabolome analyses demonstrated that the amounts of taurine and unsaturated fatty acids decreased and increased, respectively, with increasing dose. These data are useful to infer potential mechanisms underlying the radiation effect on circulatory disease.