FY2013 Highlights in Research Progress

Clinical Studies

Association of serum IL-6 level and risk of hepatocellular carcinoma: A nested case-control study

It has been elucidated that hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, obesity, alcohol consumption, smoking habit, and radiation are independent risk factors for hepatocellular carcinoma (HCC). As a mechanism underlying the association between such factors and increased HCC risk, insulin resistance and inflammation may be involved in enhancement of carcinogenesis. Among atomic bomb survivors who participated in the Adult Health Study (AHS) between 1970 and 2002, we examined the association between C-reactive protein (CRP) or interleukin (IL)-6 levels and HCC risk, using stored sera available before diagnosis for 224 HCC cases and 644 controls that were matched to the cases on gender, age, city, and time and method of serum storage, and countermatched on radiation dose.

Analyses with adjustment for hepatitis virus infection, alcohol consumption, smoking habit, body mass index (BMI), and radiation dose showed that the association of CRP levels and HCC risk was not significant. Relative risks of HCC (95% confidence interval) in the middle and highest tertiles of IL-6 levels were 3.85 (1.16–14.7) and 5.12 (1.54–20.1), respectively, compared to the lowest tertile, and the association was stronger in non-B, non-C HCC. Higher serum levels of IL-6 were associated with increased HCC risk, independently of hepatitis virus infection, lifestyle-related factors, and radiation. The interaction of obesity with IL-6 levels in relation to HCC risk was significant, but that of radiation with IL-6 levels was not observed.

Glaucoma in atomic bomb survivors

A previous paper reported that glaucoma incidence in the Adult Health Study (AHS), based on medical history information, decreased with increasing A-bomb radiation dose. While glaucoma has been found in 7–11% of patients as a complication of high radiation dose (e.g., radiotherapy), it is not understood whether radiation at low-to-moderate doses gives rise to glaucoma. Systematic ophthalmologic examinations were conducted to evaluate the possibility of excess glaucoma cases in relation to A-bomb radiation exposure.

Of the 2,699 people who underwent health examinations in the AHS clinical program between October 2006 and September 2008, the prevalence of glaucoma in relation to estimated A-bomb doses was analyzed among the 1,589 people who participated in the glaucoma study. The study's results suggested that the prevalence of normal-tension glaucoma, but not high-tension glaucoma, may have increased in association with A-bomb radiation dose. The estimated odds ratio at 1 Gy was 1.31 (95% confidential interval: 1.11–1.53, P = 0.001) for normal-tension glaucoma. However, uncertainties exist in the results because of the study's low participation rate and the resulting possibility of selection biases. The findings therefore should be interpreted cautiously until confirmed by other studies.

Radiobiology/Molecular Epidemiology

A novel *RET* rearrangement (*ACBD5-RET*) in papillary thyroid cancer from an atomic bomb survivor exposed to high-dose radiation

A novel type of *RET* rearrangement, i.e., acyl-coenzyme A binding domain containing 5 (*ACBD5*)-*RET*, was identified in an atomic bomb survivor's papillary thyroid cancer (PTC) exposed to a high radiation dose. *In vitro* and *in vivo* experiments suggested that *ACBD5-RET* rearrangement caused constitutive activation of mitogen-activated protein kinase (MAPK) pathway and had oncogenic activity, as is the case in other types of *RET/PTC* rearrangements. These findings suggest that the *ACBD5-RET* rearrangement is causatively involved in the development of PTC.

Inverse associations between obesity indicators and thymic T-cell production levels in atomic bomb survivors

Reduction of the naïve T-cell population, a hallmark of immunological aging, has been observed in relation to radiation dose in atomic bomb survivors. In the present study, we evaluated the ability to produce naïve T cells, i.e., T-cell receptor excision circle (TREC) numbers in CD4 and CD8 T cells, and obesity status among 1,073 AHS subjects. Although there was no significant association between the TREC number and radiation dose, the TREC number was inversely associated with levels of obesity indicators (BMI, hemoglobin A1c) and prevalence of diabetes or fatty liver, suggesting a possible link between obesity, attenuated T-cell immune competence, and disease development among atomic bomb survivors.

Information Technology

The Systems Technology Section of the Information Technology Department moved forward with the virtualization of its servers, continuing work from the previous year. As a result, about 50 virtual servers are currently in operation. In addition, the section replaced the network core switch and conducted updates, focused mainly on the email environment, to enable access to RERF email on tablets and handheld devices through an updated webmail system, to enhance function of long-term email storage through an updated storage system, and to improve the email security system by updating the latest version of Security Gateway.

The Library and Archives Section endeavored to improve staff services. These efforts included an explanatory meeting to present the library's services and enhancement of the collection of electronic materials beyond electronic journals. As a committee member of the Japan Medical Library Association, the section strengthened its ties with other libraries during the period from FY2012 through FY2013. Furthermore, great progress was made in the digitization of materials to cope with deterioration of archival materials in the historical material room and simplify access. The section has completed the work of digitizing archival photographs and newspaper/magazine articles. Digitization of other documents and materials is ongoing.

FY2013 Highlights in Research Progress

Genetics

An estimate of frequency of radiation-induced deletion mutations

We identified 14 deletion mutations among 148 F_1 mice derived from 4-Gy irradiated spermatogonia cells by using high-density microarrays that can scan approximately 70% of the mouse genome. Based on the parental origin, characteristics of the sequence flanking the breakpoints of deletions, and the size, we judged that 4 among the 14 are radiation-induced large deletions that arose from non-homologous end joining (NHEJ) following double-strand breaks. The results imply that the number of large deletions (>100 kb) induced in male germ cells by 1 Gy of radiation is approximately 1 among 100 genomes (4 deletions/148 genomes/70% genome/4 Gy = 1×10^{-2} deletion/genome).

Generation of recombinant mouse strains in which living mutant cells become fluorescent by intrachromosomal recombination

It has been believed that radiation induces mutations in somatic and germ cells. However, thus far little is known about when and where the mutant cells arise in the irradiated tissues. For the precise detection and observation of the mutants *in situ*, we developed recombinant mice (CAG-HPRTdupGFP knocked-in). In these mice, *in vivo*-arising mutant cells become fluorescent (GFP positive) at the whole body level, by partial deletion of the knock-in allele through intrachromosomal recombination. These mice enable us to observe the dynamic images of *in vivo*-arising mutant cells. Moreover, it is expected that with the mice we will be able to obtain new findings regarding the spread of effects in the case of mutations in tissue stem cells brought about by radiation exposure. In the small intestine, where the typical turnover of stem cells (crypt stem cells) can be observed, we found mutant cell expansions from the mutated stem cells. Radiation induced such mutations. In contrast, in tissues such as the liver and pancreas, where the somatic stem cells are not evident, most of the mutant cells arose sporadically, leading to higher spontaneous mutation frequencies. The radiation effect tended to be buried in their higher background mutation frequencies.

Epidemiology

Radiation health risks among atomic bomb survivors

Life Span Study (LSS) research has found that exposure to A-bomb radiation is associated with mortality from noncancer respiratory diseases (17% increase in risk at 1 Gy of exposure as compared with unexposed subjects); this increase in risk is especially apparent in the period 1980–2005. This relationship may, in part, be a secondary association caused by respiratory diseases developing in the terminal stages of cancer and circulatory diseases, which have been linked to radiation exposure. Considering that the biological mechanisms behind the association between radiation exposure and respiratory diseases are unclear, further examination is necessary before reaching any conclusion about the observed association.

The excess risk of radiation-associated leukemia declined after the initial increase in risk during the years immediately after the atomic bombings, but the risk has persisted throughout the 55-year follow-up period, especially for acute myeloid leukemia (in collaboration with the RERF Statistics Department). In December 2013, the Epidemiology Department hosted an international workshop for the "Evaluation of the Effects of Low-dose Radiation Exposure in the Atomic-bomb Survivors" to discuss the state of low-dose research and generate strategies to push the limits of what is known.

In the area of dosimetry, Epidemiology Department members have contributed to the Dosimetry Committee, particularly in leading to improve the precision of survivors' location at the time of bombing based on interview surveys in the early period after the atomic bombings.

Statistics

Radiation risk assessment and dosimetry

Members of the Statistics Department published several first- and second-author papers on risk assessment, including a major paper on leukemia and other hematopoietic malignancies, a paper on the risk of individual multifactorial diseases of adulthood in the offspring of the atomic bomb survivors, and a paper on dose-response models, threshold estimates, and false-negative error rates in RERF studies of cataract surgery. A member of the Statistics Department was a co-author on an invited commentary from the Epidemiology Department in a major epidemiology journal regarding missing dose estimates in the Life Span Study (LSS). Members of the Statistics Department continued collaboration with external investigators in several areas related to new radiation risk models, including mechanistic modeling of leukemia, multi-model inference for circulatory disease outcomes, model validation of a semi-parametric method for survival extrapolation using RERF data, and a semi-parametric statistical method to correct for the effects of random errors in dose estimates on risk regressions. They also collaborated on research related to causal models, such as the mediating and moderating effects of serum sex hormones on radiation risk of breast cancer.

Members of the Statistics Department continued intramural work and extramural collaboration in dosimetry, including support of the Dosimetry Committee's efforts in improvement of survivor location estimates and evaluating potential dose contamination from residual radiation. A member of the Statistics Department presented new results on the improved survivor location estimates at the annual meeting of the U.S. Health Physics Society. Members of the Statistics Department also continued to support the Genetics Department's analyses of electron spin resonance (ESR) measurements in donated teeth, and organized a small international workshop on "Remaining Issues in Shielding Calculation" for RERF cohorts.

Statistical methodology for other RERF studies

Members of the Statistics Department consulted at the inception of numerous studies, such as a major new research protocol from the Clinical Studies Department on hyperglycemia/diabetes and hypercholesterolemia, supported RERF's successful application for a new contract with the U.S. National Cancer Institute (NCI), as well as contributed to the extensive new analyses of cancer incidence under the existing contract. The Statistics Department provided analytical support for a large number of RERF studies during the year, including at least 15 published or submitted manuscripts and numerous presentations at scientific meetings.

Research Progress by Project

Cardiovascular Disease Study

The question of whether A-bomb radiation exposure causes cardiovascular diseases (CVD) has not yet been resolved and continues to be one of the critical themes in the field of radiation health effects research.

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 vice chairman, chief scientist, department chiefs, and research scientists. The group started working as a 'project team' in 2008. Thus far, the team has: (1) summarized the results of all the studies on CVD conducted at RERF; (2) streamlined specific research hypotheses to be verified; and (3) considered new studies to be conducted in the future, such as a study on arteriosclerosis and animal experiments. Based on this work, the team has planned and conducted new research, including studies of stroke, chronic kidney disease, and arteriosclerosis indices, and biomarkers related to these diseases and immunological functions, as part of the Adult Health Study (AHS). Detailed additional analyses are also being undertaken in the Life Span Study (LSS), and animal experiments using spontaneous hypertensive rats are being conducted.

In FY2013, we continued these studies and published papers on chronic kidney disease and on right bundle branch block as seen on electrocardiograms. In addition, we published a paper on the February 2013 workshop on radiation and cardiovascular disease. Research plans on chronic kidney disease and on heart disease using echocardiography have been approved for implementation.

Collaborative Immunology Study

To understand mechanisms underlying radiation-induced immunosenescence and this phenomenon's implications in the various diseases experienced by atomic bomb survivors, RERF researchers started in 2009 a five-year collaborative study with four Japanese and five U.S. institutions, with funding provided by the U.S. National Institute of Allergy and Infectious Diseases (NIAID). In the study, RERF researchers focus on five projects that analyze radiation and aging effects on 1) hematopoietic stem cells, 2) dendritic cells, 3) immune responsiveness to influenza vaccination, 4) development of an integrated scoring system of immunity, and 5) thymus architecture and function. Measurements of immune parameters using biosamples donated by Adult Health Study (AHS) participants are nearly completed with the cooperation of AHS participants and their attending physicians, medical associations, and the RERF Departments of Radiobiology/Molecular Epidemiology, Clinical Studies, Information Technology, and Statistics, and data analyses and manuscript preparations are in progress (projects 1–4). In collaboration with the Department of Epidemiology, a database on thymus autopsied specimens stored at RERF was developed and pathological analyses were initiated (project 5).

Collaborative Cancer Studies

RERF-National Cancer Institute (NCI) collaborative cancer pathology studies

In these collaborative studies, radiation risks of cancer incidence were evaluated based on the diagnoses made by consensus using standardized methods and a panel of pathologists. A paper on skin cancer incidence that occurred between 1958 and 1996 was published in *Radiation Research* in 2014. Analyses of malignant lymphoma cases that occurred between 1950 and 1995, and bone and soft-tissue tumors for cases between 1958 and 2003 were conducted. Histological review and classification of "intrinsic subtypes" of breast cancer based on intracellular hormone receptors are continuing for the cases occurring between 1958 and 2005.

Effects of *CD14* genotype and atomic bomb radiation exposure on colorectal cancer risk

In a cohort study of colorectal cancer (CRC) among atomic bomb survivors, we examined the radiation dose response of CRC risk according to *CD14* genotypes, which are determined on the basis of polymorphisms of the innate immunity-related *CD14* gene. When dividing CRC cases into proximal colon cancer (CC) and distal CRC, we found potential involvement of a particular *CD14* genotype in the development of distal CRC, but not proximal CC, among atomic bomb survivors. Other findings also imply that radiation-associated CRC risks may differ by cancer subsites.

Research Progress by Project

F_1 Clinical Study

The 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 the prevalence study's combined or individual analysis of multifactorial diseases in children showed no evidence of increased risk related to parental radiation exposure. In that 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 such diseases frequently occur. At the same time, there tended to be self-selection factors concerning whether or not to undergo health examinations. 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 continuation of the study, and the longitudinal study for the nearly 12,000 subjects was initiated on November 24, 2010.

The participation rate during the three-year period following the start of the longitudinal study was 76.8% (about 7,700 participants), improving steadily from the first year of 69.2% and the two-year period of 76.0%. As a result of advancing the study with the aim of achieving a participation rate of 80% (about 10,000 participants), the number of participants for the first round of the longitudinal study (four-year period) is expected to reach more than 9,500.

Dosimetry

One of the duties assigned to the Dosimetry Committee, established in FY2009, was the correction of survivors' exposure locations. This task was completed in FY2012, almost as planned. To undertake this work, which targeted all cohort members, we started with the correction of data input errors by reviewing archived questionnaires, including the Master Survey Questionnaire (MSQ). We then corrected the distortion of the original U.S. Army maps. We also identified anew exposure locations for about 28,000 cases for which neighborhood drawings indicating shielding histories are available, by superimposing these neighborhood drawings and aerial photos on a personal computer. We further found that the coordinates of exposure locations were entered into the original database in an unstandardized manner, using either three or four digits, and we modified these, entering all the coordinates in four digits. Since the Geospatial Information Authority of Japan's database showing detailed terrain elevation data has recently become available, in FY2013 we performed automatic calculations of terrain shielding for all the cases. Since shielding calculations were previously limited to only a portion of the cases, it seems that individual radiation dose estimates may be strongly modified for Nagasaki, which is considered to have been significantly affected by terrain shielding. In the latter half of FY2013, we also modified terrain-shielding calculations by adjusting for terrain elevation at the survivors' exposure locations, in addition to elevations of surrounding terrain. Dose-reassessment work for the Life Span Study (LSS) participants will be completed upon calculation of the relevant modifications. Reassessment work for non-LSS parents of children born to atomic bomb survivors will be carried out by the Departments of Statistics and Epidemiology, using methods similar to those adopted for LSS, and the Dosimetry Committee will be dissolved on the grounds that its duties were completed in FY2013. Around July 2014, LSS databases showing modified individual dose data will enter the public domain for use by investigators.