Departmental Overview

The Department of Molecular Biosciences is responsible for the conduct of basic science program primarily involving (1) Studies of genetic effects, (2) Studies of carcinogenic mechanisms and (3) Studies 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 1×10^{-2}/Gy per genome of relatively large size 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. Software improvements and new technologies for Long-read NGS to assess complex types of mutations i.e., large deletions and translocations, are in progress. 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-associated liver cancer, we hypothesize that chronic inflammation due to radiation exposure may be involved in 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, chromosome 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. Potential biological pathways linked to the cardiovascular disease development in the

survivors involve clonal hematopoiesis, pro-inflammatory immune cells, endogenous danger signals, and arteriosclerosis. To support studies investigating these pathways, we are also developing mouse and mathematical simulation models that can evaluate clonal expansion of hematopoietic stem cells and inflammatory phenotypes following radiation exposure.

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 DS02R1 individual doses and prove to be valuable for use in cancer risk estimation.

FY2018 Achievements

Radiation and Genetics Effects

- 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.

Radiation Dosimetry

- Tooth enamel ESR doses and cytogenetic doses of Nagasaki atomic-bomb survivors in comparison with DS02R1 doses. *Purpose:* Cancer risks for Nagasaki survivors once appeared to be lower than for Hiroshima survivors. The possibility that this was due to overestimation of the doses for the Nagasaki survivors was tested by measuring biological doses of Nagasaki survivors. Materials and methods: The electron spin resonance (ESR) method and cytogenetic method were used to estimate radiation doses for 24 Nagasaki survivors, and the results were compared to calculated DS02R1 doses. *Results:* Six factory workers and 10 other survivors showed ESR or cytogenetically estimated doses that were in reasonably good agreement with their DS02R1 doses, while one factory worker was found to have an ESR dose estimate of nearly one half of the DS02R1 dose to the eye lens (a proxy organ for teeth). A few outliers were also observed. *Conclusions:* Although apparently lower cancer risks were observed in the past for Nagasaki survivors when compared to Hiroshima survivors, the present results do not indicate the existence of a trend of overestimating the DS02R1 doses when compared with biologically estimated tooth or cytogenetic doses. This observation is in line with the recent disappearance of the city difference in cancer risks.

Radiation and Cancer

- Previous *EML-ALK* gene fusions were detected in thyroid cancers of A-bomb survivors. To explore the role of *EML-ALK* gene fusions in radiation-induced papillary thyroid cancer we created recombinant mouse model. In a line of conditional *EML4-ALK* transgenic mice with bovine thyroglobulin gene promoter, induction of *EML4-ALK* fusion gene expression by doxycycline treatment has not been observed at all, probably due to the low promoter activity in the thyroid. We therefore introduced the allele containing CMV-rtTA (the reverse tetracycline-controlled transactivator protein under the control of cytomegalovirus promoter) plasmid DNA into the mice to improve induction of *EML4-ALK* expression. Although a weak but significant expression of the

fusion gene was observed in thyroid tissues of the mice either with or without doxycycline treatment, the mice did not show any histological features of papillary thyroid cancer PTC development until at least one year of age. This result suggests that the *EML4-ALK* fusion may not be a driver mutation for PTC development.

- Increased risk of skin cancer in Japanese heterozygotes of xeroderma pigmentosum group A. *Purpose:* This study was designed as a model system to learn if asymptomatic heterozygotes with mutations in a DNA repair gene are at an increased risk for cancer. To examine this, we focused on the frequency of Japanese carriers of a founder mutation in the *XPA* gene. *Materials and methods:* Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used for the detection of this mutation. Mutation frequency was compared between normal donors and those bearing skin cancers. *Results:* The frequency of heterozygotes was 14/1698 (0.8%) in blood samples from cancer-free controls, and the corresponding frequency among skin cancer patients was 12/545 (2.2%, p=0.018) in basal cell carcinoma (BCC), and 4/383 (1.0%) in squamous cell carcinoma (SCC). Furthermore, examining BCC cases in which cancer developed in sun-exposed areas, the frequency increased to 11/440 (2.5%) (p=0.0097, and the odds ratio is 3.08 compared to normal controls). *Conclusion:* These results suggest there is a moderately increased risk for skin cancer among *XPA* heterozygotes, and request reconsideration of cancer susceptibility in heterozygotes of repair genes for radiation-damage.

Radiation and Immunologic Effects

- Impact of early life exposure to ionizing radiation on influenza vaccine response in an elderly Japanese cohort. *Purpose:* To test the hypothesis that atomic bomb radiation has accelerated the aging of immune system, we evaluated the effects of whole body radiation exposure early in life on influenza vaccination immune responses much later in life. *Methods:* A total of 292 volunteers were recruited from the cohort members of our ongoing AHS of A-bomb survivors who participated in this observational study spanning two influenza seasons (2011-2012 and 2012-2013). Peripheral blood samples were collected prior to and three weeks after vaccination. Serum hemagglutination inhibition (HAI) antibody titers were measured *Results:* We found that influenza vaccination modestly enhanced serum HAI titers in this unique cohort of elderly subjects, with seroprotection ranging from 18 to 48% for specific antigen/season combinations. Twelve percent of subjects were seroprotected against all three vaccine antigens postvaccination. Males were generally more likely to be seroprotected for one or more antigens postvaccination, with no differences in vaccine responses based on age at vaccination or radiation exposure in early life. *Conclusion:* Early life exposure to ionizing radiation does not prevent responses of elderly A-bomb survivors to seasonal influenza vaccine.
- Radiation and age-associated changes in peripheral blood dendritic cell populations among aging atomic bomb survivors in Japan. *Purpose*: To test the hypothesis that A-bomb radiation exposure induced premature aging of DCs (dendritic cells), we investigated whether the past radiation exposure reduced numbers and 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 and Conclusion*: Although there was a dose-dependent decrease in the number of plasmacytoid DCs (pDCs) in females, the numbers and functions of circulating DCs generally recovered to normal levels 65 years after radiation exposure, indicating that the role of DCs were not altered in survivors.

- Effects of age and radiation on serum iron and intracellular ROS in blood of atomic-bomb survivors. To investigate the effects of age and radiation exposure on intracellular reactive oxygen species (ROS) levels in blood cells was conducted for A-bomb survivors in the AHS cohort. We examined 2,789 Hiroshima atomic bomb survivors who underwent health examinations from 2007 to 2012. Intracellular levels of ROS (H_2O_2 and $O_2^{\cdot-}$) in blood cells were measured with fluorescent reagents carboxy-DCFDA and hydroethidine, respectively, using a flow cytometer; particularly ROS levels in T-cell subsets, which we measured using a combination of fluorescence-labeled antibodies and fluorescent reagents. Our results showed that intracellular $O_2^{\cdot-}$ levels in lymphocytes (especially memory $CD8^+$ T cells) and granulocytes increased as age and radiation dose increased, and the association between intracellular $O_2^{\cdot-}$ levels and age tended to be stronger as radiation dose increased. These results suggest that ROS levels, specifically $O_2^{\cdot-}$ levels in specific blood cells are affected by aging and radiation exposure.
- Radiation exposure and longitudinal changes in peripheral monocytes over 50 years: The Adult Health Study of atomic-bomb survivors. *Purpose:* This study investigated whether radiation exposure promoted an increase in peripheral myeloid cells, a key effector of inflammation. *Subjects a*nd *methods:* Using longitudinal hematological data over 50 years for 14,000 AHS participants, statistical modeling was performed for both leukocyte subset percentages/counts and all-cause mortality. *Results:* There were positive associations of radiation dose with monocyte percentages and counts. Radiation effects on monocytes were stronger after versus before age 60 years at health examinations. Increases in monocyte numbers were associated with higher risk of all-cause mortality. *Conclusion:* Given that previous studies among atomic-bomb survivors have shown a clonal expansion of hematopoietic stem cells, radiation exposure might accelerate aging-associated clonal hematopoiesis, which could have resulted in a long-lasting elevation of peripheral monocytes as a consequence.

Radiation and Other Noncancer Conditions

- To test the possible involvement of radiation exposure in a stroke, experiments were conducted using hypertensive and stroke susceptible rat models. The onset of symptoms related to stroke was significantly earlier in spontaneously hypertensive and stroke prone rat (SHRSP) irradiated with 0.1 Gy than in unirradiated controls, but it was not evident with 0.075 Gy. This suggested a threshold in the radiation dose effect between 0.075 and 0.1 Gy. In addition, metabolome analyses demonstrated that the amounts of some metabolites including lithocholate (a kind of bile acid) biosynthesis system were altered with radiation doses. Moreover, various cytokines, such as IL-2 and IL6, were also altered. These data are useful to infer potential mechanisms underlying the radiation effect on circulatory disease.