DEPARTMENT OF MOLECULAR BIOSCIENCES

Mission and Specific Objectives

The Department of Molecular Biosciences (MBS) supports the mission of RERF by conducting molecular mechanistic studies to elucidate and inform radiation effects observed in clinical and epidemiological studies of RERF's cohorts of A-bomb survivors and their offspring. MBS's cross-disciplinary studies are conducted in collaboration with other RERF departments and external research institutions. The department consists of four laboratories: Molecular Genetics, Cellular Genomics, Molecular Pathology, and Immunology. A few MBS researchers also hold a dual appointment with the Biosample Research Center (BRC). The main objectives of the Department are to elucidate the molecular mechanisms of the genetic effects of radiation, radiation carcinogenesis, and non-cancer diseases related to radiation exposure. Based on these objectives, the current research program is divided into four categories.

1. Analysis of radiation effects at the genomic level, i.e., macro (chromosome aberration) or micro (DNA sequencing), for somatic- and germ-cell exposure.
2. Analysis of the dynamics of cells that have survived radiation exposure, especially the relationship between clonal cell proliferation during the process of health recovery after the atomic bombing and persistent inflammation leading to disease development.
3. Elucidation of molecular mechanisms of radiation carcinogenesis with a focus on "-omics" analyses using pathological tissues and blood samples obtained from survivors, and the identification of biomarkers of radiation exposure.
4. *In vitro* and *in vivo* model experiments using cells and animals to verify hypotheses of molecular mechanisms.

Department Resources

MBS collections of blood samples of exposed parents and their children have recently been transferred to the Biosample Research Center (BRC) where they are now being centrally managed. MBS also has a large volume of data from periodic chromosome analysis and lymphocyte subset composition analysis in the Adult Health Study (AHS) over many years, as well as analysis data on blood factors such as cytokines.

Equipment and technical resources include a secure human-genome analysis room (based on IBM cloud computing), animal experiment facility, radioisotope (RI) experiment facility, P2 (Physical containment level 2) experiment facility, X-ray irradiation equipment, chromosome analysis room, and cell-sorting equipment. DNA sequencing and pathology specimen preparation rooms are also being established. These resources will be further developed to serve collaborative research across RERF and with external research institutions. With RERF's planned relocation to Hiroshima University's Kasumi Campus, the animal experiment facility and the RI facility will be discontinued and merged with the research program at the Joint Usage Facilities of Hiroshima University.

Internal and External Collaborations

The department has many years of experience in the analysis of chromosome aberrations and has contributed to the development of international biodosimetry methods and utilization of tooth-enamel, electron spin resonance (ESR) analysis, including through the Biodosimetry and Emergency Exposure Network (WHO REMPAN and IAEA-HICARE Cytogenetics Program), and the Genetic Effects Committee of the International Commission of Radiation

Protection (ICRP). Adopting recently developed whole genome analysis (WGS) technology incorporated with our own proprietary technologies, we have promoted collaborative research not only within RERF but also outside the organization, with the Institute for Environmental Sciences (Aomori), Osaka University, Nagasaki University, and other organizations in the areas of transgenerational effects using laboratory animals and whole genome analysis of exposed somatic cells. Our Trio Study design and analysis team comprises investigators from every department within RERF, as well as RIKEN, the University of Tokyo and the U.S. National Cancer Institute (NCI). Further support is being provided by Hiroshima University and Nagasaki University with respect to genetic counseling regarding incidental secondary findings from the Trio genome studies.

Preliminary studies informing the Adult Health Study-Genome Wide Association Study (AHS-GWAS) are currently underway in collaboration with the National Center for Global Health and Medicine (NCGM), Hiroshima University, and Nagasaki University, while a clonal hematopoiesis program to clarify the relationships between clonal hematopoiesis and the healing process, as well as the relationship between clonal hematopoiesis and inflammatory diseases during that process, is underway in concert with Nagasaki University, Kyoto University, and the University of Tokyo. Preliminary work using stored pathological tissues to search for biomarkers of radiation exposure has begun in collaboration with the private-sector Shimadzu Corporation and Kyoto University.

FY2023 Departmental Highlights

Radiation and Genetic Effects

- Elucidating the hereditary genetic effects of A-bomb radiation has been a key question since the establishment of RERF, and we have long planned to analyze the whole genome of exposed parents and children to answer this question. In the past year, we started to obtain consent (re-consent) for participating trios and initiated whole-genome sequencing (WGS) as well as an adjustment-of-analysis pipeline in collaboration with NCI and RIKEN based on a small number of families. Analyses will be performed using a secure external cloud server (Uchimura, RP 3-23, in progress).

- To increase awareness and acceptance of the Trio Study from research participants and the public, RERF held meetings with an external advisory committee in (Aug 2023 and the media in November 2023 (Uchimura, RP 3-23, in progress).

- We have developed new technology to detect large-scale chromosomal reorganization possibly related to human disease. Combining short-read and long-read sequencing technologies in exposed mouse families as a model experiment has allowed the capture of structural variants (SVs) with unprecedented precision, enabling the estimation of SV frequency in successive mouse generations and the effects of parental exposure (manuscript in preparation, Satoh, RP 2-13, RP S3-11, in progress).

Radiation and Cancer

- GWAS studies on A-bomb survivors can lead to a better understanding of individual radiation-related risk of carcinogenesis and other health outcomes. In preparation for the planned AHS-GWAS (Hayashi, RP P2-22, in progress), feasibility studies to evaluate genomic DNA extraction and genotyping were conducted using old blood smears and chromosome preparations obtained from A-bomb survivors. While DNA was successfully extracted from recent smear samples, the quality of the extracted DNA was much lower for samples older than 10 years. However, use of the REPL-g multi-combination system led to improved results, resulting in acceptable power even in a single nucleotide polymorphism (SNP) array testing conducted with 720,000 probes (Hayashi, RP P2-22, in progress).

- Induction of somatic cell mutations by radiation is often indirectly mediated by reactive oxygen species (ROS). Bone marrow stem cells from X-irradiated mice were extracted and cloned *in vitro* for WGS, which showed typical ROS mediated radiation-signatures of mutations, as well as broken-end rejoining between unique sequences through non-homologous end joining (NHEJ). The resulting paper was published as the world's first study to comprehensively identify radiation-induced somatic mutations occurring in vivo using WGS technology (Tanabe, Matsuda, RP P3-19, in progress).

- At the chromosome observation level, the effects of fetal exposure to A-bomb radiation disappear after birth. Fetuses are thought to have an intrinsic mechanism for the elimination of abnormal cells. However, we observed hematopoietic stem cells with translocations in

mouse fetuses one day after exposure (Hamasaki, RP P4-17, in progress, page 28). We also showed that morphogenesis and double strand break (DSB) repair are coupled during the morphogenic stage, a process that suppresses fetal malformations. These results were published (Noda, RP A4-09, in progress).

- To elucidate the mechanism of pathological radiation carcinogenesis and to explore new biomarkers, we are investigating the possibility of using old autopsy samples stored at RERF. In a preliminary analysis using formalin-fixed paraffin-embedded (FFPE) cancer tissue microarrays, we detected marker proteins in using liquid chromatography-mass spectrometry (LC/MS) and MS-imaging (marker proteins characteristic of specific cancer types). The results were published (Tsuruyama, non-RP report, page 44). In addition, MS was performed on FFPE samples with different fixation and storage conditions, and among the 1,413 proteins detected, those showing increased or decreased detection depending on specific conditions were determined (Tsuruyama, non-RP report, manuscript submitted).

- Hepatic stellate cells surrounding liver parenchymal cells are thought to play an important role in radiation-induced hepatocarcinogenesis. The expression of inflammatory cytokine CCL5 was found to be increased in liver stellate cells of irradiated mice (Taga, non-RP report, manuscript submitted).

Radiation and Immunologic Effects

- Radiation exposure decreases the proportion of naive T-cell (Tn) subpopulations and the diversity of T-cell receptor (TCR) Vβ repertoire in T cells, which might be related to impaired immune regulation of carcinogenesis. In the AHS, the proportion of $CXCR3^{high}$ Tn, a subpopulation of Tn, showed a significant increase with radiation dose and was associated with increases in blood IL6, IL7, CXCL10, and CRP, suggesting possible involvement of the Tn subpopulation in inflammation and inflammatory diseases including cancer in A-bomb survivors. The results were published (Kusunoki, RP P1-22, in progress).

- Based on published reports that lamin B1, a nuclear membrane protein, decreases with aging and radiation exposure while LINE-1 conversely increases, we plan to examine these markers in biosamples from the survivors. A decrease in nuclear lamin B1 protein and an increase in LINE-1 expression with age were suggested in a preliminary study in non-exposed volunteers, but those observations were not reproduced in CD4 T cells or other cells in another study population (Yoshida K, RP P1-21, in progress).

Radiation and Other Noncancer Conditions

- Our department's clonal hematopoiesis (CH) study was initiated using stored blood samples obtained from AHS participants to examine the occurrence of CH in the survivors and its possible relevance to the diseases. We have already obtained informed consent (IC) and are analyzing data of somatic mutations detected by whole exome sequencing (WES). A further study using stored blood samples collected over time is planned to clarify the dynamics of hematopoietic cell proliferation and expansion during the process of health recovery after the atomic bombing (Yoshida K, RP 1-23-1, in progress). Methods

to characterize and track clonal hematopoiesis following radiation exposure have been established in collaboration with outside universities (Kusunoki, RP 1-23-3, in progress).

- Oxidative stress is said to promote a decline in immunity and aging of individuals. When reactive oxygen species (ROS) were measured in 3,752 A-bomb survivors, a decrease in the number of naive CD4+ T cells and an increase in ROS in memory CD8+ T cells were observed in the high-dose exposure group. The resulting manuscript showing that ROS are involved in the long-term decline in immune function due to radiation exposure was published in 2023 (Hayashi, RP 2-75, 3-07, 4-02, in progress).

Radiation Biodosimetry

- Biological dosimetry using A-bomb survivor chromosome aberration (mainly stable translocations) frequencies as markers of radiation exposure could assist and improve individual physical dose calculations. The project, which has been in effect since 1966, incorporating fluorescent *in situ* hybridization (FISH) technology in 1989, has resulted in a total of 1,868 measurements to date. In the latest analysis utilizing recent dosimetry DS02R1, the difference in dose response between Hiroshima and Nagasaki was much less pronounced than in previous analyses, especially at doses < 1.25 Gy. However, there was still a persistent difference in dose response by shielding category, which points to remaining problems with the accuracy or precision of radiation dose estimates in some A-bomb survivors. Similar results were obtained when neutron contribution was subtracted at low doses, but when sorted out by shielding category, the mean slope of linear regression often showed a rather lower slope in Nagasaki. We published a first report in 2023, the second is in preparation (Kodama Y, RP 2-66, 3-85, 10-89, and 8-93, in progress).