DEPARTMENT OF MOLECULAR BIOSCIENCES

Departmental Overview

The Department of Molecular Biosciences (MBS) supports the mission of RERF by conducting mechanistic studies on radiation effects uncovered by clinical and epidemiological studies on the cohort of A-bomb survivors and children of survivors. These are interdisciplinary collaborative studies in collaboration with other RERF departments and outside research Institutes. The departments' studies include those on 1) genetic effects; 2) radiation carcinogenesis; and 3) non-cancer diseases associated with radiation exposure. The Department consists of 4 (Molecular Genetics, Cytogenetics, Cell Biology and Immunology) Laboratories. Currently the department is in a transition phase to a large extent toward implementation of the RERF strategic plan. Based on it, in this year, the name of the Laboratory of Cytogenetics was changed to the Laboratory of Cellular Genomics, and the Laboratory of Cell Biology was renamed to the Laboratory of Molecular Pathology. Along with this, the specially appointed research group led by Dr. Tanabe of BRC (Biosample Research Center) joined the Cellular Genomics Laboratory. The Laboratory of Molecular Pathology, led by Dr. Tsuruyama, has initiated molecular imaging approaches using pathological specimens and in situ mass spectrometry analyses in cancer tissues. This research is being developed jointly with Kyoto University and Shimadzu Corporation, and is a major research initiative in RERF's strategic plan.

Because of concerns about heritable effects following exposure to radiation from the atomic bomb (based on results in animal model systems conducted in several laboratories world-wide) the relationship between radiation exposure and heritable genetic effects has been a major concern and was among the first series of studies to be initiated at ABCC, the predecessor to RERF. Studies were performed using many different approaches, all suggesting no substantial heritable effects in the children of survivors. Such clinical and epidemiological studies continue to be conducted. There are, however, limitations to the endpoints used and the statistical power of these studies. On the other hand, a major effort led by MBS is to conduct a WGS study in trios comprised of mothers, fathers and children. In these trios one of the parents (in some cases both) were exposed to radiation from the atomic bombings. These studies are being supplemented with studies using cultured cell or experimental animal model systems to examine mechanisms. All of these studies are being linked and integrated with studies of children of survivors in epidemiological and clinical cohorts, as an F_1 umbrella multidisciplinary program project. The trio-WGS research plan is in the final approval stage, and preparations are underway to obtain informed consent from the individuals in the study.

Studies of processes involved in radiation carcinogenesis and immunological effects were also begun relatively soon after the formation of ABCC and such studies continue as we move forward with our strategic plans. These studies were originally housed in the former Department of Radiobiology/Molecular Epidemiology and are now being conducted primarily in the Molecular Pathology Laboratory and the Immunology Laboratory.

MBS is currently developing new programs examining processes involved in radiation carcinogenesis designed to interrogate radiation effects at the molecular, cellular, and tissue levels using state-of-the-art imaging, genomic, proteomic, and immunological approaches using stored blood and tissue based biosamples such as FFPE samples from survivors and their children. In addition, model systems will be used to provide additional insight into these effects and to critically test hypotheses that emerge from the biosample studies. These studies will be

integrated with data derived from RERF cohort studies as part of an institutional-wide molecular epidemiology approach.

Other studies in molecular epidemiology involve institutional-wide collaborative efforts examining genetic susceptibility and gene-environment interactions and studies of potential biomarkers. This year, preliminary GWAS study on AHS samples was initiated using Wright-stained blood smears obtained from every 2-years medical examinations of the survivors. Our immunology laboratory will be involved in these tissue-based studies as well as continuing current efforts examining mechanisms of non-cancer associated disease with an emphasis on radiation-associated clonal hematopoiesis and development of cardiovascular disease.

These areas of emphasis rely on collaborative studies within RERF and with outside experts to perform integrated analysis using genomic, transcriptomic, metabolomic, proteomic and immunologic approaches. These data will be linked with epidemiological and clinical data through the Research Resource Center. Such integrated approaches could provide new insights into radiation related disease processes that are not currently possible at other institutions.

FY2022 Molecular Biosciences Achievements

Radiation and Genetics Effects

The most important research initiative in the MBS genetics program and for RERF in general is the whole genome sequencing (WGS) study focusing on human trios consisting of atomic bomb survivors and their offspring. This is a major part of the institutional-wide F_1 umbrella program. In FY2022, we held meetings with external collaborators, developed the scientific research protocol that has been approved following external review. These studies will be conducted in collaboration with Dr Stephen Chanock (US NCI) and Dr Nakagawa at RIKEN. Mutation analysis will be performed on an external cloud server and an on-premise server, and our external collaborators will be able to perform analysis on the cloud server, but will not be able to download the data, assuring the protection of subjects' genome information. In addition, the correlation analysis between genome data, epidemiological data, and clinical data of the subjects will be performed only within RERF so that personal information will be thoroughly managed. One of the most important challenges in this WGS research has been obtaining social consensus and addressing ethical issues. On this point, we hosted an ELSI International Workshop last year, and this year, we have held an external advisory committee on the trio-WGS research in Hiroshima and Nagasaki which included survivors and F1s. The ethical procedural part of the research plan reflecting their comment has been included in our protocol which is currently under review by the IRB. When approved, we plan to obtain informed consent from the subjects as soon as possible and conduct the research. Uchimura, Satoh, Noda (MB) and Sposto (S) A part of CR162, PI; Uchimura.

- As part of the preparation for feasibility study of the Trio-WGS, human cell line was used to prepare the analysis systems. To date, we have established a WGS data analysis pipeline for detecting de novo mutations including base substitutions and indels, primarily in mouse experiments (Uchimura et al 2015, Satoh et al 2020, Uchimura et al 2022, etc.). For the human genome, this method was only evaluated in the analysis of one human cell line (lymphoblastoid cell line, GM18943). In 2022, in response to a collaborative research request from Dr. Mitsutake (Nagasaki University), our methodology was evaluated in the analysis of radiation effects using other human cell lines. Furthermore, in FY2022 we additionally built a whole genome analysis pipeline for structural variant (SV) detection using nanopore and short read NGS, and established a GPU-based analysis environment for nanopore sequencing data analysis, including methylation base calling, in our laboratory. In the collaborative study, we analyzed the WGS data of total of 23 cell clones (1Gy: 5 clones, 3Gy: 5 clones, 6Gy: 5 clones, ENU treatment: 4 clones, control: 4 clones), which were derived from hTERT-immortalized normal human fibroblast (BJ1-hTERT) cells. Our mutation detection pipeline also worked well in the analysis using BJ1-hTERT cells. Based on the accumulated mutations in each clone, we were able to reconstruct highly credible cell lineages among the clones. The results showed that ENU treatment increased de novo base substitutions and irradiation increased de novo indels. These results will be published as Mitsutake et al. In addition, the pipeline for detecting structural mutations has been successfully improved, and GPU-based nanopore sequencing data analysis (including base calling of CpG methylation) can now be performed at RERF. The results has shown that our mutation detection pipeline is stable and highly effective in mutation analysis of the human genome. Based on the studies we anticipate no technical challenges in

conducting genome analysis of human trios including atomic bomb survivors. We are ready to start as soon as IRB review is completed. We plan to conduct high-quality analyses using the latest analysis methods by collaborating with researchers at RIKEN and NCI using cloud servers. Optimizing the epigenome analysis conditions (sample preparation, etc.) is one of the important issues for the future. (Uchimura, Satoh), PI; Satoh

- Characterization of de novo mutations in F_1 mice born following radiation exposure of either male or female parent at the spermatogonia or mature oocytes stage. Thus far, we reported de novo germline mutations (mainly base substitution and small size deletions) in the mouse offspring born to irradiated parents (Satoh et al, 2020). Currently, we are working on an analysis of de novo SVs. As a first step, we investigated the incidence rate and characteristics of spontaneous de novo SVs in the mouse germline. In FY2022, we developed a data analysis pipeline for improved SV detection. We also tested optical mapping-based mutation detection methodology to determine if de novo mutations, which are difficult to detect even with long-read NGS, could be detected. We conducted WGS of long-read NGS (Pacbio and Nanopore) and short-read NGS (illumina), and optical mapping (Bionano). We analyzed four lines of the mutation accumulation (MA) mouse lines (passing more than 50 generations during 16 years of successive inbreeding). As a result of pipeline refinements, we were able to identify 77 SVs in those four lineages, which was 11 more than when we reported to the SAC last year. This includes 36 retrotransposon insertions (LINE, LTR, SINE, etc.). This indicates that 1.34 SVs (including 0.48 LINE transpositions) occur per generation in mice. Analysis using whole genome assembly revealed details of the inserted sequence of the retrotransposon and how it differs from the original sequence of the retrotranspositions. For example, it was found that the number of repeat units in the promoter region decreases during a LINE retrotransposition. The analysis using optical mapping also revealed that mutations occurring in minisatellite regions, which are generally difficult to detect with short- or long-read NGS, can also be detected. Through these studies we have succeeded in estimating the incidence of de novo SVs per generation in mice for the first time in the world using the latest sequencing technology. Our estimated rate (1.34 SVs and 0.48 LINE transpositions in C57BL/6J strain) was much higher than the previous estimates of 0.16 SVs in human and 0.13 LINE transpositions in mice. Our new results about mammalian de novo germline mutations should be helpful for the future human trio study at RERF. (Uchimura, Satoh). PI; Satoh. Satoh, 2020a, 2020b, and 2021. A summary manuscript is in preparation.

- Here, we particularly show one of our newest methods for development of novel analytical methods for de novo mutations. These studies were designed to reconstruct phylogenetic trees of ontogeny using mosaic mutations. The purpose of this study was to develop a method to reconstruct phylogenetic trees of cell lineages during early embryogenesis using mosaic mutations. This method is useful in many areas of life science, including radiation biology. For instance, this method is planned to be used in “Clonal hematopoiesis project (PI,Yoshida)” at RERF. We detect de novo mutations existing in the mosaic state in a somatic tissue with deep coverage whole genome sequencing. Then, we accurately measure their variant allele frequencies (VAFs) in several tissue samples. Finally, we use a mathematical model and the VAF data to reconstruct the phylogenetic tree during early embryogenesis. We succeeded to establish a novel method to reconstruct phylogenetic trees

(Patent has been published in 2021). We have also conducted validation experiment and several additional experiments required for completion of a full paper. In 2022, we reported the results as a scientific paper (Uchimura et al., 2022). In addition to this cell lineage analysis methodology based on mosaic mutations, the Uchimura laboratory has established pipelines to detect small-scale mutations (base substitutions and indels) as well as large-scale structural mutations. Furthermore, we have succeeded in increasing the throughput of the data analysis pipeline, making it possible to conduct many collaborative studies in parallel. Therefore, we conducted the genome analysis in collaboration with RERF researchers (research on radiation-induced mutations) and with outside researchers, including Prof. Mitsutake (Nagasaki University: genome analysis of radiation effects using cultured human cells), Prof. Wakayama (University of Yamanashi: genome analysis of somatic cell nuclear transfer mice), Prof. Gondo (Tokai University: genome analysis of the transgenerational effects of low-dose radiation exposure), Associate Professor Sugo (Osaka University: analysis of genomic mutations caused by demethylation abnormalities), and Prof. Kaminuma (Hiroshima University: genome analysis of the effects on mouse blood). Many of the studies have already yielded mostly positive results. Each of the above studies will be submitted as a co-authored paper in FY2023. (Uchimura et al., Genome Research, 32[5] 945-955, 2022, doi: 10.1101/gr.276363.121.). (PI: Uchimura, new RP)

- Radiation-induced mutations in mouse spermatogonia cells in culture. To mechanistically understand how radiation exposure induces mutations in spermatogonia stem cells and how their mutations transmit to the next generation, we have initiated an in vitro culture approach to examine mouse spermatogonia cells (hereinafter GS, germline stem cells). The cultured GS cells were X-ray irradiated and surviving cell colonies were recovered. Structural changes of the genome were analyzed by aCGH (Macrogen/Agilent standard methods) for each 5 clones of control (unirradiated), 2-Gy-irradiated and 4-Gy-irradiated GS cells. For the entire genomic sequencing, short-read WGS was conducted in control and X-irradiated GS cell clones to detect radiation-induced SNVs and small InDels and multi-site mutations. aCGH analysis revealed only one deletion in irradiated clones that appeared to be mediated by NHEJ. In the WGS, 4-Gy exposed clones showed apparent 2.5 and 4 hold increase of multi-site and deletion mutations, while SNVs and insertions showed only slight increase. Interestingly, while these InDels detected in the unexposed controls largely derived from repeat sequence, radiation-associated changes were largely occurred in the unique sequence, suggesting a role of NHEJ in the radiation-associated mutagenesis in GS cells. These results are summarized for publication. Since large size structural changes were rarely detected, we planned to artificially create large deletions and/or translocations by the use of CRISPR/Cas9 system (Noda, Hamasaki, Satoh and Uchimura). PI: Noda, partially supported by MEXT grant No. 20K12179

Radiation Biodosimetry

- In order to evaluate the dose dependent effects of A-bomb radiation on humans, a cytogenetic biological dosimetry study has been conducted over several years for a subset of A-bomb survivors in the AHS cohort. A total of 1,868 survivors (1,179 in Hiroshima and 689 in Nagasaki) were examined using the 2-color-FISH method for detecting the frequency of stable translocations involving chromosomes 1, 2, and 4. The dose response from FISH data showed a wide scattering of individual translocation frequencies in both

cities as we observed in the previous solid Giemsa staining study. Differences between the two cities remained significant but was much reduced suggesting the large city difference in the past study was mainly due to different aberration detection rates between Hiroshima and Nagasaki laboratories. The city difference was not significant when Nagasaki factory workers were excluded from the analysis. The results suggested that radiation-shielding was still a significant dose-effect modifier but neither sex, city, nor smoking was significantly associated with background rate. We published these analyses this year. Sposto et al., Radiation Res., in press. (Kodama, Hamasaki, Cordova, Cullings). PI; Kodama.

Radiation and Cancer

- Epidemiological studies have demonstrated that radiation exposure has led to an excess relative risk of hepatocellular carcinoma (HCC) among A-bomb survivors. Certain mouse strains are also susceptible to radiation-induced HCC. We have established a new isolation method of hepatic stellate cells from 1-week-old mice to evaluate the direct effects of irradiation on the expression of inflammatory cytokines and senescence-related molecular markers in the cells using *in vitro* irradiation experiments. As a result, real-time PCR and ELISA analyses of isolated stellate cells indicated the elevated levels of inflammation marker, CCL5, in a dose-dependent manner after irradiation. In 2022, we have set up an experimental system to histologically analyze mouse livers. By using this experimental system, increased expression of CCL5 was directly observed with glial fibrillary acidic protein (GFAP)-positive hepatic stellate cells in mouse liver sections 1 week after X-irradiation. We now plan to prepare new RP to investigate the morphological changes of inflammatory hepatic stellate cells and macrophages in liver steatosis and fibrosis using mouse model systems. (Taga, a manuscript in preparation).

- Preliminary studies of chromosome aberration frequencies in hematopoietic stem cells (HSCs) following fetal irradiation of mice were conducted to test the hypothesis that radiation effects on the induction of persistent chromosome translocations may change in the stages of fetal development, namely, whether or not the stem cells have already settled into their niche. As a first step, we examined whether reciprocal translocations are induced in fetal HSCs soon after irradiation. Pregnant mice (E12.5-15.5d) were exposed to 2Gy of X rays and sacrificed 1 day later to collect fetal liver. The isolated HSCs were aliquoted into the wells of a 96-well plate (1 cell/well) to obtain single-cell derived colonies. Proliferated colonies were collected, and the cells were prepared for karyotype analyses using mFISH to determine translocation frequencies. mFISH result showed that 9 out of 43 (21%) fetal clones contained translocations. Our previous studies have shown that translocations in hematopoietic cells are rarely observed in adult after in utero exposure, but this result showed that translocations are observed in fetal hematopoietic stem cells soon after exposure. However, their frequency tended to be lower than that of mothers. Such abnormal cells bearing translocations start to be cleared at very soon after exposure, resulting in a state in which almost no cells with translocations are observed in the adult, except for cases of clonal expansion. A summary report based on the current data was published in the end of 2022. (Hamasaki et al., Journal of Radiation Research, doi.org/10.1093/jrr/rrac078). (Hamasaki). PI; Hamasaki.

- Since the blood specimens of approximately 25,000 A-bomb survivors who were all subjects of the Adult Health Study, including those who developed cancer early after the A-bombings, have been stored since the health examinations in 1958, genome analysis using these specimens, initially for GWAS studies, will enable us to elucidate the mechanisms of radiation-related cancer development in detail and to identify individual differences in susceptibilities to these cancers. There are complete blood samples from all AHS subjects, and a large amount of smear samples from blood tests performed during every 2-year examination are stored. To determine the feasibility of this approach, it is necessary to investigate the availability of smears prepared from trace amounts of blood specimens. In this study, we compared the ability of the Axiom Japonica Array NEO (AJAN) and the Infinium Japanese Screening Array (IJSA) to accurately identify SNPs in DNA extracted from smears prepared from blood specimens of six in-house volunteers. The call rates and concordances of the SNPs in these two SNP arrays were examined and compared using DNAs obtained from fresh-blood specimens (W-DNA) and DNAs extracted from smears prepared from the blood specimens and amplified with the QIAGEN REPLI-g DNA amplification kit (amplified DNA). As a result, the average call rates of W-DNA and amplified DNA for two SNP arrays were more than 99% and 96%, respectively, and concordances were more than 93%, but IJSA showed more reliable results with an average concordance of 99.7%. This preliminary study was completed in September 2022. Subsequently, a new preliminary study is being conducted using DNA extracted from fresh and previously stored blood specimens. DNAs were extracted from smears stored 10, 30, and 50 years ago, paper discs stored 20 years ago, and Giemsa-stained specimens stored 30 years ago. A whole genome amplification method using the REPLI-g amplification kit is currently under investigation.
  (Hayashi, Yoshida K, Ohishi, Yoshida N, Kato, Sposto, Tokunaga, Ueki, and Ozasa, RP-P, terminated by September 2022, and Hayashi, Ohishi, Brenner, Kato, Cologne, Yoshida N, Hamasaki, Kodama, Tokunaga, Ueki, Matsuura, Yoshida K, Tanabe, and Noda). PI: Hayashi.

- Preliminary studies (RP-P) have been conducted on the possible role of oxidative stress responses in protection against radiation-induced mutagenesis and oncogenesis. The aim of this study is to identify roles of the oxidative stress response pathway controlled by a transcription factor NRF2 in protection against radiation-induced mutagenesis using mou se models. Identification of factors that can protect against radiation mutagenesis could lead to the elucidation of molecular mechanisms of the radiation oncogenesis and the inter-individual variability in radiation risks, and could lead to the development of protective measures to reduce the health risks. In case of γ- and X-ray irradiation, the primary mechanism of the radiation mutagenesis is believed to be the DNA damages induced by reactive oxygen species (ROS) derived from ionized water. Reportedly, activation of NRF2, the master transcriptional activator of antioxidant genes, significantly reduces the acute radiation toxicity. We examine the mutagenic effects of X-irradiation on wild-type control mice and two mutant mouse lines, one with the NRF2 null mutation and the other with the constitutive NRF2 activation due to diminished expression of a Keap1 protein, which is an endogenous inhibitor for NRF2. Thereby we determine whether or not modulation of NRF2 activity in mouse can affect the mutagenic effects of X-irradiation. Wild-type mice and the two mutant mouse lines are exposed to whole-body X-irradiation, and then single

hematopoietic stem cells (HSCs) are isolated by fluorescence-activated cell sorting from bone marrow for clonal propagation. DNA samples extracted from the clonal cell populations derived from single HSCs are subjected to whole genome sequencing (WGS) for identification of somatic mutations using sequence data of matched tails as a reference to differentiate germline variants. From these analyses, the mutagenic effects of X-irradiation are determined, and the effects of NRF2 loss or activation will be evaluated. From these analyses, we should be able to test whether or not the oxidative stress response pathway controlled by NRF2 plays any role in protection against radiation mutagenesis. As an initial step toward these goals, we elucidated spectra and frequencies of spontaneous and X-ray-induced somatic mutations in HSCs from wild-type mice. Single nucleotide variants (SNVs) and small indels were the most common types of somatic mutations, and increased up to 2 to 3-fold by whole-body X-irradiation. Analysis of base substitution patterns in the SNVs actually indicated a role of ROS in radiation mutagenesis. Most of spontaneous small deletions were shrinkage of tandem repeats, and X-irradiation specifically induced small deletions out of tandem repeats (non-repeat deletions). Presence of microhomology sequences in non-repeat deletions suggested involvement of microhomology mediated end-joining repair mechanisms as well as nonhomologous end-joining in radiation-induced DNA damages. We also identified multisite mutations and structural variants. The radiation-specificity of each mutation type was evaluated from the spontaneous mutation rate and the per-Gy mutation rate estimated by linear regression, and was highest with non-repeat deletions, followed by multisite mutations and structural variants; these types of somatic mutations were thus revealed as mutational signatures of ionizing radiation. A manuscript describing these results was submitted for publication. This study is also expected to be a model for future studies to characterize radiation-induced somatic mutations in human by WGS analysis of preserved blood samples donated by A-bomb survivors. (Tanabe O, Matsuda Y, Kajimura J, Yoshida N, Sposto R, Kato N). PIs: Tanabe and Matsuda. Partially supported by MEXT grant No. 19K12338 and 22K12388 (to O.T.).

- A histopathological study using autopsy specimens long stored in RERF is planned. For this, a pathological specimen preparation device and mass spectrometer were introduced, and a protein analysis protocol for formalin-fixed tissue is being trialed. Experimentally, animal tissue specimens are prepared, and a protocol is developed to detect differences in tissue quality due to the effects of fixation conditions. Shimadzu's MALDI-TOF MS 8030 was installed this year in the laboratory to evaluate the oxidation and degradation of proteins in tissues due to aging and the effects of radiation exposure. For these initial studies we have been able to detect oxidation of methylene bridges in fixed samples of model animals. We are currently quantifying the oxidized protein using LC/MS spectrometry. We are investigating if oxidation is available as an index for evaluating sample deterioration. The primary mass spectrometry imaging (MSI) technique will be developed with the Shimadzu corporation. Results are summarized in the latter half of 2023. Chemical blotting onto the conductive slide-glass from the usual slide-glass promotes MSI, and we improved the sensitivity in imaging. We used a cervical cancer HeLa cell line exposed to radiation as a model and found that cell injury was detectable by MSI. Phosphatidylserine was one of the candidates for cell injury. (Tsuruyama, Ito, new RP or SOP in 2023)

Radiation and Immunologic Effects

- Reactive oxygen species (ROS) play an important role in immune responses, yet excessive production and accumulation of ROS may increase the risk of inflammation-related diseases. We previously found that elevated levels of ROS in certain blood cells with increasing radiation dose in atomic bomb (A-bomb) survivors were associated with increased inflammatory status. In this study, we examined the effects of aging and radiation exposure on intracellular ROS levels and percentages of T-cell subsets in a total of 10,540 samples obtained from repeated biennial examinations of 3,963 Hiroshima and Nagasaki A-bomb survivors who participated from 2008 to 2016. The percentages of T-cell subsets and intracellular ROS levels were measured by flow cytometry using a combination of both fluorescently labeled antibodies and fluorescent reagents, Carboxy-DCFDA and hydroethidine. The results showed that the percentage of naïve $CD4^+$ or $CD8^+$ T cells decreased with age and radiation dose and that intracellular $O_2^{\cdot-}$ levels in certain $CD8^+$ T-cell subsets increased with age and radiation dose. In addition, when the subjects were divided into three groups according to the percentage of naïve $CD4^+$ T cells, the intracellular $O_2^{\cdot-}$ level of certain $CD8^+$ T-cell subsets increased significantly with age and radiation dose only in the group with a low percentage group of naïve $CD4^+$ T cells. These results suggest that radiation-induced decrease in naïve $CD4^+$ T-cell pool size may lead to decreased immune function, resulting in increased intracellular ROS levels in certain $CD8^+$ T-cell subsets.
  (Hayashi, Kato, et al. RPs, terminated in 2018.). PI: Hayashi, a manuscript in preparation.

Radiation and Other Noncancer Conditions

- Clonal hematopoiesis (CH), potentially associated with radiation exposure and increased risks of inflammatory diseases, is of substantial interest to the scientific community. To develop strategies for assessments of CH linking to radiation-associated noncancer diseases, specifically arteriosclerosis as a part of CH program project, we conducted preliminary experiments to establish one or more mouse models that can test the hypothesis that CH in irradiated mice is involved in pro-inflammatory phenotypes and can promote atherosclerosis formation. Preliminary mouse experiments ensured an extremely high prevalence of CH in 3-Gy whole-body irradiated mice, and CH in each of the irradiated mice contained multiple clones that expanded to collectively comprise 60-80% of a whole population of bone marrow nuclear cells, suggesting that high-dose radiation can induce massive hematopoietic cell generation and proliferation from a tiny number of stem/progenitor cells. We also examined longitudinal trajectories of CH mutations in irradiated mice by using longitudinally-collected blood cells. The blood of irradiated mice exhibited elevated levels of both pro-inflammatory myeloid cells and red blood cell distribution width (RDW), which is often observed in human populations having CH. These preliminary study results have been published in Scientific Reports (2022). To assess a feasibility study to examine CH and atherosclerosis formation in LDLR-knockout mouse model, we have initiated a preliminary experiment assessing clonal hematopoietic cell populations in the bone marrow, the peripheral blood, and the aorta of LDLR-knockout mice irradiated with 3 Gy and fed with a high-fat diet (Yoshida, Kusunoki et al., CR155). PI; Yoshida. Scientific Reports 12: 17276 (2022) doi: 10.1038/s41598-022-21621-6

- To investigate whether radiation-associated T-cell alterations are involved in reduced cancer immunosurveillance and hence in elevated cancer risk in A-bomb survivors, we are analyzing an association between the percentages of lymphocyte subsets including naïve CD4 T (Tn) cells and cancer development up to 2015, using longitudinal (1988-2011) and baseline (1992-1995) data among 700-800 AHS population. However, recent reports suggest that a subset of Tn cells expressing a high level of CXCR3 act differently from conventional Tn cells, i.e., $CXCR3^{high}$ Tn cells contribute to inflammatory response that may be related to reduced cancer immunosurveillance. Thus, we examined relationships between age, radiation dose, and the proportion of $CXCR3^{high}$ cells in Tn cells among these AHS population and found that the $CXCR3^{high}$ Tn proportion was positively associated with age and radiation dose as well as the plasma levels of proinflammatory cytokines, CXCL10 and IL-6 (manuscript in preparation). This result suggests that radiation and aging expand Tn cells poised for inflammation potentially impeding cancer immunosurveillance and that $CXCR3^{high}$ and $CXCR3^{low}$ Tn subsets should be separately investigated for our cancer risk assessments based on T-cell data. Information to be obtained from the preliminary cancer immunosurveillance study will be utilized to design a future full-scale immunosurveillance study among 3,000 AHS participants. (Yoshida, Kusunoki et al.,, manuscript in preparation). PI; Kusunoki.