DEPARTMENT OF MOLECULAR BIOSCIENCES

Departmental Overview Page 1

The Department of Molecular Biosciences is responsible for the conduct of basic science program involving (1) Studies of genetic effects and (2) Studies of carcinogenic mechanisms.

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 mutations at hyper-variable mini- and micro-satellite loci and at about 1,000–2,500 loci per genome did not indicate any significant genetic effects of parental exposure to radiation. Our recent animal study using a high-density microarray comparative genomic hybridization (CGH) method identified relatively large deletion/amplification mutations throughout the genome and indicated the mutation rate, 1×10^{-2}/Gy per genome for induced deletions. This method is currently used to estimate the trans-generational effects of radiation in the children of A-bomb survivors. We are also beginning whole genome sequencing-based genetic studies using next-generation sequencing technology that will provide the capability to detect the entire spectrum of mutations. We are also developing a green fluorescent protein (GFP) mouse model for quantitative measurement of germ-cell mutations. In addition to Dr. Uchimura who will be assigned at the beginning of April 2017 as Chief of Molecular Genetics Lab., one or two young scientists will be newly recruited to promote these studies.

In the studies of carcinogenic mechanisms, we aim to clarify mechanistic relationships between radiation exposure and cancer development. Early molecular events in cancer development have been examined with thyroid, colorectal, and lung cancer tissue specimens in the LSS, and we found that gene rearrangements involving *RET* or *ALK* frequently occurred in papillary thyroid cancer cases exposed at young ages to high radiation doses. The carcinogenic potential of altered genes found in these radiation-associated cancers are also being assessed using in vivo and in vitro experiments. We are also examining genetic factors in breast, thyroid, and skin cancers. 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, to test the hypothesis that chromosomally aberrant fetal stem cells were negatively selected.

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 among A-bomb survivors. We are developing longitudinal study designs to fully utilize data that have been and will have been obtained from the AHS population. Unrepairable DNA radiation damage, DNA methylation and transcription are being analyzed to seek epigenetic mechanisms that lead to increased risks of diseases 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 frequency of stable-type chromosome aberrations (translocations) examined using fluorescence *in situ* hybridization (FISH) indicates a wide scatter of individual translocation frequencies against physical dose but a somewhat smaller scatter against another independent biodosimeter, electron spin resonance (ESR) using tooth enamel. We anticipate

that such biodosimetric data will provide information on possible random and systematic dose uncertainties in individual doses calculated by DS02 and prove to be valuable for use in cancer risk estimation.

FY2016 Molecular Biosciences Achievements

Radiation and Genetics Effects

- Mouse CGH study determined that the mutation rate was 1 x 10^{-2}/ Gy per genome for induced deletions. The results indicated that the mean response to transgenerational effects of radiation is far lower than expected from the mean response of the mouse 7-locus tests (Asakawa et al., Radiat. Res., 186:568-76, 2016). The junction sequences of the deletions were also determined.
- CGH study on offspring of A-bomb survivors and their parents identified 5 *de novo* deletions and 6 duplications and determined the parental origin of 5 deletions and 5 duplications. The junction sequences were also determined.
- In WGS study of irradiated human cell clones, we had a proof of concept for whole genome analysis and validated the array-CGH data of the same samples in accordance with suggestions from an external collaborator.
- WGS identified more small indels in F1 mice born to irradiated spermatogonia cells than those born to controls.

Radiation Dosimetry

- Fluorescent in situ hybridization (FISH) analysis showed a wide scatter of individual translocation frequencies against physical dose as seen in the previous solid Giemsa staining study.
- ESR dose and cytogenetic dose were compared in the same donors, and we found that these two doses agreed well to each other..
- We have identified and characterized new proteins that co-localized in repair foci complex in cells bearing radiation-induced unrepairable double strand breaks (DSBs).

Radiation and Cancer

- To clarify the biological significance of the *EML4-ALK* fusion gene in radiation-related PTC, we made conditional transgenic mice bearing *EML4-ALK*and initiated doxycycline treatment experiments for production of thyroid cancer.
- The greatest relative risks (RRs) of lung cancer (LC) and breast cancer (BC) were found in the group of *IL6R-A/G* or *IL6R-G/G*, combined with the highest dose category ($\geq$700 mGy): RR = 2.08 (95% CI: 1.11-3.90) for LC; and RR = 6.61 (95% CI: 2.03-21.6) for BC, as compared to the reference group (non-exposed, *IL6R-A/A*), suggesting involvements of *IL6R* genotypes in individually-differing risks of certain types of radiation-related cancers among A-bomb survivors.
- A paper describing chromosome aberration frequency in mouse thyroid cells following fetal irradiation has been published (Hamasaki et al., *Radiat Res.* 2016; 186: 360–366). A new study testing hypotheses on stem cell selection and replacement during fetus development is planned, and preliminary experiments have been initiated.

- In the screening of *XPA* founder mutation carriers, we found 14 heterozygotes among 1,698 individuals in a control population and 14 heterozygotes among 714 squamous or basal cell carcinomas developed in areas exposed sunlight. The individuals who developed carcinomas showed a significantly increased frequency of the *XPA* mutation (Fisher's exact test, $P = 0.02$). This study has been terminated in 2016, and a scientific report is being prepared.
- We started a feasibility study for radiation carcinogenesis, using *p53-GFP* transgenic/knock-in mice that we created for the *in vivo* detection of radiation-induced forward mutations in the *p53* locus.

Radiation and Immunologic Effects

- The hypothesis that past radiation exposure exacerbates age-associated deterioration in immune response to influenza vaccination was tested in 292 AHS subjects over two influenza vaccination seasons. Gender or advanced age did not give a consistent and significant impact on strain-specific flu vaccine response. Individuals exposed to 1 Gy or higher were more likely to seroconvert to two antigens than lower dose exposure, suggesting that more than 65 years after the A-bombing, there is no negative effect of radiation exposure on vaccine response in individuals.
- Using a cell-sorting-based colony formation assay, we demonstrated that Notch signaling in combination with IL-7 induced differentiation of human hematopoietic progenitor cells (HPCs) into innate lymphoid cell expressing NKp44 (NKp44+ILC3) but conversely suppressed IL-15-dependent NK generation, indicating Janus-faced roles of Notch signaling in the fate decision between NKp44+ILC3 and cNK lineages at different stages of human HPCs. Numerical and functional analyses of circulating dendritic cell (DC) populations among 229 AHS participants indicated 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.
- Deep sequencing enabled a robust analysis of longitudinal T-cell receptor (TCR) repertoire changes in in-house volunteers' samples cryopreserved over 20 years. We found 1) TCR repertoire diversity decreased ($p=0.0008$) and frequencies of clonal populations increased ($p=0.0015$) linearly with age in CD8 T cells; 2) CD4 T cells retained fairly diverse TCR repertoires along with relatively low clonality; and 3) some of the major T-cell clones persisted and even expanded over 20 years.
- A detailed morphometric analysis of thymus activity and architecture among autopsy thymus tissues obtained from 165 A-bomb survivors indicated that hallmarks of thymic involution increased in individuals exposed to both low (5 – 200 mGy) and moderate to high (>200 mGy) doses of ionizing radiation compared to non-exposed individuals (< 5 mGy). (Ito, *Radiat Res*, in press).

Radiation and Other Noncancer Conditions

- A genome-wide DNA methylation assay, reduced representation bisulfite sequencing

(RRBS), detected more than 600 genes whose methylation levels at CpG site are different between young and elder volunteers. Gene ontology and pathway analyses of the RRBS data indicated that the IL-2 receptor pathway is associated with the genes harboring different methylation levels in terms of aging effects.

- The onset time of symptoms related to stroke in irradiated spontaneously hypertensive stroke prone rat (SHRSP), even at 0.1 Gy were significantly earlier than that of the control. Based on this result, we have obtained a grant from the Japanese Ministry of Environment (MOE) and we started the studies, where we examine the rats irradiated with 0.05 Gy. Metabolome analyses for SHR's samples demonstrated that the levels of some metabolites were altered with radiation doses and those were closely related with cause of cardiovascular diseases (CVD)..