FY2011 Highlights in Research Progress

Clinical Studies

Initiation of a new study on cognitive function among the Adult Health Study (AHS) population

We recently initiated a study of late-life neurocognitive function among AHS subjects in Hiroshima and Nagasaki who had been exposed to A-bomb radiation *in utero* or at 12 years of age or younger. The fetal stage and childhood are the most important periods in terms of brain growth and development, and previous studies have reported significant impact of radiation on intellectual capacity. However, the previous cognitive function studies targeted only subjects exposed at 13 years of age or older. The present study examines cognitive function by way of person-to-person interviews in health examinations as well as a self-administered questionnaire in the mail survey phase of the AHS. Assessment results regarding two types of cognitive function in about 1,000 subjects are expected to be obtained from this study.

A prospective follow-up study of the association of radiation exposure with fatal and non-fatal stroke among atomic-bomb survivors in Hiroshima and Nagasaki (1980–2003)

We investigated stroke incidence in relation to atomic-bomb radiation dose based on a 24-year follow-up (1980–2003). Study subjects were 9,515 atomic-bomb survivors (males: 34.8%) who were free from prevalent stroke in 1980, when the follow-up started. Stroke events and deaths were reviewed to confirm first-ever events, and subtypes (ischemic/hemorrhagic stroke) were categorized based on established criteria according to the definitions of typical/atypical stroke symptoms. We confirmed 235 hemorrhagic stroke cases and 607 ischemic stroke cases. The risk of hemorrhagic stroke incidence for men (after adjustment for risk factors such as age, blood pressure, and smoking) increased in a linear dose-response relationship from <0.05 to ≥2 Gy (11.6/10,000 person-years [PY] → 29.1/10,000 PY, $p = 0.009$), and incidence also rose within the dose range <1 Gy ($p = 0.004$). In women, no increased risk was observed within the dose range <1.3 Gy (13.5/10,000 PY), while it increased to 20.3/10,000 PY for doses 1.3–2.2 Gy and to 48.6/10,000 PY for doses ≥2.2 Gy ($p = 0.002$). In both sexes, dose was unrelated to ischemic stroke risk.

Genetics

Irreparable radiation-induced DNA damage

Effects from radiation exposure are caused by cellular tissue damage including injury of somatic and germ cells in irradiated individuals. Such cell damage is attributable to irreparable radiation-induced genome damage that persists as long as the cells or tissues exist in the body. Using normal human cells in our analysis, we determined that irreparable radiation-induced DNA damage after exposure had remained in cells for the duration of our examination (at least one year of continual observation). In animal experiments, we also detected such irreparable DNA damage in various tissue types over a fairly significant period of time after irradiation. Biochemical discrimination between irreparable damage (permanent damage) and repairable damage (typically, complete repair within 24 hours) may provide in the future novel opportunities to re-examine the archival tissue samples of A-bomb survivors and detect traces of such DNA damage that remain from the time of the bombings.

Pilot study of comprehensive analysis of radiation-induced mutations via whole genome sequencing

The recent remarkable progress in sequencing technologies has reduced the cost of commercially available whole human genome sequencing (WGS) services (¥500,000/sample). RERF's Laboratory of Biochemical Genetics planned a comprehensive analysis using WGS of radiation-induced mutations, from single base substitutions to large gene deletions. Experimental testing using cultured cells obtained from Japanese subjects was conducted. WGS was carried out for pre-irradiated cells and post-irradiated cultured cell lines. The human genome consists of a huge number of as many as three billion bases. With WGS services, such a huge genome is provided as 1.5 billion sets of short 90-base sequences (equivalent to at least 30 times the size of the human genome). These 1.5 billion short-sequence sets need to be joined together using a computer program to reconstruct the original complete three-billion-base genome. This work is currently carried out under the instruction of Dr. Akihiro Fujimoto of the Institute of Physical and Chemical Research. Before too long, the entirety of radiation-induced mutations may be fully understood.

Information Technology

The Information Technology Department has further carried out the server virtualization since last fiscal year, and there are 23 virtual servers currently in operation, including such platform servers as external WWW servers and Adult Health Study database servers. Server virtualization is expected to help reduce the expenses required for the update of hardware and achieve greater durability of service.

We have introduced Linux (x86) servers to replace the Unix (Solaris OS) servers wherever feasible because Unix servers, which were the main technology used in the past, required high operating costs. We also virtualized non-migratable Unix servers based on Solaris platforms, thereby greatly reducing operating costs.

Furthermore, all servers installed at the Nagasaki Laboratory were moved to the Hiroshima Laboratory, and the auto-recovery functions in the Nagasaki's server room were strengthened to safeguard the data in case of major disasters.

All of the Ethernet network layer-2 switches, which were installed in each of the Hiroshima Laboratory buildings, were replaced with new Ethernet gigabit switches in preparation for anticipated future increases in traffic. At the Nagasaki Laboratory, replacement of such switches was completed last fiscal year.

Since the use of cloud computing, an emerging mainstream computer environment, is expected to reduce operating costs, we will carefully watch such technologies and related market trends from now on.

FY2011 Highlights in Research Progress

Radiobiology/Molecular Epidemiology

Evaluation of systemic markers of inflammation in atomic-bomb survivors with special reference to radiation and age effects

Using the newly developed automated plasma reactive oxygen species (ROS) assay system, we measured ROS levels in the plasma of Adult Health Study participants. By comprehensively evaluating eight inflammation-related cytokines/markers including the previously measured plasma levels of interleukin (IL)-6, tumor necrosis factor (TNF)-α, C-reactive protein (CRP), IL-4, IL-10, immunoglobulins (Igs), and erythrocyte sedimentation rate (ESR), we examined the combination of cytokines/markers that can best represent asymptomatic inflammatory status of A-bomb survivors. Our results indicate that the linear combination of ROS, IL-6, CRP, and ESR offers the best representation of inflammatory status, and that the scoring of this group of cytokines/markers can show effects of radiation and aging on inflammatory status more clearly than the conventional analysis using a single marker. The results strongly support our hypothesis that radiation exposure together with natural aging may accelerate the persistent inflammatory status observed in A-bomb survivors.

Improved polymerase chain reaction (PCR) amplification for molecular analysis using DNA from long-term preserved formalin-fixed, paraffin-embedded (FFPE) tissue specimens

Archival tissue specimens have become an invaluable source of material for molecular analyses in retrospective studies, especially for diseases associated with exposure to uncommon environmental events (e.g., radiation exposure). Although successful PCR amplification is essential for molecular analysis of DNA from archival FFPE tissue specimens, we too often encounter problems with poor PCR amplification quality. To overcome this issue, we have established an improved method for efficient PCR amplification by pre-treatment of DNA with borate buffer (pH 11.0) at 100°C for 30 minutes.

Epidemiology

The health risks of radiation among the atomic-bomb survivors

The risk of radiation for all solid cancer death increased linearly compared to non-exposure by 42% at age 70 after exposure to atomic-bomb radiation of 1 Gy at age 30 in the Life Span Study (LSS) subjects, and the risk was higher among those exposed at a young age. The risk of radiation for urothelial cancer death increased linearly by about 100% per Gy, but no positive interaction between effects of radiation and lifestyle including smoking was observed, which was different from the observation for lung cancer. Confounding of smoking and other lifestyle factors for radiation risk of urothelial cancer was not observed in the LSS subjects. The risk of radiation for bone sarcoma death increased linearly by about 750% per Gy at the dose range higher than the threshold of 0.85 Gy (collaboration with University of Hong Kong). The risk of radiation for chronic renal failure death increased by 9% at 1 Gy, and this result may explain a part of the increased risk of radiation for cardiovascular diseases (collaboration with Rochester University). The associations of radiation with noncancer respiratory and digestive diseases require further investigation into whether they are causal or artifactual.

Statistics

Radiation risk assessment and dosimetry

In the area of risk assessment, Statistics researchers completed a new manuscript on risk of hematopoietic malignancies, published a paper on long-term risk of thyroid cancer in collaboration with researchers at the U.S. National Cancer Institute, and played major roles in a new risk analysis of cataract surgery and a study of the interaction of smoking and radiation in causation of lung cancer by histological type. They also continued work on multi-model inference in collaboration with investigators from the Helmholtz Centre Munich, Germany. They contributed to a new Clinical Studies research protocol (RP) to study whether radiation plays a role in the effect of weight cycling on mortality, which will include collaboration with Kurume University on statistical research related to the use of functional covariates in proportional hazards regression. Members of the Statistics Department also contributed to a number of other papers on risk assessment using established statistical methods.

In the area of dosimetry, members of Statistics continued to support the Dosimetry Committee's efforts, particularly in improvement of survivor location estimates, including methods for improved calculation of terrain shielding of survivors in new locations estimated by precisely locating survivors' shielding history neighborhood diagrams on specially constructed mosaics of pre-bombing aerial photographic maps of the cities. Members became active in initiating geospatial and other analyses to support the Committee's efforts to address topical issues raised by outside researchers, regarding residual radiation sources (neutron-activated soils near the hypocenters and local radioactive fallout) and continued collaborative work with external investigators on dose error, biodosimetry, and neutron dose weighting factors.

Statistical methodology for other RERF studies

Other statistical methodological research included analyses comparing effects of *p53BP1* gene polymorphisms on cancer and somatic mutation (glycophorin A) using multiple imputation of missing data for haplotype estimation, a paper on choice of time scales in Cox regression of epidemiologic follow-up data, which is in press, and a paper providing a comprehensive review of conventional methods for case-cohort study design and analysis (such as the Radiobiology/Molecular Epidemiology study of immunogenes and cancer). Members of Statistics also continued collaborative research with external investigators on a method of extrapolating survival estimates. Members of Statistics assisted other scientists in the use of specialized statistical methods in analyses for numerous manuscripts and provided statistical consultation on designs and power calculations for several new RPs.

Research Progress by Project

Cardiovascular Disease Study

The issue of whether A-bomb radiation exposure causes cardiovascular diseases has attracted much attention from those concerned, leading to many of RERF's research results being detailed in Attachment B of a 2006 report by the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR, 2008).

To address this issue comprehensively based on research in the areas of epidemiology as well as clinical and basic medicine, RERF established the Cardiovascular Disease Study Working Group, consisting of the Chief of Research, chief scientists, department chiefs, and research scientists. The group started working as a 'project team' in 2008. By FY2011, the team began putting up results in a wide variety of work, including (1) summarizing of the results of all the studies conducted at RERF and streamlining the specific research hypotheses to be verified; (2) initiation of review of new studies to be conducted in the future by examining details of a study on arteriosclerosis at the Department of Clinical Studies and the possibility of animal experiments; (3) reporting at the Scientific Council meeting held at the end of FY2009 of research plans regarding stroke, chronic kidney diseases, arteriosclerosis markers, valvular heart diseases, and related biomarkers and immunological functions; and (4) in FY2010 initiation of animal experiments using spontaneous hypertensive rats and a clinical study to investigate in more detail the possible involvement of cytokines.

In FY2011, we continued the above project (4) and completed writing of a new research protocol on radiation exposure and valvular heart disease using echocardiography. The RP is currently under internal RERF review.

Collaborative Immunology Study

Immunosenescence is the gradual deterioration of the immune system with aging and a contributing factor in the increased frequency of morbidity and mortality observed among the elderly due to numerous aging-related diseases. Based on the evidence accumulated from RERF's immunology studies, we hypothesize that radiation exposure accelerates aging of the immune system and may lead to augmentation of immune dysfunction and other adverse health outcomes in A-bomb survivors. With funding provided by the U.S. National Institute of Allergy and Infectious Diseases (NIAID), RERF researchers started in 2009 a five-year collaborative study with four Japanese and five U.S. institutions, with the aim of enhancing their efforts to elucidate mechanisms underlying radiation-induced immunosenescence and understanding this phenomenon's implications in the various diseases experienced by A-bomb survivors.

The research program consists of five focused projects that aim to elucidate radiation and aging effects on 1) hematopoietic stem cells and their microenvironment in T-cell development, 2) the role of dendritic cells in T-cell responsiveness and function, 3) immune responsiveness to influenza vaccination, 4) a multivariable assessment of immune parameters to create an integrated scoring system of immunity, and 5) thymus architecture and function. The achievements of this collaborative study in FY2011 were as follows: i) microassay systems to evaluate phenotypes and functions of human hematopoietic stem cells were established, and analysis using AHS blood samples was initiated (project 1); ii) microarray assays to examine *in vitro* dendritic cell functions were established, and analysis using AHS blood samples was initiated (project 2); iii) the FY2011 influenza vaccination full-scale study was conducted in collaboration with RERF's Department of Clinical Studies and the Hiroshima City Medical Association, using paired blood samples collected pre- and three weeks post-vaccination from 150 AHS subjects (project 3); iv) an assay measuring intracellular reactive oxygen species in the various lymphocyte subsets was established as part of project 4; and v) a database on thymus autopsied specimens stored at RERF was developed in collaboration with the Department of Epidemiology, and assessments of the availability and quality of the specimens for pathological and molecular analyses were initiated (project 5).

Collaborative Cancer Studies

RERF-NCI collaborative cancer pathology studies

Thyroid cancer cases occurring between 1996 and 2005 have been histologically reviewed in addition to the already reviewed cases occurring between 1958 and 1995. Based on the results, analysis was conducted of radiation risk on thyroid cancer by histopathology occurring between 1958 and 2005. An investigation started for radiation effects on "intrinsic subtypes" of breast cancer based on intracellular hormone receptors. A study of radiation effects on breast cancer-related serum biomarkers among cancer-free female survivors has been published, with a suggestion that radiation may differentially affect pre- and post-menopausal hormone levels (Grant EJ et al., *Radiation Research* 2011; 176:678–87). A study on the effect modification of smoking on radiation risk for lung cancer by histological type has been completed.

Genetic susceptibility to radiation-induced cancers

A series of host immune responses are thought to be involved in the course of disease after hepatitis C virus (HCV) infection (HCV clearance or development into chronic hepatitis), and progression of chronic hepatitis into hepatocellular carcinoma (HCC), with the assumption being that course of infection depends in part on inter-individual variance in immune responses. In A-bomb survivor cohort (Adult Health Study cohort), we found that *NKG2D* gene haplotype (being involved in immunological host defense against virus and cancer development and also in inter-individual difference in natural killer [NK] activity) was associated with the presence or absence of persistent HCV infection as well as HCC development. In addition, it was also suggested that radiation exposure as well, not only *NKG2D* haplotype, was involved in development of HCC.

Research Progress by Project

F_1 Clinical Study

A clinical study of the F_1 offspring of A-bomb survivors was conducted from 2002 through 2006 to examine the effects of parental radiation exposure on prevalence of adult-onset multifactorial diseases among F_1 subjects. The results of this study were published in a 2008 report. In the study, however, the average age of the F_1 subjects who underwent health examinations was young, at 49 years, just at the beginning of the age range in which diseases frequently occur. At the same time, the possible presence of cross-sectional study bias could not be ruled out. Thus, the Scientific and Ethics Committees for the Health Effects Study of the Children of A-bomb Survivors, the Scientific Council, and the Senior Review Panel recommended that a longitudinal study should be conducted. Based on these recommendations, RERF's F_1 Clinical Study Working Group, consisting of the Chief of Research, an executive director, chief scientists, department chiefs, and research scientists, had discussions to develop a new research plan, which was completed in FY2009. This research plan was approved by the first meeting of the Scientific and Ethics Committee for the Clinical Study of the F_1 Offspring of A-bomb Survivors in July 2010, and the longitudinal study commenced in November 2010, with the collaboration of the Department of Epidemiology, Department of Information Technology, and other departments.

The second meeting of the Scientific and Ethics Committee for the Clinical Study of the F_1 Offspring of A-bomb Survivors in January 2012 opened with a report on the first-year progress of the longitudinal clinical study and concluded with explanations about the present health examination items and draft revisions of an informed consent form and its explanatory notes concerning storage/use of biosamples, with the relevant revisions approved after the committee's deliberations. Additionally, based on the results of additional analyses examining individual multifactorial diseases with use of data from the original study examining disease prevalence, there was reportedly no evidence proving parental radiation exposure-related increases in respective disease risks.

Dosimetry

For estimation of radiation doses to which A-bomb survivors were exposed, maps created by the U.S. Army immediately after the war were used by RERF to identify hypocenter coordinates and survivor locations, but the issue of distortion in these maps has been raised for many years. Additionally, doses for about 7,000 people in the Life Span Study population were treated as "unknown" because DS02 could not be applied to their shielding conditions. To solve such problems in dose estimation, the Dosimetry Committee was established.

Last fiscal year, aerial photographs taken immediately before the A-bombings were modified based on their exposure angle, height, and lens aberration, as well as altitude of the photographed images to prepare one flat orthogonal aerial photograph consolidating all of the areas in question, with which locations in the standard coordinate system were identified. Based on reference points held in common between these maps and the U.S. Army maps, we formulated equations to convert coordinates on the U.S. Army maps to those on the aerial photographs covering both of the cities in their entirety. Further, with respect to proximally exposed survivors for whom neighborhood drawings showing their shielding histories were available, we established methods to reconfirm their exposure locations on the orthogonal aerial photographs, using geospatial information system (GIS) software.

This fiscal year, in addition to the abovementioned achievements, we established methods for considering effects from elevation and terrain shielding for the new locations and also have started the work of identification of individual exposure locations.