DEPARTMENT OF MOLECULAR BIOSCIENCES

Departmental Overview

The new Department of Molecular Biosciences (formerly departments of Genetics and Radiobiology/Molecular Epidemiology) that has commenced last year, now embarks on the first year upon the official approval by MLHW. This department will be responsible for the conduct of basic science program involving (1) Studies of genetic effects and (2) Studies of carcinogenesis mechanisms.

In the studies of genetic effects, the frequency and nature of heritable mutations in members of survivor families (mother, father, and offspring) have been examined with screening of mutations at hyper-variable mini- and micro-satellite loci and at about 1,000–2,500 loci per genome. None of those studies indicated statistically significant genetic effects of parental exposure to radiation. Recently, high-density microarray comparative genomic hybridization (CGH) methods using over one million probes have been introduced to detect relatively large deletion/amplification mutations throughout the genome. This method was first used to estimate the trans-generational effects of radiation in the offspring of model animals and currently 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 the studies of carcinogenesis mechanisms, we aim to clarify mechanistic relationships between radiation exposure and cancer development. Toward this end, we are analyzing early molecular events in thyroid, colorectal, and lung cancer development in the LSS and are also assessing the carcinogenic potential of altered genes found in these radiation-associated cancers 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 potentially related to radiation-induced attenuation of immune function and to enhanced risks of chronic diseases among A-bomb survivors. We are also examining the genetic basis for inter-individual differences in immune functions and the impact of genetics on susceptibility to radiation-associated diseases. 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. 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.

FY2015 Molecular Biosciences Achievements

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Radiation and Genetics Effects

- Completed mouse CGH study and determined the parental origin of the mutations. 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.

- Completed CGH experiments on 667 DNA samples from offspring of A-bomb survivors and their parents using high-density microarrays with 1.4 million probes. We identified 6 *de novo* deletion mutations and 6 duplication mutations and determined the parental origin of 6 deletions and 4 duplications by haplotyping of each mutated chromosome. Additional haplotypings are currently underway on remaining duplication mutations.

- Summarized WGS study of irradiated human cell clones. The scientific report as a proof of concept for whole genome analysis is almost ready for internal review.

- Started whole genome sequencing of F1 mice born to exposed male spermatogonia cells since we have obtained a MEXT grant. Each of six male mice born before and after exposure to male germ cells has been subjected to whole genome sequencing. We have already obtained raw sequence data.

- Published the first paper regarding the generation and characterization of the HPRT-dup-GFP mice for detecting radiation-induced somatic and germ cell mutations in vivo. (Noda, *PLoS One* 2015; 10(8): e0136041).

Radiation Dosimetry

- Completed fluorescent in situ hybridization (FISH) analysis in A-bomb survivors. A wide scatter of individual translocation frequencies against physical dose was observed as seen in the previous solid Giemsa staining study.

- A total of 298 molars donated by 228 Hiroshima AHS participants and 26 molars donated by 25 Nagasaki A-bomb survivors have been measured by ESR. We are doing statistical analysis to compare them with DS02 doses and cytogenetic- estimated doses from the same donors.

- We have found that a subset of unrepairable double strand breaks (DSBs) is associated with the nuclear membrane, making the nuclear membrane dysfunctional. A summary report was published (Noda, Gene Environ, 2015; 37(13):1-12).

- Using transcriptome and proteome analyses, we are determining potential novel genes/proteins that may characterize past-irradiated cells. One of the candidates appeared to be a transcription factor protein involved in early development.

Radiation and Cancer

- To clarify the biological significance of the *EML4-ALK* fusion gene in radiation-related PTC, we made a construct using cDNA of the *EML4-ALK* fusion gene and bovine thyroglobulin gene promoter using pTet-On system (CLONTECH). We contracted out production of the conditional transgenic mice to a commercial laboratory, UNITECH Co, and got 5 lines of founder mice. We initiated doxycycline treatment experiments for production of thyroid cancer using one F1 line that shows high thyroid-specific and high level expression of EML4-ALK. Histological changes are also being analyzed using HE-stained sections of thyroid tissue in transgenic mice treated with doxycycline.

- Completed preparing the manuscript describing results following irradiation at different fetal stages. These data indicate different translocation frequencies in adult mouse thyroid cells when compared between early and late post conception irradiations. Additionally we have started preliminary experiments that examine radio-sensitivity on the induction of chromosome aberrations in fetal hematopoietic stem cells.

- Completed screening of *XPA* founder mutation carriers among 1170 non-melanoma skin cancers and 680 chromosome slides as controls（lymphocytes in microscopic slides that were used for the past F1 cytogenetic study）We are doing statistical analysis to see if there is an increased risk of nonmelanoma skin cancer among the *XPA* heterozygotes.

- We have created p53-GFP transgenic/knock-in mice for the in vivo detection of radiation-induced forward mutations in the p53 locus. We are planning for radiation carcinogenesis experiments.

Radiation and Immunologic Effects

- Shortened DNA telomeres, which are an indication of cellular aging, may also confer risk for cancer and other chronic diseases. The long lasting detrimental effects of radiation exposure on telomere length in leukocytes were both dose- and age-at exposure dependent. Alterations of biomarkers such as uric acid metabolism, inflammatory

cytokines, and T-cell counts were also observed.

- Age- and radiation-related changes in different subtypes of circulating hematopoietic stem and progenitor cells (HSPCs) were evaluated in 231 Hiroshima A-bomb survivors. Many years after radiation exposure and with advancing age, the number and function of HSPCs in living survivors as a whole appear to have recovered to normal levels. (Kyoizumi, *Radiat Res,* 2016; 185(1):69-76).
- A negative interaction effect between the radiation dose and the frequency of γH2AX foci was observed in a proportion of a subset of HSPCs as assessed by the cobblestone area-forming cell assay, suggesting that the effect of DNA damage on the self-renewability of HSPCs may be modified by A-bomb radiation exposure (Kajimura, *Mutat Res-Gen Tox En*, 2016; 802:59-65).
- Interaction effects were found between radiation dose and metabolic indicators (hemoglobin A1c and fatty liver disease) on B-cell and NK-cell percentages among A-bomb survivors, supporting the hypothesis that the long-term effects of radiation exposure on lymphocyte subsets may be modified by metabolic status.
- There were associations between shorter T-cell telomeres and higher hemoglobin Alc levels or fatty liver development, and also interaction effects of radiation dose and CRP or HDL cholesterol levels on T-cell telomere length among A-bomb survivors, suggesting that long-term radiation effects on the maintenance of T-cell telomeres may be modified by the metabolic condition of individuals.
- Improvements of immunofluorescence techniques provided high-quality images with low background and high contrast from paraffin blocks of thymus tissue that were prepared up to 60 years ago. Using the techniques, an efficient immunofluorescence-based analysis has become available for investigation of radiation effects on thymic function in autopsy thymus specimens long-term stored at RERF. (Kajimura, *J Histochem Cytochem,*2016; 64(2):112-24).
- The relative risk of breast cancer was the largest for the *ATM-111 G/G* group exposed to the highest dose category, as compared to the reference group (non-exposed *ATM-111 A/A or ATM-111 G/A*), suggesting a possible involvement of the *ATM* genotypes in the inter-individual variance of incidence risk of radiation-related breast cancer among A-bomb survivors.
- The relative risks of distal and proximal colorectal cancer (CRC) were higher in the high-dose-exposed group with*CD14–911A/A* and *IL18–137 G/G* genotype, respectively. In phenotype–genotype analyses, the *CD14–911A/A* genotype presented significantly higher levels of membrane and soluble CD14 compared with the other two genotypes,and theIL18–137 G/G genotype tended to be lower levels of plasma IL-18 compared with the other two genotypes, suggesting a possible involvement of CD14-mediated inflammatory response in the development of distal CRC and an IL18-mediated inflammatory response in the development of proximal colon cancer among A-bomb survivors. (Hu, Human Genome Variation, 2015; 2(15035) 2015; 1-9).

Radiation and Other Noncancer Conditions

- A genome-wide DNA methylation assay, using a method called "reduced representation bisulfite sequencing (RRBS)" was assessed in collaboration with University of Copenhagen, using DNA samples of naïve CD4 T cells from non-AHS volunteers. DNA methylation changes between young and elderly healthy volunteers were detected in hundreds of genes, especially in the inflammatory IL-1 signaling pathway including *IRAK1* gene.
- The symptoms related to stroke in irradiated spontaneously hypertensive stroke prone rat (SHRSP), even at 0.25 Gy, were significantly earlier than that of the control, and lifespan was significantly shortened with increasing dose. Pathological findings such as fibrosis and inflammation in cardiac muscles, increased systolic blood pressure level, and retardation of body weight increase were significantly associated with radiation doses.