FY2009 Highlights in Research Progress

Clinical Studies

Association of lymphotoxin-alpha genotypes and radiation exposure with gastric cancer: A nested case-control study using stored biosamples

A nested case-control study was carried out in the Adult Health Study cohort using sera and blood cells stored prior to diagnosis of gastric cancer. Enrolled were 287 noncardia gastric cancer cases of diffuse and intestinal types and three controls per case selected from cohort members matched on age, gender, city, and time and type of serum storage and countermatched on radiation dose. *LTA 252GG* and *GA* genotypes were associated with the prevalence of *H. pylori* IgG seropositivity and higher antibody titer against CagA in controls, and the genotypes were an independent risk factor for noncardia gastric cancer of diffuse type (relative risk [RR] = 2.8, 95% confidence interval [CI]: 1.3–6.3, p = 0.01, and RR = 2.7, 95% CI: 1.5–4.8, p < 0.001, respectively), but not for intestinal type, after adjusting for *H. pylori* IgG seropositivity, CagA antibody titers, chronic atrophic gastritis, smoking, and radiation dose. Radiation dose was associated with noncardia gastric cancer in subjects with both the *LTA 252G*-allele and current non-smokers.

The *LTA 252* genotype is associated with noncardia gastric cancer of diffuse type in Japan and interacted with radiation dose.

Fatty liver incidence and predictive variables

Using abdominal ultrasonography, we followed biennially through 2007 (mean follow-up, 11.6 ± 4.6 years) 1,635 Nagasaki atomic-bomb survivors (606 men) without fatty liver at baseline (November 1990 through October 1992) and examined longitudinal trends of potential predictive variables. In all, 323 (124 men) new fatty liver cases were diagnosed. The incidence was 19.9/1,000 person-years and peaked in the sixth decade of life. After controlling for age, sex, and smoking and drinking habits, obesity (RR = 2.93, 95% CI: 2.33–3.69, P < 0.001), low high-density lipoprotein-cholesterol (RR = 1.87, 95% CI: 1.42–2.47, P < 0.001), hypertriglyceridemia (RR = 2.49, 95% CI: 1.96–3.15, P < 0.001), glucose intolerance (RR = 1.51, 95% CI: 1.09–2.10, P = 0.013), and hypertension (RR = 1.63, 95% CI: 1.30–2.04, P < 0.001) were predictive of fatty liver. In multivariate analysis including all variables, obesity, hypertriglyceridemia, and hypertension remained predictive. Radiation dose did not predict fatty liver. In fatty liver cases, body mass index and serum triglycerides, but not systolic or diastolic blood pressure, increased significantly and steadily up to the time of diagnosis. Obesity, hypertriglyceridemia, and, to a lesser extent, hypertension might serve as predictive variables for fatty liver.

Radiobiology/Molecular Epidemiology

Increases in the percentage of less competent memory T cells in A-bomb survivors

It is suggested that radiation exposure reduces the diversity of repertoires of memory T cells. To evaluate the maintenance of T-cell memory in A-bomb survivors, we examined functionally distinct memory CD4 T-cell subsets (mature memory cells, cells weakly responsive to antigenic stimulation, and functionally anergic cells) in their peripheral blood lymphocytes. These subsets were identified by differential CD43 expression levels and measured using flow cytometry. Percentages of functionally weak and anergic subsets increased with radiation dose, whereas the percentage of functionally mature memory cells decreased. These results suggest that the steady state of T-cell memory may have been perturbed by prior radiation exposure.

ALK gene rearrangement found for the first time in papillary thyroid cancer (PTC) among A-bomb survivors

Through molecular analysis of adult-onset PTC in A-bomb survivors, we recently found a new type of rearrangement in PTC cases with non-detected gene alterations that carried no mutations in *RET*, *NTRK1*, *BRAF,* or *RAS* genes. This rearrangement of the *ALK* (anaplastic lymphoma kinase) gene was found for the first time in PTC, although identification of the partner genes is needed.

Statistics

Radiation risk assessment and dosimetry

Contributions for this year included analyses of F_1 clinical data for individual multifactorial disease outcomes considered as multivariate data with potential correlation, analyses of data on hormonal factors in a nested case-control study of breast cancer, analyses of cataract data specific to location within the eye, prediction of future radiation-related excess events in risk assessment, and application of generalized additive and multiplicative models to analyze joint effects of radiation and smoking on risk of lung cancer. Statistics continued to collaborate with Epidemiology in improvement of individual survivors' exposure data through map work and photogrammetry and in evaluation of available data on survivors' exposure to residual radiation, and with Genetics on biodosimetry measurements. Collaboration with external investigators included new studies with mechanistic models of carcinogenesis.

Statistical methodology for RERF studies

Active research continued in causal (joint) models involving intermediate variables, with initiation of new research on applications to nested case-control studies. Collaboration with external investigators included several approaches to quantifying and adjusting for uncertainty in radiation dose estimates and related analysis of biodosimetry data, and a new project on validation of a semi-parametric survival extrapolation method.

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Epidemiology

Risk on health in atomic-bomb survivors, those exposed *in utero*, and children of survivors

The risk of radiation in skin cancer incidence significantly increased at a level of around 270% per Gy with a linear dose-response relationship for basal cell carcinoma among the survivors, and also with estimated threshold of around 0.6 Gy. An elevated risk of lung cancer due to radiation has previously been observed among the survivors, and the effect was modified by smoking habits (collaboration with the Department of Statistics). The risk of myelodysplastic syndrome (MDS) significantly increased at a level of around 430% per Gy with a linear dose-response relationship among the survivors in Nagasaki (collaboration with Nagasaki University and the Department of Statistics).

The risk of a second primary cancer among the survivors was high but was not higher than the excess risk associated with a person's primary cancer (collaboration with the University of Washington, U.S.).

The risk of cancer and non-cancer disease mortality for those exposed *in utero* did not differ from that for those exposed in early childhood as a whole, but the risk of cancer mortality was higher at younger attained ages for those exposed *in utero* compared to those exposed in early childhood. The risk of cancer and non-cancer diseases was not associated with paternal or maternal doses among the children of the survivors conceived after radiation exposure. Both of these studies continue to collect follow-up data.

New mail survey

A new mail survey on the survivors (Life Span Study subjects) has been completed for the 5,200 people eligible for recruitment to the Adult Health Study, and the response rate was around 83% for those for whom we have current address information. Questionnaires are scheduled to be sent to an additional 14,000 subjects.

Genetics

The lack of a dose response for chromosome translocations following fetal exposure is tissue-dependent

We have previously reported that survivors who were exposed *in utero* showed almost no dose response in the translocation frequency in their lymphocytes when examined at 40 years of age, which was confirmed by subsequent animal experiments. To investigate tissue specificity, we examined chromosome aberration frequencies in rat mammary epithelial cells following fetal irradiation, and found that the translocation frequency was nearly the same as that of their mothers, whereas lymphocytes did not record the chromosome damage as observed in mice experiments. Thus, present results indicated that the lack of translocation dose response following fetal exposure is tissue-dependent.

An animal model for human female exposure: No indication for the transgenerational effects of radiation following exposure of immature oocytes of rats to 2.5 Gy of gamma rays

The estimate on radiation risk of mutation induction in mouse spermatogonia served as a good model for human male exposure to radiation and has been used for the estimation of genetic risk of radiation in humans. In contrast, it has not been possible to use female mice as the model for human female exposure to radiation because the target cells, immature oocytes, are extremely sensitive to the killing effect of radiation. We found that female rats were indeed resistant to radiation and prepared F_1 rats derived from 2.5-Gy gamma irradiated SD female mated with BN male rats. We examined the spleen DNA of 750 F_1 animals each from the exposed and the control groups by two-dimensional electrophoresis. We selected some 1,500 good spots each from SD and BN for mutation analyses. Thus far, we have analyzed about 2.2 million spots (loci) each derived from maternal SD or paternal BN strains. We found a total of 24 mutations; 13 in the control group and 11 in the exposed group. The majority of the mutations (20/24) occurred at microsatellite repeat sequences and were not regarded as radiation related. Two deletion mutations were found in the exposed group but both occurred on the paternal alleles. In summary, we have observed no indication for the transgenerational effects of radiation following exposure of immature oocytes of rats to 2.5 Gy of gamma rays.

Information Technology

Guidelines on appropriate handling of personal information at medical and related organizations have been introduced by the Ministry of Health, Labour and Welfare. The guidelines, containing various instructions on handling of information, indicate how to protect personal information and stipulate that all records must be maintained for appropriate investigation in the case of leaked personal information. We at RERF have conducted system changes in order to appropriately store information, and as an example, installed an email archive system this fiscal year that allows storage of all email passing through RERF.

Data were previously backed up on magnetic tapes and regularly sent to the Nagasaki Laboratory in order to store the information at both the Hiroshima and Nagasaki Laboratories, and eventually at an underground bank vault of Hiroshima Bank. However, we decided to directly forward the data over the Internet to a backup server installed at the Nagasaki Laboratory and have since started necessary preparations. It is expected that stable operation of the new system will be achieved early next fiscal year.

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Cardiovascular Disease Study

Whether A-bomb radiation exposure causes cardiovascular diseases has attracted much attention from those concerned, and many of RERF's research results were detailed in Annex 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 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. This group has been reviewing the issue as a project team since 2008. Starting in FY2008, the team summarized the results of studies conducted at RERF and spent considerable time in a review process aimed at streamlining hypotheses to be verified. In FY2009, the team initiated review of new studies to be conducted in the future, examined details of a study on arteriosclerosis to be started by the Department of Clinical Studies, and have been discussing the issue of possible animal experiments. In addition, because the Scientific Council decided to conduct a focused review of the Department of Clinical Studies, their planned study on cardiovascular diseases was chosen as the main presentation theme, and the project team became involved in the relevant preparations. Overall, the research projects evaluated by the Scientific Council included stroke, chronic kidney diseases, arteriosclerosis markers, valvular diseases, and related biomarkers and immunological functions.

F_1 Clinical Study

A health effects study on the F_1 offspring of A-bomb survivors was conducted from 2002 through 2006 to examine from an epidemiological perspective the presence or absence of effects of parental radiation exposure on adulthood development of multifactorial diseases among F_1 subjects. The results of this study were published in a 2008 report. In the study, however, average age of the F_1 subjects who underwent health examinations was young, at 49 years, just beginning 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 Committee for the Health Effects Study of the F_1 Offspring of A-bomb Survivors, the Scientific Council, and the Senior Review Panel recommended that a longitudinal study be conducted. Based on these recommendations, RERF's F_1 Clinical Study Working Group, consisting of the Chief of Research, a permanent director, chief scientists, department chiefs, and research scientists, held many meetings to start development of a new research plan and establish the framework necessary for conducting the study. As a result, preparation of a research protocol, with Research Scientist Waka Ohishi (Department of Clinical Studies, Hiroshima) as the responsible investigator, was completed this fiscal year. At the same time, after a series of discussions with organizations of the children of A-bomb survivors, members of the Scientific and Ethics Committee for the Clinical Study of the F_1 Offspring of A-bomb Survivors were officially determined (Chairman: Dr. Tadao Shimao, Vice Chairman: Dr. Hiraku Takebe). The foundation for commencement of the study in FY2010 has thus been established.

Dosimetry

The maps created by the U.S. Army immediately after the war and used as a coordinate system indicating the hypocenter and survivor locations have long been pointed out as having distortion. In addition, shielding conditions of about 7,000 Life Span Study subjects are categorized under "unknown dose" because they did not match the standards for DS02 application. The Dosimetry Committee was established to improve such problems involving dose estimates.

This fiscal year, we reentered all the information as data to two decimal places based on the original questionnaire records in order to amend the problem of mixed one- and two-decimal-place data depending on the timing of data entry. Exposure location error of up to and even greater than 100 m has thus been corrected.

Regarding distortion of the maps, we obtained aerial photographs taken just before the A-bombings and corrected the shooting angle, altitude, lens aberration, and elevation of the photographed objects to create an orthophotograph integrating the entire area into one planimetric map. We established as many control points as possible that are the same in the U.S. Army maps and these aerial photographs, based on which we will create a coordinate transformation formula for conversion of the entire area of both cities from the U.S. Army maps to aerial photographs with the aim of conversion of the coordinate data. In addition, we made preparations to identify the exposure location of each survivor using the aerial photographs, if the survivor had shielding history with available neighborhood drawings, and to reconfirm the locations of the proximally exposed survivors.