放影研 RERF update
Radiation Effects Research Foundation News and Views
Hiroshima and Nagasaki, Japan
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Radiation Effects Research Foundation

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This newsletter is published by the Radiation Effects Research Foundation (RERF; formerly the Atomic Bomb Casualty Commission), established in April 1975 as a private, nonprofit Japanese foundation. It is supported by the government of Japan through its Ministry of Health, Labour and Welfare and by the United States through its Department of Energy (DOE), in part by DOE contract DE-HS0000031 with the National Academy of Sciences. RERF became a public interest incorporated foundation on April 1, 2012.

RERF conducts research and studies—for peaceful purposes—on medical effects of radiation and associated diseases in humans, with a view to contributing to maintenance of the health and welfare of the atomic-bomb survivors and to enhancement of the health of all humankind.

Editor-in-Chief: *Evan B. Douple, Associate Chief of Research*
Technical Editor: *Fumie Maruyama, Public Relations & Publications Office*

Editorial Policy

Contributions to RERF Update *receive editorial review only and do not receive scientific peer review. The opinions expressed herein are those of the authors only and do not necessarily reflect RERF policies or positions.*

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From the Editors

Konnichiwa (hello and good afternoon) and welcome to *RERF Update*!

Over a year has passed since the 2011 Great East Japan Earthquake and tsunami and in this issue you will read that RERF scientists continue to provide information regarding risks of long-term health effects following exposures to radiation based on RERF's studies of the health effects in the atomic-bomb survivors. Inquiries regarding the potential implications for health from the Fukushima accident have resulted in an enhanced RERF public relations effort with information disseminated by our scientists through several venues including RERF's website, interviews with the news media, public lectures, and training workshops for visiting students, public health workers, and officials. Some RERF scientists contribute by their membership in a number of advisory groups. Those activities are consistent with RERF's qualifying as a public interest incorporated foundation (PIIF) which did occur when RERF registered as a PIIF on April 1, 2012 (no fooling; see cover photo).

Although the winter was colder than normal and continued for what seemed to be an unusually long time, eventually things returned closer to "normal" at RERF. For example, the 39th Scientific Advisory Committee (SAC) met to assess RERF's progress, and the research activities and accomplishments of the two departments that received focused SAC review—Genetics and Radiobiology/Molecular Epidemiology—are highlighted in overviews written by the respective department chiefs in this issue along with the SAC's report. And, although the sakura (cherry) blossoms were delayed due to the cool winter weather, eventually Hijiyama was a burst of flowers and color. The cheerful sounds of ohanami celebrations, which were conspicuously missing or subdued last year, began to return to Hijiyama (as shown in the photograph on this page) along with the distinctive and pleasant call of the uguisu (Japanese Nightingale).

Unfortunately, we must report that since the last issue of *Update*, three of the most influential and important contributors to the scientific work of ABCC and RERF have died; James F. Crow, Itsuzo Shigematsu, and Seymour Jablon. Please take time to read about the major impacts that each of these three men had on the Foundation.

Lastly, let me thank profusely *Update*'s Technical Editor, Yuko Ikawa, who retired at the mandatory retirement age in December 2011. Ms. Ikawa joined RERF in April 1985 as an executive secretary who supported the offices of the Chief of Secretariat, Chairman, and Permanent Directors. She was transferred to the Editorial and Publications Section of RERF in July 1999 and became Section Chief in October of 2001. In January 2006, the office was reorganized into the Public Relations and Publications Office. *RERF Update* had been inaugurated in the spring of 1989 by Ms. Beth Magura. In the spring of 2001, Ms. Ikawa became involved in editing of *Update* and in the next year, became the Technical Editor of *Update* which was published once per year. I joined Ms. Ikawa in January of 2008, and she and I have been working together to produce two issues per year since that time. She is well known for her attention to detail, hard work, dedicated service to RERF, and high productivity. Fortunately, she will continue to work part-time at RERF as an Adjunct Specialist so will still be available to provide assistance to me and her successor. The other fortunate thing is that I get to introduce her successor, Fumie Maruyama, who was recruited as the new Technical Editor. A long-time employee of RERF, Ms. Maruyama had been working with me in the Office of the Associate Chief of Research since my arrival in 2008. She has the attributes of a good editor, so in the photograph on the next page I appear very confident as I'm handing over the Technical Editor reins!

A group of people enjoying cherry blossoms on Hijiyama

We hope that you enjoy this, Maruyama-san's first issue, and

don't hesitate to let us know how we might improve our reporting of RERF's many activities. Mata oidekudasai (goodbye and please come again),

Evan B. Douple
Editor-in-Chief

Fumie Maruyama
Technical Editor

Transitioning of *Update*'s technical editors in the RERF's Japanese garden; from left, Yuko Ikawa (former), Evan B. Douple, and Fumie Maruyama (current)

Report on the 39th Scientific Advisory Committee Meeting, 2012

The 39th Scientific Advisory Committee (SAC) met from March 5–7, 2012 in Hiroshima, Japan to review RERF's scientific programs. It was co-chaired by **Kazuo Sakai** and **Sally Amundson**. **Shunichi Yamashita**, Vice President, Fukushima Medical University, joined the SAC with the expiration of the term of **Takashi Yanagawa**. Three additional experts were invited to be special councilors to assist the SAC in an in-depth review of the Departments of Genetics and Radiobiology/Molecular Epidemiology (RME). They were **Tetsuya Ono** of Tohoku University, **Toshio Suda** of Keio University, and **William Morgan** of Pacific Northwest National Laborabory.

RERF Chairman **Toshiteru Okubo** provided a warm welcome to the SAC members and guests and expressed how important the SAC's work is to the staff of the RERF. He also explained the basics of the now-completed change of the Act of Endowment with RERF becoming a public interest incorporated foundation (PIIF).

Responses to last year's SAC general recommendations and highlights of research accomplishments during 2011 were summarized by Vice Chairman **Roy E. Shore**. In response to the 2011 SAC recommendations, we have streamlined the research protocol (RP) review process and are employing a method to provide an early concept review of potential RPs that helps focus and improve them. Program-based structures have been implemented to overlay departmental work so as to facilitate thematic interdisciplinary research. The programs pertain to radiation research in cancer, circulatory disease, other noncancer endpoints (e.g., cataract), immunologic effects, genetic (transgenerational) effects, and dosimetry, plus the "platform protocols" for the basic data collection and methodologic developments. A special RERF committee was created to review the status of information technology at RERF and to develop guidance regarding the needs for integrated databases of research information and biospecimens. We are working to strengthen our collaborations with various universities and to strengthen our fledgling post-doctoral program. We also are working with department chiefs and members to develop high-quality publications on radiation-related health assessments. The Committee on Biological Samples has been tasked to develop a plan to consolidate our biospecimen processing, storage, and integrated biosample database management.

Among the highlights of accomplishments during FY2011 were publications on a number of topics related to radiation risk, most especially a major update of cancer and noncancer mortality in the Life Span Study (LSS) cohort. Other radiation-related publications included findings regarding risk of hemorrhagic stroke, risk of myelodysplastic syndrome, levels of breast-cancer related hormones, various subtypes of lung cancer, chronic kidney disease mortality, effects on age at menopause, and development of a new method to detect *in vivo* mutations associated with radiation damage and genetic risk. Regarding radiation dosimetry, 14 papers from our international conference on RERF organ/tissue dosimetry were published, as were papers on A-bomb fallout exposures: a geospatial analysis of cesium-137 in Hiroshima soil samples, and ESR (electron spin resonance) tooth enamel measurements of distal A-bomb survivors in rela-

tion to fallout exposure.

RERF scientists have organized or participated in a number of radiation-related activities to communicate our findings or conduct training. Invited lectures were given at the International Congress on Radiation Research by Chief Scientist **Kazunori Kodama**, Epidemiology Department Chief **Kotaro Ozasa,** and Associate Chief of Research **Evan B. Douple**. Chief Scientist **Nori Nakamura** is a member of the ICRP (International Commission on Radiological Protection) and Dr. Kodama of UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation). In January 2012 we conducted an international workshop on radiation and stem cell effects. During FY2011 we also conducted a radiation epidemiological training workshop for biologists from a number of institutions, and had over 180 trainees who spent from a day to over a month receiving training at RERF. We also have intensified our efforts to provide press conferences and press releases regarding new research findings.

Scientists at RERF have been active participants in Fukushima-related matters. We dispatched experts to train local public-health personnel in making personal radiation measurements, quickly developed a Fukushima-oriented webpage with information and FAQs about radiation effects, and provided expertise for a biological dosimetry working group. RERF members are continuing to provide advice to the Japanese central government and local governments regarding possible health risks and to the Fukushima long-term epidemiological study team regarding study planning, health assessments, and biological measurements. RERF members also are serving on Fukushima risk-assessment committees of UNSCEAR and the WHO (World Health Organization).

The SAC believes "that the RERF is the preeminent leader in radiation risk research in the world and has the expertise, populations, and data sets to conduct [unique] fruitful investigations…" They appreciated the sustained support of the Japanese Ministry of Health, Labour and Welfare (MHLW), the U.S. Department of Energy (DOE), and the U.S. National Academy of Sciences (NAS). They also stated "The Fukushima disaster dramatically highlights the importance of the RERF mission; a mission made only more crucial by increased worldwide power needs, increased use of medical radiation, and increased threats of radiological or nuclear terrorism."

The general recommendations by the SAC included the following:

- RPs "should address clearly articulated testable hypotheses that contribute to the overall goals of RERF." In adopting new technologies or systems, "care should be taken that such experiments are designed to address high priority questions in an appropriate way." A transparent system should be in place to ensure that biosamples are used to address the most important biological questions.
- Interactive discussions in evaluating RPs can be used as an opportunity for education and training of young investigators.
- The efforts of RERF in support of activities related to Fukushima are an ideal example of delivery of a valuable RERF "product," the transfer of RERF scientific and methodological knowledge to a pressing public concern.
- RERF must continue to give the highest priority to the reassignment and renovation of space for the secure and accessible storage of the biological samples, and to the development of a database providing sample identification, location, and other information.
- The SAC continues to strongly recommend that the varied databases of participant information and biological samples be integrated into a central database for the use by all departments. Access to the complete data on every subject is crucial to RERF's central mission.
- Investigators should not depend solely upon internal research funding, and should continue to be encouraged to obtain competitive external grants.
- The SAC appreciates the efforts made to resolve the issues surrounding the potential impact of "black rain" fallout on dosimetry and health outcomes in the face of current public concern. We understand that full resolution of these questions may not be possible due to many uncertainties in the data, but we are confident that RERF is undertaking appropriate analyses where possible and encourage publication of the findings.
- To meet the bioinformatics needs of high-throughput technologies in basic science and epidemiology, the Statistics Department should explore the possibility of developing links with outside collaborators before initiating any bioinformatics recruitment.
- The SAC commends the ongoing seminars and interactions between and within departments but also encourages the development of several focused research discussion groups to help reduce the fragmentation of research effort.
- The ongoing collaborations with external organizations are commendable, but the SAC suggests that these and new collaborations should seek to include some level of RERF financial support, such as through subcon-

tracts.
- More high quality publications are essential for the continued success of RERF.

Highlights of some of the recommendations for individual departments or offices are:
- Some standardization of protocols should be developed for archival tissues. This includes detailed protocols for preparation of samples for analysis as well as quality control standards.
- The images of somatic and germ line mutations *in vivo* or in explants from the GFP-mutant mouse are stunning and spectacular. The targeted issue to address with those studies seems to be germ cell mutation, which is a neglected facet in estimating the genetic doubling doses of radiation.
- The atomic-bomb survivor studies on cardiovascular disease (CVD) incidence and on the association between chronic kidney disease and CVD risk factors should be strengthened and summarized as full papers. A careful attempt should be made to detect the possible role of rare diseases in the etiology of the more commonly appearing disease phenotypes.
- As the younger epidemiologic and clinical cohorts have fewer "events" and need to be followed for a long time, efforts need to be implemented to maintain high participation and reduce loss to follow up. Also, efforts to obtain up-to-date denominators for population studies of cancer incidence should continue.
- The Statistics Department has been evaluating the basic LSS dosimetry with several publications and extensive collaborations. The department is encouraged to quickly make the needed adjustments to the DS02 system based upon the Epidemiology Department's re-evaluation of the original maps and aerial photographs.
- Opportunities could be sought to reach students at local universities and even secondary schools with the grandeur and excitement of radiation research. Public lectures for adult learners might be offered in other major cities beside Hiroshima and Nagasaki.

In summary, the SAC highlighted the unique role of RERF in informing the world about radiation effects, and they called for additional attention to research priorities, careful development of research hypotheses and focus, integrated databases, and publication productivity.

RERF Scientific Advisors

Dr. Kazuo Sakai, Co-chairperson, Director, Research Center for Radiation Protection, National Institute of Radiological Sciences

Dr. Sally A. Amundson, Co-chairperson, Associate Professor of Radiation Oncology, Center for Radiological Research, Columbia University Medical Center

Dr. Katsushi Tokunaga, Professor, Department of Human Genetics, Division of International Health, Graduate School of Medicine, The University of Tokyo

Dr. Kiyoshi Miyagawa, Professor, Laboratory of Molecular Radiology, Center for Disease Biology and Medicine, Graduate School of Medicine, The University of Tokyo

Dr. Kazuo Tajima, Director, Aichi Cancer Center Research Institute

Dr. Shunichi Yamashita, Vice President, Fukushima Medical University

Dr. Marianne Berwick, Professor and Chief, Division of Epidemiology, Associate Director, Population Sciences, University of New Mexico

Dr. John J. Mulvihill, Children's Medical Research Institute/Kimberly V. Talley Chair in

Participants of the 39th Scientific Advisory Committee meeting held at Hiroshima RERF

Genetics; Professor of Pediatrics; Head, Section of Genetics, University of Oklahoma Health Sciences Center

Dr. Michael N. Cornforth, Professor and Director of Biology Division, Department of Radiation Oncology, University of Texas Medical Branch (Absent)

Dr. David G. Hoel, Distinguished University Professor, Department of Medicine, Medical University of South Carolina and Principal Scientist, Exponent Inc.

Special Advisors

Dr. Tetsuya Ono, Professor, Division of Genome and Radiation Biology, Department of Cell Biology, Graduate School of Medical Sciences, Tohoku University

Dr. Toshio Suda, Professor, Department of Cell Differentiation, The Sakaguchi Laboratory of Developmental Biology, School of Medicine, Keio University

Dr. William F. Morgan, Director of Radiation Biology and Biophysics, Biological Sciences Division, Pacific Northwest National Laboratory

Second Public Lecture Program Held in Hiroshima

On December 10, 2011, RERF held its second public lecture program in the Memorial Hall in the basement of the Hiroshima Peace Memorial Museum's East Building, from 14:00 to 16:30. The objectives of this lecture program were to explain in a straightforward manner to the general public, including A-bomb survivors, about RERF's long years of research findings, health effects of radiation, and a topic of increasing interest following the Tokyo Electric Power Company's Fukushima Dai-ichi nuclear power plant crisis in March 2011. An additional objective was to provide an opportunity for the public to communicate with RERF. About 200 citizens participated in the lecture, a program that was initiated last year.

At the beginning of the program, following opening remarks by Chairman Toshiteru Okubo, Mr. Sunao Tsuboi, President of the Hiroshima Prefectural Confederation of A-bomb Sufferers Organizations and the honored invited guest of the program, extended his greetings. Two lectures followed. First, Chief Scientist Nori Nakamura spoke on "Thinking about low-dose radiation exposure," explaining cancer risks from exposure to low-dose radiation and chronic radiation exposure, the types of radiation exposures typically seen in Fukushima, based on results from epidemiological studies of A-bomb survivors. Next, Genetics Department Assistant Chief Asao Noda, in his lecture titled "Methods for radiation dose assessment," briefed the audience on the basics of radiation and methods of radiation dose measurement.

Following the lectures, Dr. Hiroo Dohy, then President of the Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital and of the Hiroshima International Council for Health Care of the Radiation-exposed (HICARE), gave special remarks as well as a general overview of the lectures. In the question-and-answer session that followed, so many questions were collected by questionnaire from the audience after each of the lectures that not all of them could be responded to in the time available. Several audience members remained in the hall eagerly asking questions even after the close of the lecture.

Assistant Department Chief of Genetics Asao Noda giving a lecture during the second public lecture program

Fourth Meeting of the RERF Board of Directors Held in Hiroshima

Since RERF's transition of status to an exceptional incorporated foundation with councilors in April 2011, the RERF Board of Councilors has replaced the Board of Directors (BOD) as the highest decision-making body at RERF. The BOD has changed its structure and functions, and its meetings are now attended by resident Directors and Auditors. Four such meetings were held during FY2011.

The fourth meeting of the BOD was held at the Hiroshima Laboratory on March 9, 2012. With Mr. David Williams joining via teleconference from his office in South Carolina, USA, all Directors and Auditors attended the meeting.

Dr. Toshiteru Okubo reported on the Directors' job performance and significant personnel matters. The BOD then deliberated and unanimously approved the activities plans and budget estimates for FY2012, the first fiscal year after RERF's transition to a public interest incorporated foundation (PIIF; see the following article). In addition, there were reports on the status of progress in the PIIF transition process and on RERF's responses to Deloitte Touche Tohmatsu(RERF's external auditor)'s internal controls review report, as well as finalization of the dates and venue for the second meeting of the Board of Councilors.

Participants

Directors:
Dr. Toshiteru Okubo, Chairman (Representative Director)
Dr. Roy E. Shore, Vice Chairman and Executive Director
Mr. Takanobu Teramoto, Executive Director

Auditors:
Mr. Takashi Kohno, Hiroshima General Law/Accounting Office (CPA/licensed tax accountant, Takashi Kohno Office)
Mr. David Williams, Senior Financial Advisor, National Academy of Sciences

RERF Attendees:
Dr. Evan B. Douple, Associate Chief of Research
Dr. Nori Nakamura, Chief Scientist
Dr. Kazunori Kodama, Chief Scientist
Mr. Eiji Akimoto, Chief of Secretariat
Mr. Douglas C. Solvie, Associate Chief of Secretariat

Completed Transition to Public Interest Incorporated Foundation

After becoming an exceptional incorporated foundation with councilors in April 2011, a resolution required to file for authorization of RERF's change of status to a public interest incorporated foundation (PIIF) was adopted at the June 2011 Board of Directors and Board of Councilors meetings. RERF filed an application for the said transition with Japan's Prime Minister on August 31, 2011. Following review of the application by the Cabinet Office's Public Interest Corporation Commission, a certificate of transition authorization was granted by the Prime Minister on March 22, 2012. RERF was registered as a PIIF on April 1, 2012 (Sunday).

Even after transition to PIIF status, the organization's English name (and abbreviation)—the Radiation Effects Research Foundation (RERF)—will not change. Under the new designation, what was formerly called the Board of Directors is replaced by the Board of Councilors. The new Board of Directors now includes Toshiteru Okubo, Roy E. Shore, and Takanobu Teramoto. What had been the Scientific Council is now called the Scientific Advisory Committee.

See the cover photograph: RERF's new sign with the official Japanese name "Public Interest Incorporated Foundation" on it. Shown in the photograph are Chairman Toshiteru Okubo (right) and Chief of Secretariat Eiji Akimoto.

Japan's Ambassador to Iceland Masayuki Takashima Visits RERF

On January 13, Japan's Ambassador to Iceland Masayuki Takashima paid a visit to RERF's Hiroshima Laboratory. Ambassador Takashima spent two days in Hiroshima with the aim of studying issues involving peace in the city and touring the Hiroshima Peace Memorial Museum and other peace-related facilities as well as RERF. At the laboratory, Chairman Toshiteru Okubo and Vice Chairman Roy E. Shore provided Ambassador Takashima with a brief overview of RERF and an outline of the foundation's research efforts. The Ambassador was then led on a tour of the facilities by RERF's Directors, who explained the organization's biosample storage facilities and permanent exhibitions.

Ambassador Masayuki Takashima (far left) being briefed on RERF and its research efforts

Visit to RERF by Two Ukraine Officials

On 24 February, two officials from Ukraine visited RERF. Dr. Dimitry Anatolijovich Bazyka is the Director-General and Head of the Department of Clinical Immunology in the Research Centre for Radiation Medicine of the National Academy of Medical Sciences of Ukraine. He was accompanied by Dr. Valery Alexandrovich Kashparov, Director of the Ukraine Institute of Agricultural Radiology of the National University of Life and Environmental Sciences of Ukraine. This was their first visit to RERF and they included their stop in Hiroshima after a visit to Fukushima.

The two visitors received greetings from Chairman Toshiteru Okubo. Vice Chairman Roy E. Shore then explained the history of ABCC and RERF and described the current foundation, its cohorts, funding support, facilities and staff, and the focus of its research. He proceeded to review the highlights of RERF's most recent findings, especially focusing on the studies of the Life Span Study and Adult Health Study and the work of the Departments of Clinical Studies, Epidemiology, and Statistics. Associate Chief of Research, Evan B. Douple concluded RERF's presentation with a discussion of RERF's basic science research and a review of the activities underway in the Department of Genetics and the Department of Radiobiology/Molecular Epidemiology (RME). He emphasized that the basic science research at RERF explores mechanisms responsible for radiation-induced health effects observed in humans, conducts experiments in model systems or in biosamples from humans that cannot be conducted directly in humans, and identifies radiation effects that might be future epidemiological and clinical studies in humans. All of the previously mentioned studies are conducted within the mission of RERF.

Dr. Bazyka then described the composition and research activities underway at the Research Centre for Radiation Medicine in Kiev, Ukraine. He summarized the results of the studies of the Chernobyl clean-up worker cohorts conducted by the research center and described the studies' biosample resources and available dosimetry information. This was followed by an exchange of opinions regarding the findings from our two institutions obtained studying two different populations of radiation-exposed victims. The visitors presented RERF scientists a copy of the 2011 book, *Health Effects of the Chernobyl Accident: A Quarter of Century Aftermath*, for which Dr. Bazyka served as one of the editors-in-chief.

Dr. Dimitry Anatolijovich Bazyka (left) and Dr. Valery Alexandrovich Kashparov

At the conclusion of the discussions, the guests were led on a tour of the RERF facilities by Drs. Shore and Douple. Being an immunologist, Dr. Bazyka was especially interested in the cell-sorting facilities of the RME Department and the storage of cells in the deep freezers and liquid nitrogen tanks. The tour also included a visit with Dr. Kotaro Ozasa, Chief of the Department of Epidemiology and Dr. Yoshiaki Kodama, Chief of the Department of Genetics.

U.S. TV Science Correspondent Visits RERF

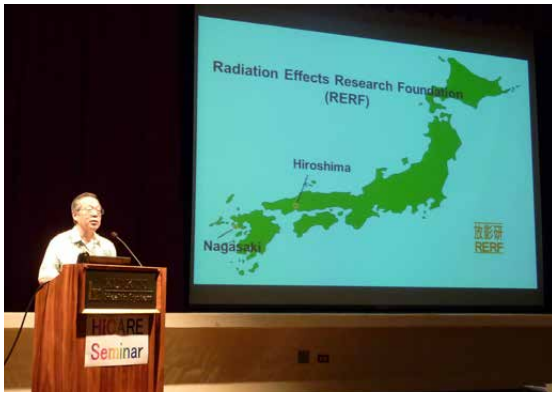
As the March 11 anniversary of the Fukushima nuclear reactor accident approached, many foreign reporters returned to Japan to prepare stories on the status of the recovery. On February 17, Miles O'Brien, science correspondent for the U.S. Public Broadcasting Service (PBS), visited RERF after his trip to Fukushima, where he had been documenting the clean-up and scientists were deciding whether the residual radiation in the affected area could cause harmful health effects. He spoke with RERF scientists and was given a tour of RERF facilities. His story, "*Near Fukushima, a Big 'Guessing Game' Over Radiation's Long-Term Risks,*" was aired on prime-time U.S. TV on the PBS *NewsHour* March 9, 2012. In the program Mr. O'Brien said, "Given their [Japan's] history, they do not have to look far to get the most accurate scientific data on radiation exposure and its long-term effects on human beings. After all, the atomic age began here. I went to Hiroshima to learn more about the famous Radiation Effects Research Foundation." As of this printing, the archived footage was available at:

http://www.pbs.org/newshour/bb/science/jan-june12/fukushima_03-09.html

PBS correspondent Miles O'Brien (left) being shown the liquid nitrogen tanks containing RERF's important biosamples by Evan B. Douple, Associate Chief of Research

RERF Chief Scientist Dispatched to Seminars in Hawaii on Medical Care of Atomic-bomb Survivors

RERF Chief Scientist Kazunori Kodama was dispatched to deliver three seminars in Hawaii, the U.S., hosted by the Hiroshima International Council for Health Care of the Radiation-exposed (HICARE). The seminars, intended for physicians, medical students, A-bomb survivors, and the general public in Hawaii, were held on February 23 and 24. It was the third HICARE seminar series held overseas, following others held in the continental U.S. and Brazil. The objectives were to help deepen understanding of A-bomb survivor medical care and to establish collaborative relationships with local medical specialists. With the Fukushima Dai-ichi nuclear power plant accident as a backdrop, Hiroshima's radiation emergency medical



Dr. Kazunori Kodama delivering seminars in Hawaii on medical care of A-bomb survivors

care system and its experiences in radiation emergency support were also discussed.

In addition to Dr. Kodama, Dr. Hiroo Dohy, then President of HICARE and of the Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, and Dr. Koichi Tanigawa, Vice President of the Hiroshima University Hospital, were also dispatched to the seminars.

Staff News

Yoshiaki Kodama, Chief of the Department of Genetics, retired under the mandatory age limit on December 31, 2011. He was reappointed as chief of the same department on January 1, 2012. On January 1, **Eric J. Grant**, Senior Scientist in the Department of Epidemiology, and **Ayumi Hida**, Chief of the Division of Medicine in the Department of Clinical Studies in Nagasaki, were promoted to the position of assistant chief of their respective departments.

In Japan, March 31 is the last day of the fiscal year, and the new fiscal year starts on April 1 for most organizations, including schools and companies. At RERF, **Saeko Fujiwara**, Chief of the Department of Clinical Studies, and **Akihiko Suyama**, Chief of the Department of Epidemiology in Nagasaki, resigned under the early retirement system on March 31, 2012. On April 1, Dr. Fujiwara assumed her duties as Vice Director of the Health Management and Promotion Center at the Hiroshima Atomic Bomb Casualty Council. Dr. Suyama got a fresh start in April as an internist at the Osaka Saiseikai Izuo Hospital. **Waka Ohishi**, Assistant Chief of the Department of Clinical Studies in Hiroshima, concurrently serves as acting department chief. Dr. Fujiwara was appointed consultant of the same department in May and is expected to continue contributing to RERF. **Kotaro Ozasa**, Chief of the Department of Epidemiology in Hiroshima, will concurrently serve as Chief of the Nagasaki Epidemiology Department for the time being.

Truong-Min Pham, Postdoctoral Scientist in the Department of Epidemiology, and **Robert D. Abbott**, Senior Scientist in the Department of Statistics, left RERF on March 30 and May 9, respectively. Dr. Pham moved to Toronto, Canada. Dr. Abbott returned to the United States to assume his previous position as professor of statistics at the University of Virginia. It is unfortunate that RERF lost four research scientists this spring. However, on April 1, **Eiji Katsurada** was hired by the Division of Health Examinations at the Department of Clinical Studies in Hiroshima and **Keiko Ueda** by the Division of Clinical Laboratories at the same department. The two research scientists newly hired by RERF introduce themselves in the following section.

At RERF, employees who have worked for 10, 20, and 30 years are commended for long service in April every year. In 2012, four employees were commended for 30 years of service, 14 employees for 20 years, and three employees for 10 years, respectively. Among them, **Masataka Taga**, Research Scientist of the Cell Biology Laboratory, Department of Radiobiology/Molecular Epidemiology, and **Kanya Hamasaki**, Research Scientist of the Cytogenetics Laboratory, Department of Genetics, were commended for 10 years of service. In Nagasaki, three employees were commended for 30 years of service, three for 20 years, and two for 10 years, respectively.

Eiji Katsurada, M.D.

Eiji Katsurada

I have been given the honor of taking a position at the Department of Clinical Studies (Hiroshima) thanks to the support of Professor Nobuoki Kohno of the Department of Internal Medicine 2 (formerly composed of the groups of respiratory diseases, diabetes, circulatory diseases, and kidney diseases), Hiroshima University. I graduated from the Hiroshima University Faculty of Medicine, like other physicians at RERF's Department of Clinical Studies. As a college student, I would often go on trips to various countries in East, South, and Southeast Asia by myself while busily working part-time. After graduating from the university, I served as a clinician of internal medicine in various areas in Hiroshima prefecture for 15 years, working day and night (including as an on-duty emergency physician). I was fortunate to be supported by the staff at each workplace, and my clinical experience gained through contact with many patients has been meaningful and precious to me. I was mainly involved in measures to combat community-acquired infections (common infectious diseases) and severe or chronic infections, as well as treatment of lifestyle diseases including hypertension, mild angina, arrhythmia, diabetes, abnormal lipid metabolism, early detection of malignant

neoplasms, and prevention of arteriosclerotic (cardiovascular) diseases, focusing on anti-obesity initiatives and smoking-cessation advice, among other activities. I am certain that such experience will prove useful in health examination activities at RERF. Significant advances have been made in the field of drug therapy over recent years such as with ARB/ACE antihypertensive medication (discovery of the renin-angiotensin-aldosterone system), statins (used to lower cholesterol), and the diabetes medication "Metformin." I have realized from my experience, however, that diet and exercise are even more important. For health reasons, I myself have reduced my sodium intake and continue an exercise regimen that includes biking. I also would like to add that I am qualified as a care manager.

Even though I try to catch up on the news to understand what has happened since the accident at the Fukushima Dai-ichi nuclear power plant, I never dreamed of being able to engage in research at RERF, the principal research institute of radiation health effects, an opportunity for which I feel truly honored. My immediate goal is to correctly understand the research results obtained at RERF thus far, since I am still new and have much to learn. Your support would be greatly appreciated.

Keiko Ueda, M.D., Ph.D.

Keiko Ueda

Greetings to the staff of RERF. I am Dr. Keiko Ueda, and I joined the Division of Clinical Laboratories of the Department of Clinical Studies as a research scientist on April 1 this year.

Let me briefly tell you my career history. After graduating from the Hiroshima University Faculty of Medicine in 1998, I worked at the Hiroshima University Hospital and the Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital as an internal medicine resident. I then was employed at the First Department of Internal Medicine, Faculty of Medicine (presently the Department of Medicine and Molecular Science at the Hiroshima University Graduate School of Biomedical Sciences). Subsequently, after working at the Division of Gastroenterology, Department of Internal Medicine, Chugoku Rousai Hospital, for three years, I entered the Hiroshima University Graduate School, where I pursued my research interest of investigating possible association between non-alcoholic steatohepatitis (NASH) and impaired glucose tolerance. To this end, I performed mainly *in vitro* experiments, culturing hepatic stellate cells (HSC), which play a central role in liver fibrosis and inflammation, and adding advanced glycation end products (AGE) to such cells to examine HSC activation. After receiving my Ph.D., I worked at the Department of Hepatology, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital, for three years. I then took one year off last year to concentrate on raising my child, and was offered the chance to work at RERF.

My image of RERF comprised merely some buildings I used to see along a running course used by the university sports team I was on, a memory that reminded me of the tough training I underwent as a university student. I had no connection with RERF nor expected to work here or be engaged in research ever again. My recent career involved working as a clinician specialized mainly in liver diseases. The life in research that I will experience at RERF is certain to be quite different from my clinical work, and thus I have mixed feelings of both hope and anxiety. Last year, I gave birth to a daughter, and this first experience at childrearing took all my time. This year, as I have become more comfortable with childrearing over the past 12 months, I plan to restore my strength while taking up tennis as a hobby and start working on radiation effects studies with new aspirations. Having said that, I still know almost nothing about RERF and will have to ask for help from the staff of various departments and sections. I look to all of you for your guidance and support.

Visiting Student Researchers

Dr. Wong Kit Fai, a hematologist working for the Department of Pathology, Queen Elizabeth Hospital in Hong Kong, underwent training for biological dose estimation at the Cytogenetics Laboratory of the RERF Department of Genetics, during the period from December 5, 2011 through December 16 of that year. Even though the Queen Elizabeth Hospital plans to establish a biological dose estimation laboratory in preparation for possible radiation accidents, there was no staff member who had experience in this field of research. Therefore, the objective of Dr. Wong's training at RERF was to learn about methods of chromosome analysis best suited for biological dose estimation.

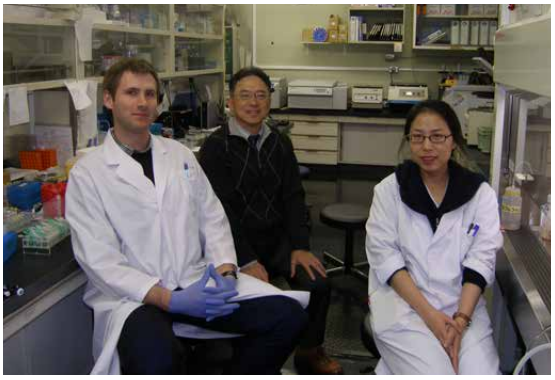
Furthermore, Ms. Hye-Jin Shin, a researcher at the National Cancer Center (NCC), Korea, and Mr. Georgijs Moisejevs, a laboratory assistant and medical student at the Laboratory of Biochemistry, Riga Stradins University, Latvia, underwent training at the RERF Department of Radiobiology/Molecular Epidemiology during the period from January 5, 2012 through February 24 of the same year. The following are self-introductions of Ms. Shin and Mr. Moisejevs and their remarks about experiences at RERF.

Hye-Jin Shin

I am from Seoul, Korea, where I earned Bachelor of Science and Master of Science degrees in Genetic Engineering from Sungkyunkwan University. I am now working at the Radiation Medicine Branch in the National Cancer Center (NCC), Korea as a molecular biologist conducting basic research related to radiation and molecular oncology.

Until 10 years ago, I often considered that Japan is so close, yet so far away. However, nowadays, I feel more familiar with, and closer to, Japan as a result of the active cultural exchanges between the two countries. And I often thought of a journey to Japan during the summer vacation season. Just in time, I was offered participation in the RERF International Exchange Program from my institution and I was able to come here as a trainee. I'd like to express my thanks to RERF for offering me these wonderful opportunities to share research experiences accumulated by RERF scientists over several years as well as to experience Japan's unique culture.

While at RERF I was trained for two months in the Department of Radiobiology/Molecular Epidemiology (RME) to measure intracellular and plasma ROS (reactive oxygen species) levels in regard to investigating the immune function, as well as to quantify DNA methylation for analysis of epigenetic status. In addition, I was able to conduct HLA (human leukocyte antigen) genotyping and SNP (single nucleotide polymorphism) assays with genomic DNA samples from cervical cancer patients of the NCC, Korea, advised and assisted by Dr. Tomonori Hayashi and the staff in RERF's Immunology Laboratory.



Georgijs Moisejevs (left) and Hye-Jin Shin (right) with Dr. Tomonori Hayashi (center) at the Immunology Laboratory

I think that the training in RERF will give me several good ideas for my future studies. Once again, I want to convey gratitude to all of the RME staff and I'll never forget my valuable experience here in RERF.

Georgijs Moisejevs

I am in my last year as a medical student at Riga Stradins University, Latvia. In addition, I have worked as a laboratory assistant almost for five years in my host university's Laboratory of Biochemistry where I studied redox processes. As is well known, our area in 1986 experienced the Chernobyl nuclear power plant accident. Many people were exposed to the radiation, including several people from Latvia, who worked as liquidators or clean-up workers during the disaster. My laboratory, in collaboration with many institutions, participated in the health-effect investigations of those people.

Even now when the world is peacefull, people still need to conduct research on radiation effects, because humanity is using nuclear power to produce energy and no one knows when some kind of accident may appear, such as the Fukushima disaster one year ago.

For me it was a great opportunity to participate in this kind of scientific exchange program at the RERF's Radiobiology/Molecular Epidemiology Department (RME). During my stay here, I spent a lot of time with my supervisor, Dr. Tomonori Hayashi, conducting experiments related to radiation-induced apoptosis. It was very interesting to learn new methods that could be used in my laboratory.

After graduating from the medical university, I want to become a medical oncologist and conduct research in the field of cancer biology. So the knowledge taken from my RERF experience will help me in my future career. In addition, I made several very useful contacts with the Japanese scientists.

I would like to thank my supervisor, Dr. Hayashi, as well as all colleagues from RME, who were such excellent hosts and who gave me excellent explanations, training, and practical skills. Thank you!

Second Meeting of the Scientific and Ethics Committee for the Clinical Study of the F_1 Offspring of A-bomb Survivors

Waka Ohishi, Acting Chief
Department of Clinical Studies, Hiroshima

The second meeting of the Scientific and Ethics Committee for the Clinical Study of the F_1 Offspring of A-bomb Survivors was held at Hiroshima RERF's Auditorium on January 12, 2012. The meeting included reports on the progress of the longitudinal clinical study of the F_1 offspring of A-bomb survivors and on the analysis of the prevalence of individual multifactorial diseases among the F_1 offspring of A-bomb survivors, with discussion taking place regarding the draft of revisions to the written explanation and consent form used at the clinical examinations.

The objective of the original cross-sectional health effects study of the children of A-bomb survivors, conducted from 2000 through 2006, was to study the association between parental radiation exposure and the prevalence of multifactorial diseases among their offspring, based on a mail survey (of 24,673 persons) and health examinations (11,951 persons). As a result of the study's analysis combining six multifactorial diseases (hypertension, hypercholesterolemia, diabetes mellitus, angina pectoris, myocardial infarction, and stroke), no evidence was observed indicating that risk of these diseases increased with parental radiation exposure. The average age of the participants in the original study was approximately 49 years, a relatively young age at which disease prevalence typically just begins to increase. In addition, existence of selection bias in terms of who decided to participate in the health examinations could not be denied in the study. Therefore, changing from a cross-sectional to a longitudinal study was recommended as necessary by the then Joint Scientific and Ethics Committee for the Health Effects Study of the Children of A-bomb Survivors, the Scientific Council, and the Senior Review Panel.

Based on these recommendations, Vice Chairman **Roy E. Shore**, Executive Director **Takanobu Teramoto**, Chief Scientist **Kazunori Kodama**, Chief Scientist **Nori Nakamura**, and the F_1 Clinical Study Working Group, consisting of Clinical Studies Department Chief **Saeko Fujiwara** and other department chiefs and research scientists, met repeatedly to review the issue. In addition, at its first meeting held on July 7, 2010, the Scientific and Ethics Committee for the Clinical Study of the F_1 Offspring of A-bomb Survivors deliberated and approved the research protocol for the "Longitudinal Clinical Study of the F_1 Offspring of A-bomb Survivors," for which I served as principal investigator. Preparations for this longitudinal clinical study's implementation were made by gaining support from all the sections in the Clinical Studies Departments in Hiroshima and Nagasaki, and other departments including Epidemiology and Information Technology, and on November 24, 2010, the study was initiated involving approximately 12,000 participants.

The agenda of the second meeting started with RERF Chairman **Toshiteru Okubo**'s opening remarks and introduction of the committee members, and after Committee Chairman **Tadao Shimao**'s greetings, I reported on the progress during the one year since initiation of the longitudinal study. Chairman Shimao then moderated a Q and A session, and in terms of the issue of the participation rate of 70%, including the number of expected participants, as opposed to the targeted rate of 80%, the committee members provided a number of meaningful proposals to improve participation. Associate Senior Scientist **Yoshimi Tatsukawa** of the Department of Clinical Studies next reported on the latest analysis of individual multifactorial diseases employing data from the original study, as follows: "No evidence was observed for the increase of disease risk related to parental radiation exposure, but it is necessary to continue the study by following the participants until they become elderly, when development of multifactorial diseases tends to increase." Regarding ethical matters for deliberation, I explained the draft revisions to the consent forms and explanatory notes on clinical examinations and storage/use of biological samples, and following a Q and A session moderated

The second meeting of the Scientific and Ethics Committee for the Clinical Study of the F_1 Offspring of A-bomb Survivors held at the Hiroshima Laboratory

by Committee Vice Chairman **Hiraku Takebe**, the draft revisions were approved. The meeting concluded with Chairman Shimao's summary report, followed by Dr. Shore's closing address and words of gratitude to the participants.

A high participation rate for such incidence studies is a requirement for improved quality and reliability in estimation of disease risk. We would like to improve the participation rate in the clinical examinations by quickly putting into practice the proposals provided at the latest committee meeting. We also will continue our efforts to emphasize to participants the health-related benefits from participation in the examinations and the significance of the longitudinal study by actively contacting the study participants by letter and/or telephone.

Members of the Scientific and Ethics Committee for the Clinical Study of the F_1 Offspring of A-bomb Survivors

Tadao Shimao (Chairman), Consultant, Japan Anti-Tuberculosis Association
Hiraku Takebe (Vice Chairman), Fellow, Kinki University Atomic Energy Research Institute
Hirotsugu Ueshima, Special Contract Professor, Lifestyle-Related Disease Prevention Center, Shiga University of Medical Science
Takashi Kawamoto, Professor, Graduate School of Education, The University of Tokyo
Shinsuke Kimura, Attorney, Kimura Shinsuke Law Office
Takashi Gojobori, Vice Director and Professor, National Institute of Genetics
Hideo Sasaki, Director, Health Management and Promotion Center, Hiroshima Atomic Bomb Casualty Council
Steve Wing, Associate Professor, Department of Epidemiology, School of Public Health, University of North Carolina
Kazuo Tajima, Director, Aichi Cancer Center Research Institute
Masao Tomonaga, Director, Japanese Red Cross Nagasaki Atomic Bomb Hospital
Taisei Nomura, Professor Emeritus, Osaka University
Norihiko Hayakawa, Professor Emeritus, Hiroshima University
Katsumi Furitsu, Visiting Lecturer, Hyogo College of Medicine
Eiji Maruyama, Professor, Graduate School of Law, Kobe University

RERF International Workshop: Radiation Effects on Mutation in Somatic and Germline Stem Cells

Asao Noda, Assistant Chief
Department of Genetics

The above workshop was held in the RERF Auditorium on January 18–19, 2012

Stem cells play a principal role in the continuous provision of new cells in the body. As long as stem cells are healthy, old cells can be replaced by new cells, whereby tissue homeostasis is maintained. Dramatic progress has been made recently in the area of research into somatic and germline stem cells, and consideration of radiation effects on such stem cells is important for investigation of radiation effects on human health. The international workshop, aiming at debate about the current status of stem cell research as well as effects of radiation on stem cells, was attended by four stem cell researchers from abroad and five from Japan (experts related to stem cells of the small intestine, skin, testis, and bone marrow).

At the workshop, lectures were provided by **Catherine Booth** (keynote lecturer; Epistem Ltd., UK), **David T. Breault** (Harvard Medical School, USA), **Jolyon H. Hendry** (Christie Hospital NHS Foundation Trust, UK, and member of the International Commission on Radiological Protection [ICRP]), **Toshio Suda** (Keio University), **Takashi Shinohara** (Kyoto University), **Claudia E. Ruebe** (Saarland University, Germany), **Emi Nishimura** (Tokyo Medical and Dental University), **Hiroshi Mitani** (The University of Tokyo), **Asao Noda** (Assistant Chief, RERF Department of Genetics), **Nori Nakamura** (RERF Chief Scientist), and **Ohtsura Niwa** (Professor Emeritus, Kyoto University). The workshop concluded with a comprehensive discussion regarding how data related to radiation effects on stem cells would contribute to RERF's ongoing research into cancer risk estimation.

The following questions were introduced at the workshop and identified key problems for consideration by all participants:

(1) Where do tissue stem cells reside and function

in tissues undergoing typical cell turnover? Is it possible to locate stem cells within tissue, by characterizing tissue stem cells?

(2) What characteristics do stem cell niches maintaining tissue stem cells have?

(3) What effects does radiation exposure have on stem cells, or, how do stem cells behave in response to radiation exposure?

(4) What effects does radiation response of stem cells have on tissue radiosensitivity and recovery from radiation exposure?

(5) Functional deterioration of stem cells is suggested as the cause of aging. Is it possible to successfully observe such histological (pathological) changes? Does radiation exposure affect the aging of tissues?

(6) Is it possible to explain risks from fetal or childhood radiation exposure in terms of the behavior of tissue stem cells?

(7) Is it possible to explain risks of radiation carcinogenesis in terms of the radiosensitivity of tissue stem cells?

The following summarizes my thoughts regarding the questions and issues listed above, after taking into account reports by workshop participants:

The small intestine is a target organ of radiation effects. In the body, the small intestine is composed of the most active tissues in terms of cell division, with digestive and absorptive cells newly born in small-intestinal villi completing their roles in approximately five days, upon which they are replaced by new cells. The intestinal basal membrane contains intestinal crypt structures, which in turn house intestinal stem cells. Dr. Booth outlined previous intestinal stem cell studies, and Dr. Breault introduced the latest findings, concluding that Tert (+) cells, which reside at the +4 crypt position from the crypt base and undergo cell division extremely slowly, are true stem cells (multipotent intestinal stem cells: ISCs). Future tasks include determination of whether or not it is possible to explain intestinal radiosensitivity and acute radiation symptoms observed at the individual level in terms of radiosensitivity of ISCs.

The most radiosensitive stem cells are those of the bone marrow. Radiation therefore causes symptoms of anemia. Dr. Suda discussed a maintenance mechanism for bone marrow stem cell properties and concluded that hypoxia in niches and hypoxic signaling (HIF-1α signaling) are indispensable as part of this mechanism for maintaining stem cell quiescence. Dr. Hendry stated that there was a correlation between degree of differentiation, hypoxia, and radiosensitivity of bone marrow stem cells, adding that the more undifferentiated/hypoxic cells have greater radio-resistance. Discussions engaged in by the four researchers mentioned above suggested the possibility that a hierarchical stem cell organization governing 'stemness' is present and that more primitive stem cells are more quiescent (slowly cycling) and more radio-resistant. This would represent a new development transcending the conventional wisdom that self-renewing stem cells are highly sensitive to radiation.

Spermatogonia are the original germ cells, from which spermatozoa are produced, and hence the possibility exists that radiation effects on spermatogonia are expressed as hereditary, or transgenerational, effects. Dr. Shinohara has successfully cultivated spermatogonia extracted from mice testis, while maintaining stem-cell properties. If *in vitro* reproduction of meiosis and spermatogenesis can be realized in the future, the field of radiobiology would benefit significantly. Dr. Ruebe, who investigated the recovery process (damage repair) in sper-

Participants of the international workshop

matogonia from radiation exposure, reported that the initial phase of spermatogonia radiation response was different from that of somatic cells.

The skin is composed of tissue cells that repeatedly divide over a lifetime and contains stem cells. The skin is one of the target organs of radiation disorders and is suited for observation of the aging process. Dr. Nishimura, who analyzed radiation-accelerated senescence mechanisms in melanocyte stem cells, reported that these stem cells differentiated into pigment cells in stem cell niches, leading to the aging of such cells. This suggests that, since stem cell niches cannot maintain melanocyte stem cells as stem cells, aging of the skin may very well start at this point.

With regard to the development of model systems for research into radiation effects on stem cells, Drs. Mitani and Noda reported on *medaka* (*Oryzias Latipes*)- and mice-based experimental systems, respectively. Both aimed at visualizing effects on stem cells (apoptosis and mutations) *in vivo*. Those systems are likely to make visible and observable the origins of radiation carcinogenesis in the body.

Sometimes effects of adulthood radiation exposure persist over a lifetime, while in some cases effects of fetal exposure are not detected postnatally. Dr. Nakamura reported that no effects of fetal radiation exposure observed using chromosomal aberrations as a marker were detected in adult lymphocytes.

Finally, Dr. Niwa reported on changes in tissue stem cells and stem cell niches through ontogeny, growth, and aging, adding that the relevant changes affect risk of radiation carcinogenesis.

In relation to this workshop, I would like to take this opportunity to express my deep appreciation for the support we received from the Ministry of Health, Labour and Welfare as well as the cooperation from so many people in line with the plan prepared by the RERF Department of Genetics. A more detailed account of the workshop has been published and is available at the *International Journal of Radiation Biology*.

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Highlights of RERF Departments: Research Activities in the Department of Genetics

Yoshiaki Kodama and Jun-ichi Asakawa

Department of Genetics, RERF

Summary

The Department of Genetics consists of two laboratories, the Biochemical Genetics Laboratory and the Cytogenetics Laboratory. The Biochemical Genetics Laboratory has conducted research to investigate whether mutation rates have significantly increased among children born to A-bomb survivors and has developed the necessary techniques for such research. Extensive research conducted thus far has not demonstrated evidence of genetic effects from A-bomb exposure.[1,2] The laboratory has also collected blood samples from families containing at least one A-bomb survivor (mother-father-child) to secure DNA samples necessary for molecular biology research and is establishing Epstein-Barr virus (EBV)-transformed cell strains (B cells). Since genome analysis became possible in the late first decade of the 2000s, the laboratory is now conducting research on radiation-related germline mutations in both animal models and humans, using high-density microarrays (1–2 million probes). Further, with recent rapid advances in technology, whole-genome base sequencing has become possible.

The Cytogenetics Laboratory has screened chromosome aberrations in somatic cells from A-bomb survivors and focused on evaluation of biological responses with the aim of carrying out biological dosimetry. The laboratory has also utilized electron spin resonance (ESR) spectroscopy to identify trace substances that were generated by radiation exposure and have remained in tooth enamel. The purpose of these studies is to obtain information to confirm individual radiation dose estimates used for evaluation of risk of cancer and noncancer diseases due to exposure to A-bomb radiation and improve the quality of such estimates. The laboratory is also conducting in genetic research on breast cancer and skin cancer as well as research involving the detection of irreparable DNA damage. Furthermore, the laboratory developed a new animal model system for estimation of genetic effects of low-dose radiation and succeeded in creating mice with cells that generate green fluorescence upon mutation. Recent research activities and the future plans of the Department of Genetics are summarized below.

Department Staff

The Department of Genetics has a chief, an assistant chief, two laboratory chiefs, a senior scientist, two research scientists, 13 technicians, and two administrative staff members.

Research Scientists of Genetics (first row from left) Mieko Kodaira, Yoshiaki Kodama (Department Chief), Nori Nakamura (Chief Scientist), Asao Noda, (second row from left) Yuko Hirai, Jun-ichi Asakawa, Yasunari Satoh, Kanya Hamasaki

Biochemical Genetics Laboratory

Introduction of high-density microarray technique

Since genomic base sequencing has been completed for the human and mouse genome, laboratories can now conduct genetic research at the whole-genome level using high-density microarrays. In 2009, a high-density microarray system was introduced into the Biochemical Genetics Laboratory at RERF. With the comparative genomic hybridization (CGH) technique using the microarray system, copy number variations (CNVs; gene amplifications or gene deletions) in two DNA samples for a single species can be examined. Since mutations induced by radiation are considered to be mainly gene deletions resulting from DNA double-strand breaks, CGH research using high-density microarrays is now considered to be an appropriate technique for studying radiation-induced genetic effects.

As a result of improvements in CGH experi-

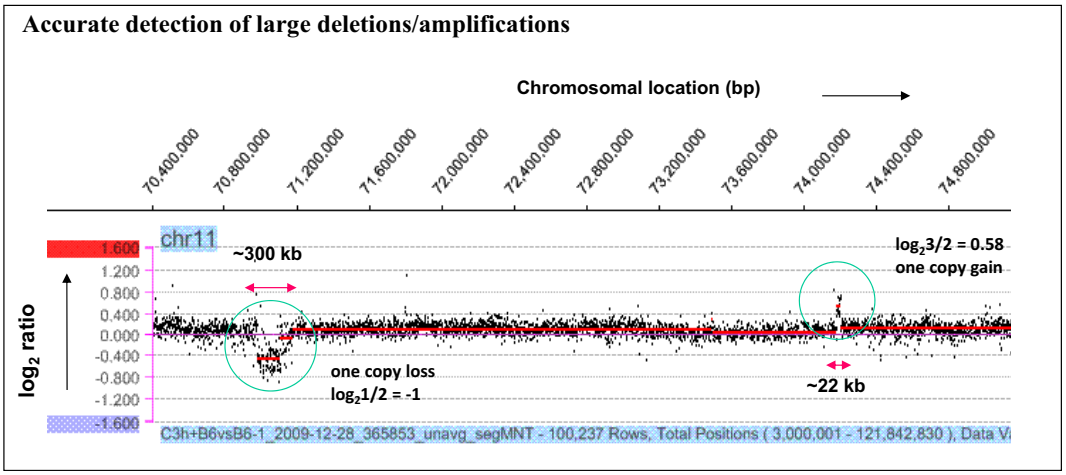


Figure 1. Copy number variations at 22 kb and 300 kb on the mouse genome detected by the microarray CGH method. Each of the points in the figure indicates the relative ratio of the copy numbers of the two DNA samples. When the copy number is the same, $\log_2$ equals "0." However, when the copy number in one DNA sample changes, the absolute value of $\log_2$ changes by at least 0.4.

mental and analytical techniques, it has become possible to detect from small to large CNVs (as illustrated in Figure 1), enabling the conduct of the following three studies using high-density microarrays: (1) study of offspring of exposed male mice as a model for human male exposure, (2) study of offspring of exposed female rats as a model for human female exposure, and (3) study of children born to A-bomb survivors in Hiroshima and Nagasaki.

(1) Study of offspring of exposed male mice as a model for human male exposure

This is a joint study carried out with Dr. Yoshiya Shimada's group at the National Institute of Radiological Sciences. His group was responsible for the study's gamma-irradiation, animal breeding, and extraction of F_1 DNA samples. Male C57BL/6 (B6) mice were exposed to 4 Gy of gamma rays and mated with female C3H mice eight weeks later (F_1 of exposed group). As the controls, F_1 mice born to non-exposed male B6 mice and female C3H mice were used. CGH experimentation was conducted for a set of DNA samples from one F_1 mouse in the exposed group and one in the non-exposed group. As a result of CGH analysis conducted on 80 such sets, 26 candidate CNV mutations were detected. Analysis of the DNA of the parents of the F_1 mice in which these CNVs were detected showed that five of the CNVs were also detected in the parents. Thus, these CNVs in the F_1 were inherited from their parents. The remaining 21 CNVs were *de novo* mutations (Figure 2). Of these 21 mutations in total, nine were detected in the exposed group (six gene deletions found in six F_1 mice, three gene amplifications in three F_1 mice), and 12 in the control group (seven gene deletions found in seven F_1 mice, five gene amplifications in three F_1 mice, three gene amplifications in one F_1 mouse). Judging only from the number of mutations, no genetic

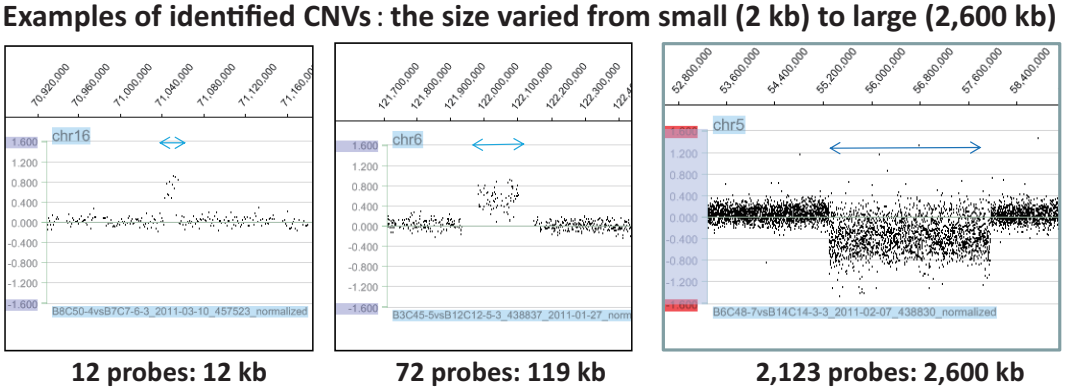


Figure 2. Examples of identified CNVs. The two right cases were *de novo* mutations observed in F_1 mice only. The left case was a hereditary mutation because the same CNV was observed in the male parent.

effects of radiation were suggested. Analysis is now under way for clarifying the mechanism of production of the abovementioned mutations and their characteristics at the molecular level.

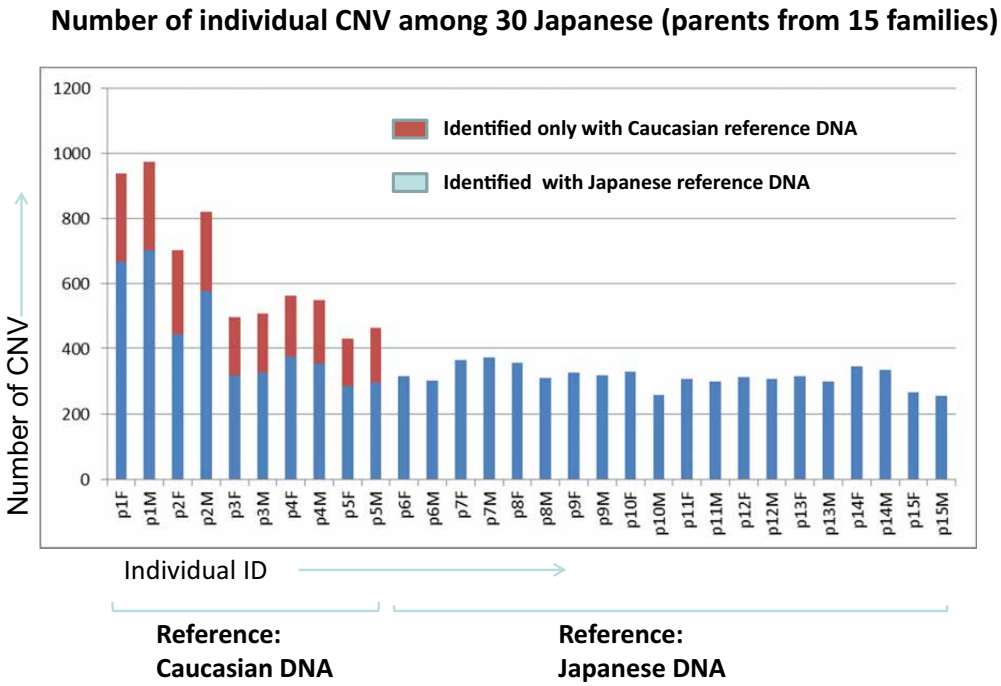
(2) Study of offspring of exposed female rats as a model for human female exposure

In comparison with the male animal model, the female animal model was problematic. This was due to the fact that female mice become sterile (due to depleted ova) after radiation exposure as a result of extensive death of immature oocytes (target cells at risk), because such cells in mice are extremely sensitive to the killing effects of radiation,[3] and therefore an F_1 generation is not possible. After investigation of several non-mouse animal species, we identified the rat as a good model. We conducted large-scale mutation screening with DNA 2-dimentional electrophoresis (DE) technique of 750 F_1 rats derived from irradiated immature oocytes (2.5 Gy of gamma rays) and of 750 F_1 rats from unirradiated females as a control group. We selected about 1,500 spots each derived from female parents and male parents deemed to be appropriate for detection of mutations, and searched for mutation candidate spots showing qualitative changes (gene copy number changes) and positional changes. As a result of analysis of 1,500 images each from the exposed group and the control group, totaling 3,000 images (equivalent to a total of 2.3 million gene locus tests), it was suggested that genetic effects of radiation in female exposures are smaller than those in spermatogenous cells of male mice. In a new research protocol using high-density microarrays, with F_1 rats from the exposed group and control group comprising a set, screening of CNV mutations using the CGH technique is being conducted for 200 sets of DNA samples from 200 rats from the 2.5 Gy exposure group and 200 rats from the control group, all of which were used in the DNA 2-DE study. As of April 2012, CGH experimentation has been completed for about 160 sets of the rats. Experimentation will be completed soon for all the sets.

(3) Study of children born to A-bomb survivors in Hiroshima and Nagasaki

Genetic effects of A-bomb radiation (trans-generational effects) still have not been fully elucidated due to the low rates of spontaneous and radiation-induced mutations in germ cells. Therefore, we are conducting CGH research based on high-density microarray technology for a total of 688 persons, i.e., both parents of 184 families with one of the parents exposed to high doses of A-bomb radiation and their 320 children (160 from paternally exposed families and 160 from maternally exposed families). We plan to use a 3 × 1.4 M high-density microarray that contains three identical sets of 1.4 million probes (mean interval: 2.2 kb) on a single slide. The microarray permits us to analyze DNA samples from three subjects at a time on a single array. We do not expect to discover a sufficient number of mutations in the 160 children of the maternally exposed families to detect an elevated rate, because the mutation rate obtained in irradiated rat immature oocytes appeared to be considerably lower than that in rat spermatogonia. Nonetheless, analysis of this group is indispensable because the paternal alleles were not exposed to radiation and hence serve as controls for the abovementioned paternally exposed families. The analyses will provide far more information than is currently available on risks associated with maternal and paternal radiation exposure.

In the model experimentation using mice or rats, the genomes of such animals used are almost the same and CNVs are extremely rare. However, in the human genome there are more than several hundred different CNVs.[4] In the CNV mutation search using the CGH technique, CNVs with high frequency (polymorphic CNVs: same mutations observed in at least two persons out of 50) are usually considered to be hereditary. Therefore, even if such CNVs are detected in children, they are not considered to be candidate mutations. First, to comprehensively search for CNVs in study subjects, CGH experimentation was conducted in 50 father-mother-child trios using specific persons as controls. DNA from a Caucasian male used worldwide as a control and DNA from the first Japanese, a male, to undergo whole-genome sequencing were studied. To enhance quality, CGH experimentation was performed using the dye-swap method, in which replacement of fluorescent dyes is conducted for marking two samples in a single trio set with the relevant experiments conducted twice. To date (as of December 2011), CGH experimentation has been completed for 26 trios. As a result of comparison of CNVs among 15 trios, a total of 10,639 CNVs were detected in 30 fathers and mothers and classified into 3,070 different types of CNVs. Slightly more than 50% of these CNVs were as small as around 3 kb, including only two probe regions. As shown in Figure 3, there are about 300 different CNVs in each individual when the Japanese male was used as reference. However, when the Caucasian male was used, additional roughly 200 unique interracial CNVs were identified (Figure 3, the red areas of the bar graph). Based on the above results, we decided to use the said DNA for the subsequent experiments, since the Japanese DNA, being of the same ethnic group as that of the subjects, was considered to be appropriate for use as reference for mutation searches. Using the abovementioned Japanese DNA as a



- **The use of a Japanese DNA as the reference was a good choice in our CGH study.**

Figure 3. CNVs identified in 30 Japanese. The term "p1F" indicates the paternal DNA of pair 1 and "p1M" the maternal DNA of pair 1. At least half of about 300 CNVs in any individual are only about several kb. As the size of such CNVs increases, CNV numbers decrease.

control, analysis of 50 trios in Hiroshima and Nagasaki will be conducted to identify polymorphic CNVs. If CNVs different from those CNVs are detected only in the children of the trios, we plan to analyze such rare CNVs as candidate *de novo* mutations. By so doing, it is expected to be possible within a few years to understand at the whole genome level the problems of genetic effects that are as yet unresolved.

Current status and problems of whole-genome base sequencing

Genome analysis, especially base sequencing, continues to make remarkable progress with advances in IT technology. There are now private companies and research organizations that provide whole genome sequencing (WGS) services, making whole genome information available at relatively low cost. However, since the genome of mammals, including humans, consists of a large number of bases ($2–3 \times 10^9$), 30,000 erroneous base sequences may be obtained even with analysis that is 99.999% accurate. In reality, about 30 spontaneous point mutations actually occur in the human genome. It would be next to impossible to differentiate these actual mutations from the said 30,000 false-positive base sequences. In addition, software used for analysis of such large amounts of information is not appropriate for identifying gene deletions (especially heterozygotes) mainly induced by radiation. Furthermore, individual differences in human genome sequences is a major factor that complicates the analysis. In fact, we performed (commercially available) WGS using cultured cells derived from a Japanese male and detected about 3.2 million single nucleotide polymorphisms (SNPs), 100,000 deletions/insertions of 1 bp, 70,000 deletions/insertions of from 2 bp to 30 bp, and 5,000 genomic structural abnormalities/copy number variations, in comparison with human reference sequences. For the above reasons, it is at this time not practical to use WGS for evaluation of genetic effects in A-bomb survivors' children.

To establish a system for accurately and efficiently identifying radiation-induced mutations by WGS, the Biochemical Genetics Laboratory is planning to conduct analysis of radiation-induced mutations using WGS and cultured cells from an individual with the same genetic background and animal models previously used. In particular, we obtained a Grant-in-Aid for Scientific Research for a WGS-dependent study of radiation's genetic effects using rats in a female exposure model and are making preparations for conduct of the study. Analysis of WGS data will be carried out under the guidance of Dr. Tatsuhiko Tsunoda's group at RIKEN, the organization that performed whole genome analysis in a Japanese participant for the first time.

Cytogenetics Laboratory

Cytogenetics study of A-bomb survivors

Since chromosome aberrations show high specificity for radiation, it is well known that frequency of chromosome aberrations can be used as an indicator of level of radiation exposure. The purposes of the chromosome study for A-bomb survivors are to conduct biological dose estimations using chromosome aberrations as markers, to investigate biosamples from each survivor, and to evaluate physical dose estimates arrived at by computation.

The chromosome study for the Adult Health Study (AHS) population was initiated in the late 1960s. Because more than 20 years since the bombings had already passed at that time, most unstable chromosome aberrations, including the easily identifiable dicentric chromosomes and rings, had nearly completely disappeared from the peripheral blood of A-bomb survivors. In this chromosome study, therefore, only stable chromosome aberrations (translocations or inversions) were studied. Giemsa staining was used from the time of initiation of the study through 1993. Using this Giemsa staining method, about 2,000 cases in Hiroshima and about 1,000 cases in Nagasaki were studied. The study showed a wide scattering of frequency of chromosome aberrations compared with the actual physical radiation doses calculated for both cities, and a difference in the dose-response relationship between Hiroshima and Nagasaki. At doses of 1.5 Sv or less, the response was significantly larger in Hiroshima than in Nagasaki (Figure 4).[5] Since research using Giemsa staining was independently conducted at the laboratories in Hiroshima and Nagasaki, the possibility of detection errors dependent on the laboratory (observer) could not be negated. This is an especially important point in considering differences between Hiroshima and Nagasaki.

In the late 1980s, a new chromosome analysis technique called fluorescence *in situ* hybridization (FISH) was developed. With this technique, specifically targeted chromosomes can be stained using DNA probes specific to selected chromosomes. This technique enables accurate and speedy detection of exchange-type abnormalities, including translocations. We started to use the FISH technique in 1994 in a chromosome study of A-bomb survivors. At the same time, to resolve the possibility of detection errors between the two laboratories, all work related to the chromosome testing was conducted exclusively at the laboratory in Hiroshima. To date, analysis was completed for about 900 cases in Hiroshima and about 500 cases in Nagasaki. Like in the study using the Giemsa method, the study using FISH revealed a wide scattering of chromosome aberration frequencies compared with physical doses. Differences in the dose-response relationship, presumably due to shielding status, were also observed. The above findings suggest that there are errors in the dose estimates for some A-bomb survivors. On the other hand, the differences between Hiroshima and Nagasaki were remarkably reduced using FISH, suggesting that the city differences previously observed were due to differences in abnormality detection rates between the two laboratories (observers).

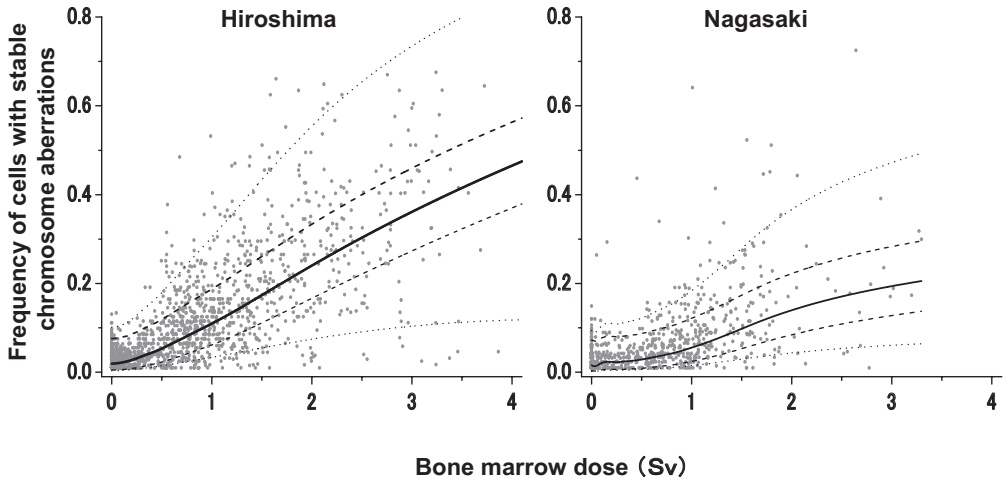


Figure 4. Physical dose estimates and chromosome aberration frequencies. Each point indicates data of individual A-bomb survivors (1,980 cases in Hiroshima and 1,062 cases in Nagasaki). The solid line shows dose-response relationship and the dashed lines represent 95% confidence interval. The dotted lines are an assumed coefficient of variation of 50% of the estimated doses beyond the confidence interval.[5]

Lack of cytogenetic dose response in the in utero *population*

Based on epidemiological research conducted in England in the 1950s concerning the development of cancer among children, it had been considered that the fetus is highly sensitive to radiation. However, in the chromosome study of those exposed *in utero* to A-bomb radiation, almost no dose response was observed with respect to frequency of translocations in the peripheral lymphocytes of such subjects. On the other hand, clear dose-response relationships were observed regarding translocation frequencies in the lymphocytes of the mothers of those exposed *in utero* (Figure 5a).[6] This finding was reproduced in a study of hematological cells in irradiation experiments later conducted using mice (Figure 5b).[7] To study whether this finding was unique to hematopoietic cells, rat fetuses were exposed to radiation, and frequency of chromosome aberrations in mammary epithelial cells was examined. Examinations conducted 6–45 weeks after irradiation showed that in the exposed fetuses, like in the mothers, radiation damage remained (manuscript in preparation). Therefore, such results suggest that the lack of a translocation dose response after exposure *in utero* was tissue-dependent. At present, to confirm the results of this study, which used mammary cells, a similar study using mouse thyroid epithelial cells is currently under way.

Estimation of exposure dose using tooth enamel

By measuring the amount of CO_2^- radicals remaining in tooth enamel, radiation exposure doses for individuals can be estimated. For such an estimation, enamel is separated from a tooth extracted for therapeutic reasons, and the amount of CO_2^- radicals is measured using ESR spectroscopy. Since ESR signal intensity is proportional to the radiation dose received and does not change over time, exposure dose can be measured directly, irrespective of the mode of exposure. This technique has been used in dose assessment for those exposed to radiation in the Chernobyl accident and other exposures. Recently, a method was also developed for use in making direct measurements of teeth in the mouth, not extracted teeth. The purposes of the study are to estimate using the ESR method, individual doses using tooth enamel and to compare the results with DS02 doses and the frequency of chromosome aberrations in lymphocytes from the same individuals. ESR signal intensity is converted to gamma rays using the calibration curve from cobalt gamma-ray irradiation.

One of the problems with ESR measurement was how to treat exposure to diagnostic dental X rays. However, because a long time had passed since the atomic bombings, obtaining all diagnostic records was considered a difficult task. Furthermore, it was thought that medical exposure doses may be different by production year and model of

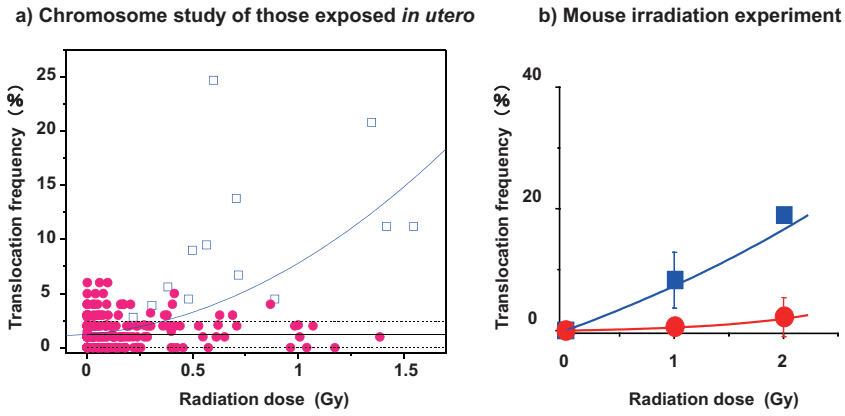


Figure 5. *In utero* exposure and chromosome aberrations

a) Results of the chromosome study of those exposed *in utero*. No dose-response relationship was observed regarding frequencies of translocations in blood lymphocytes among those exposed *in utero* (●) (age at examination: 40 years). However, dose response was observed in the mothers (□) of those exposed *in utero*.[6] The curve shows the frequency of dicentric chromosomes resulting from *in vitro* irradiation (dicentric chromosomes and translocations are thought to occur at the same frequency).

b) Results of irradiation experimentation using mice. Mice which were pregnant for 15.5 days were exposed to radiation. Lymphocytes of the offspring were examined on the 20th day after birth. Symbols indicate the mice exposed *in utero* (●) and their mothers (■). Results similar to those from the chromosome study of humans exposed *in utero* were obtained.[7]

diagnostic equipment, and therefore accurate evaluation of diagnostic dental X rays was expected to be difficult. Therefore, we measured each of the teeth obtained by dividing it into an outer part (buccal side) and an inner part (lingual side). Since X-ray diagnosis at dental clinics mainly involves radiation exposure on the outer sides, we thought there may be differences between the outer and inner teeth. When we actually conducted measurements, however, we found little difference between the outside and the inside of the back teeth, while the signal from the enamel of the outside of the front teeth was rather large.[8] The large signal commonly found in the outside of front teeth may be due to ultraviolet ray effects, but no clear reasons have yet been found. Since this problem became apparent, the back teeth (large molars) are now used in our ESR measurements.

In Figure 6, for 61 A-bomb survivors, frequency of translocations in lymphocytes (Y axis) was compared with physical dose (left panel, X axis) and ESR dose estimates obtained from the large molars (right panel, X axis).[8] In the left panel, a wide scattering can be seen in the relationship between the physical dose and the frequency of translocations, while in the right panel, the distribution of plots is smaller, indicating good correlation between the dose information obtained from teeth and the frequency of translocations. The one exception in the right panel is the "wisdom tooth" (indicated by an arrow). The subject was 15 years old at the time of the A-bomb exposure, and the estimated dose from analysis of the tooth was 0 Gy. However, the frequency of translocations in this subject is more than 20%, suggesting exposure to a high level of radiation. It was only later that this tooth was found to be a wisdom tooth. It is known that there is large individual variation in the timing of formation of wisdom teeth. As for this subject, the tooth for which the measurement was made had not fully developed at the time of A-bomb exposure. For confirmation, we requested this person to donate another tooth other than a wisdom tooth, but we have not yet obtained the said donation. Thus, although dose estimation using tooth enamel is an effective method, difficulties in collecting samples (teeth) is a major problem.

Dose estimation for distally exposed persons using tooth enamel

As for the radiation dose (gamma rays and neutrons) directly released from the atomic bombs, consensus was reached among the physicists concerned (DS02 dose estimates). However, exposure to so-called residual radiation, including exposure to secondary radiation due to activation of the soil by neutrons or nuclear fission products contained in radioactive fallout, is a controversial topic because of insufficient measurement data. To provide new information for discussion of this issue, we conducted an evaluation of exposure doses using ESR for teeth donated from A-bomb survivors in Hiroshima who were exposed to the bomb at distances of 3 km or greater from the hypocenter and whose DS02 dose estimates were less than 5 mGy. We evaluated 49 persons who were exposed to the bomb at the ages of 10 years or older using a total of 56 donated molars. To examine the effects of diagnostic dental X rays, these teeth were cut into outer and inner parts, and an ESR measurement was conducted separately for each part. Dose estimates of the 56 large molars ranged from –200 mGy to 500 mGy (gamma rays). The mean dose of the outer side of the teeth was 70 ± 157 mGy (median value: 17 mGy), and that of the inner side 34 ± 127 mGy (median value: 13 mGy) (Figure 7).

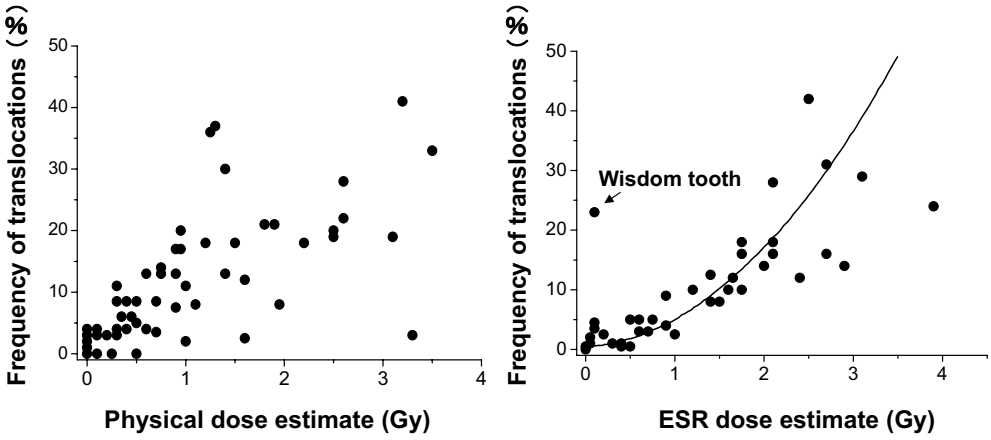


Figure 6. Results of a comparison of the frequencies of translocations in lymphocytes with physical dose estimates (left panel) and ESR dose estimates (right panel) for 61 A-bomb survivors. The curve in the right panel shows a dose response for dicentric chromosomes from an *in vitro* irradiation experiment. The arrow indicates a "wisdom tooth" from a person exposed to A-bomb radiation at the age of 15.[8]

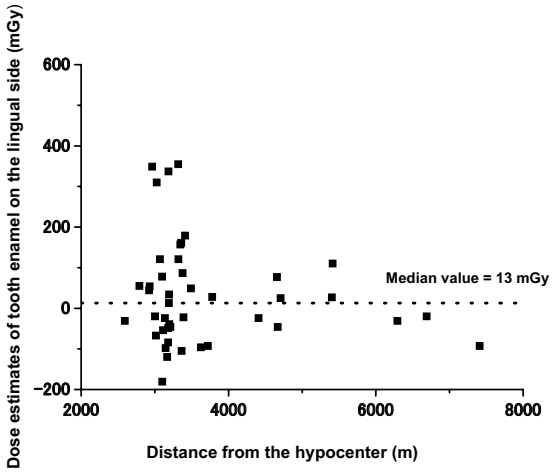


Figure 7. Dose estimates obtained from tooth enamel ESR and distance from the hypocenter for distally exposed individuals in Hiroshima[9]

Four of the 49 persons were exposed to A-bomb radiation in the "black rain" area, but did not exhibit extremely high dose information. In three donors, exposure doses of both the outer and the inner parts of the teeth were as high as 300–400 mGy. These donors may have been exposed to some type of radiation, but the exact reason for these high doses is unknown. Nevertheless, the exposure dose estimated by ESR for the 49 distally exposed individuals did not indicate evidence for exposure to high levels of radiation (for example, more than 1 Gy) from residual radiation, as suggested by some parties.[9]

Genetics study concerning breast cancer

Epidemiological research at RERF has shown that breast cancer among A-bomb survivors has a high mean excess relative risk (ERR), strongly suggesting association of the disease with radiation exposure. Moreover, it is assumed that the ERR of early-onset breast cancer cases (age at the time of bombing: less than 20 years old, age at diagnosis: less than 35 years old) is especially high. We hypothesized that the risk of early-onset breast cancer risk is high in A-bomb survivors because normal gene functions were lost due to radiation exposure in those inheriting breast cancer-related gene variants (heterozygotes). To investigate this possibility, we examined specific SNPs considered to be associated with early development of breast cancer. The study indicated that specific genotypes exist in large numbers in the early-onset breast-cancer group among A-bomb survivors (exposed group, age at diagnosis: less than 45 years old). This study is ongoing.

Genetics study concerning skin cancer

The frequency of patients (homozygote) with cancer-prone recessive hereditary disorders, such as xeroderma pigmentosum (XP), is usually low, at several cases per 100,000 people, but carriers (heterozygote) are not rare, with a frequency on the order of one percent. However, there is little data regarding cancer risk in such heterozygote carriers, as they are generally difficult to identify. But, the examination of carriers of an inactive mutant allele is most appropriate as a model in establishing cancer risk in carriers, on the assumption that the specific gene function decreases about 50%. The aim of this research is to evaluate the cancer risk in such carriers by comparing the frequency of carriers bearing a founder mutation in the *XPA* gene in non-melanoma skin cancer patients against a control population, by taking advantage of the fact that a founder mutation at the *XPA* gene (inactive mutation) exists at a relatively high frequency in the Japanese population and can be found easily using the polymerase chain reaction-restriction fragment length polymorphisms (PCR-RFLP) method. In this research, we plan to conduct screening of 1,000 non-melanoma skin cancer cases and compare the frequency with that in a control population of 1,500 individuals. This research is suitable for a model in establishing cancer risk in heterozygous carriers of gene mutations related to cancer-prone recessive hereditary disorders.

Detection of irreparable DNA damage

It is known that some DNA double strand breaks (DSBs) induced by radiation cannot be completely repaired and exist for a long period of time. It is considered that such irreparable genome damage is involved in cell death and functional changes in tissues. However, since it is impossible to biochemically differentiate such irreparable damage from repairable damage, the frequency and biological effect of such irreparable damage cannot be precisely assessed. We consider repair foci that are formed after radiation exposure and persist within the nucleus for a long period of time or permanently to be irreparably damaged sites, and are conducting their characterization and review of their biological effects. Eventually, we want to quantify irreparable DSBs within cells and tissues exposed to radiation in the past.

Development of animal model system for evaluation of genetic effects of radiation

To study genetic (transgenerational) effects of radiation, we are creating model mice in which germ-cell mutations can be detected easily, instead of using F_1 individuals born to irradiated parents. Multiple detection systems are being examined. One of the systems is based on the idea that cells generate fluorescence upon reversion.[10] Another system allows cells to generate fluorescence by for-

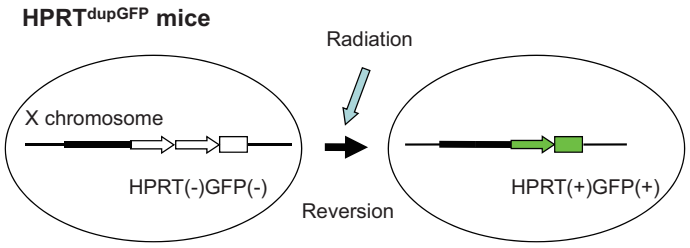


Figure 8. Cells become GFP(+) by reversion from partial duplication of endogenous gene.

ward mutation in the body.[11] Thus far, the former system has reached the stage of feasibility. Using mouse embryonic stem (ES) cells as host cells, a partial duplication region was introduced into the *HPRT* gene and connected with the green fluorescent protein (*GFP*) gene (Figure 8). Because of recombination arising in the duplication region, it was confirmed that the reversion cells became GFP positive (Figure 9). Therefore, knock-in mice were produced using these ES cells (injection of ES cells into fertilized eggs and transplantation of those cells into the fallopian tubes of pseudopregnant mice were outsourced). Although the promoter activity of the endogenous *HPRT* gene was sufficient in ES cells, it was not strong enough at the cellular level of a mouse, and GFP-positive somatic cells were not discovered. Therefore, we replaced the *HPRT* gene promoter of the ES cells with a CAG promoter, and again produced mice. In the mice thus created, GFP-positive cells were identified at the cellular level in many systems, including the pancreas, small intestine, liver, and testis. In other words, we created mice in which mutations in cells throughout the body can be measured *in situ*. In these mice, only recombinant mutations in the tandem duplication region can be identified. However, cells with mutation in somatic cells and germ cells in such mice can be very easily detected as cells generating green fluorescence. Furthermore, mutation frequencies in hematological cells can be obtained by flow cytometer. At present, with consideration given to the method of measurement of mutation frequencies, measurements of mutation rates in the cells of various organs are being conducted.

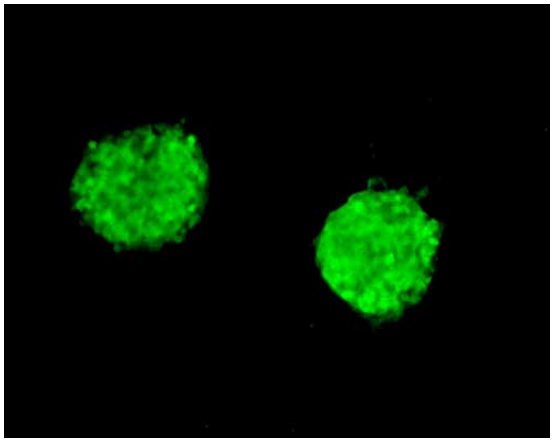


Figure 9. HPRT(+) GFP(+) ES cell colonies obtained from spontaneous mutation.

Information on the genetic risks from radiation in humans largely depends on the results from mutation studies using the mouse specific-locus method. However, as those studies required as many as several million F_1 animals, information on low-dose radiation is limited. Use of the above-mentioned model mice may make it possible to evaluate genetic effects from low-dose radiation relatively easily without using a large number of F_1 animals. Furthermore, if the frequency of somatic mutations in an animal can be determined *in vivo*, new information could be provided for cancer risk evaluation for low-dose radiation, which to this point has been difficult. In addition, it would be possible to determine the frequency of mutations in the somatic cells of an F_1 mouse born to irradiated mice and examine whether genetic instability can be observed.

Future Studies

Major activities to be conducted in the future by the Department of Genetics are as follows:

1) Evaluation of genetic effects of radiation on A-bomb survivors' children

   Biosamples (blood cells) from about 1,000 families (father-mother-child) are stored in liquid nitrogen. These biosamples will be analyzed by the latest techniques, including high-density microarray technique, multicolor FISH technique, and whole-genome base sequencing using a next-generation sequencer, for evaluation of genetic effects of A-bomb radiation.

2) Evaluation of effects of radiation exposure using an animal model system

   Using genetically modified mice in which cells generate green fluorescence by mutation (GFP mice), efforts to detect and quantify radiation-induced mutations *in vivo* will be carried out, and evaluation of genetic effects by radia-

tion exposure, elucidation of the mechanism of cancer development, and research on genetic instability will be performed.

3) Evaluation of exposure dose using biological dose estimates as indicators

A-bomb survivors' exposure doses will be reassessed using chromosome aberration data and ESR data from tooth enamel.

References

1. Kodaira M, Izumi S, Takahashi N, Nakamura N. No evidence of radiation effect on mutation rates at hypervariable minisatellite loci in the germ cells of atomic bomb survivors. *Radiat Res* 2004; 162:350-6.
2. Kodaira M, Ryo H, Kamada N, Furukawa K, Takahashi N, Nakajima H, Nomura T, Nakamura N. No evidence of increased mutation rates at microsatellite loci in offspring of A-bomb survivors. *Radiat Res* 2010; 173:205-13.
3. Straume T, Dobson RL, Kwan TC. Neutron RBEs and the radiosensitive target for mouse immature oocyte killing. *Radiat Res* 1987; 111:47-57.
4. Conrad DF, Pinto D, Redon R, et al. Origins and functional impact of copy number variation in the human genome. *Nature* 2010; 464:704-12.
5. Kodama Y, Pawel DJ, Nakamura N, et al. Stable chromosome aberrations in atomic bomb survivors: Results from 25 years of investigation. *Radiat Res* 2001; 156:337-46.
6. Ohtaki K, Kodama Y, Nakano M, et al. Human fetuses do not register chromosome damage inflicted by radiation exposure in lymphoid precursor cells except for a small but significant effect at low doses. *Radiat Res* 2004; 161:373-9.
7. Nakano N, Kodama Y, Ohtaki K, et al. Chromosome aberrations do not persist in the lymphocytes or bone marrow cells of mice irradiated in utero or soon after birth. *Radiat Res* 2007; 167:693-702.
8. Nakamura N, Miyazawa C, Akiyama M, et al. A close correlation between electron spin resonance (ESR) dosimetry from tooth enamel and cytogenetic dosimetry from lymphocytes of Hiroshima atomic-bomb survivors. *Int J Radiat Biol* 1998; 73:619-27.
9. Hirai Y, Kodama Y, Cullings HM, Miyazawa C, Nakamura N. Electron spin resonance analysis of tooth enamel does not indicate exposures to large radiation doses in a large proportion of distally-exposed A-bomb survivors. *J Radiat Res* 2011; 52:600-8.
10. Noda A, Kodama Y, Cullings HM, Nakamura N. Radiation-induced genomic instability in tandem repeat sequences is not predictive of unique sequence instability. *Radiat Res* 2007; 167:526-34.
11. Noda A, Hirai Y, Kodama Y, Kretzschmar WW, Hamasaki K, Kusunoki Y, Mitani H, Cullings HM, Nakamura N. Easy detection of GFP-positive mutants following forward mutations at specific gene locus in cultured human cells. *Mutat Res* 2011; 721:101-7.

Highlights of RERF Departments: Research Activities in the Department of Radiobiology /Molecular Epidemiology

Yoichiro Kusunoki

Department of Radiobiology/Molecular Epidemiology, RERF

Abstract

To address the question of how atomic-bomb (A-bomb) radiation affects human health and causes diseases, the Department of Radiobiology/Molecular Epidemiology (RME) takes two approaches—immunological and molecular oncological. The goal is to clarify the mechanisms linking radiation exposure and the development of various diseases, including cancer among A-bomb survivors, as well as genetic susceptibility to diseases. Mechanistic understanding is needed as a foundation for more robust risk assessments of radiation-associated diseases along with improved prevention and treatment of these diseases.

Immunology studies investigate immunobiological alterations caused by radiation and aging, in relation to disease development among A-bomb survivors. Accumulating evidence suggests that past radiation exposure accelerates immunosenescence. To elucidate mechanisms of radiation-related immunosenescence, we focus on radiation and age effects on hematopoietic stem cells (HSCs), dendritic cells (DCs), and thymic structure and function. Along those lines, we evaluate radiation- and aging-related alterations in human HSC and DC phenotypes and functions using assay systems established in collaboration with experts outside RERF. Studies on influenza vaccine response and development of an immunological scoring system were also initiated to seek possible links of radiation-related immunosenescence and disease development. In addition, we are investigating immunogenetic susceptibility to radiation-associated diseases. To date, polymorphisms of the following genes were found to underlie individually-differing and radiation dose-dependent risks of diseases among A-bomb survivors: *EGFR* (lung), *IL18* (colon), *IL10* (stomach), *NKG2D* (HCV-related hepatitis and hepatocellular carcinoma), and *HLA- DQA1* and *DRB1* (diabetes mellitus).

Oncology studies aim to clarify mechanistic relationships between radiation exposure and development of selected cancers among A-bomb survivors. A papillary thyroid cancer (PTC) study among the Life Span Study (LSS) cohort revealed that *RET/PTC* and anaplastic lymphoma kinase (*ALK*) rearrangements, rather than *BRAF* point mutations, were early events in PTC that were closely associated with radiation. We thus are investigating the mechanisms of radiation-induced thyroid carcinogenesis and are focusing on the molecular pathways involving rearranged *RET* and *ALK* genes. Results of a colorectal cancer study suggest that increased radiation dose is associated with microsatellite-unstable colorectal cancers carrying various epigenetic and genetic alterations. Analyses of epigenetic alterations in peripheral blood were initiated to assess age and radiation effects, and a preliminary result suggests that aging effects on methylation status may differ between blood cell populations.

Department Staff

The RME Department is currently comprised of a chief, an assistant chief (concurrently, a laboratory chief), a laboratory chief, eight research scientists, twelve technicians, and two administrative assistants.

Research Scientists of Radiobiology/Molecular Epidemiology (first row from left) Seishi Kyoizumi (NIAID Project Research Scientist), Tomonori Hayashi, Yoichiro Kusunoki (Department Chief), Kei Nakachi (RERF Consultant), Evan B. Douple (Associate Chief of Research), Kiyohiro Hamatani, (second row from left) Kengo Yoshida, Reiko Ito, Norio Takahashi, Junko Kajimura, Kazue Imai, Yasuharu Niwa, Masataka Taga

Research Projects and Recent Progress

1. Immunology Studies

Effects of A-bomb radiation on the immune system are being investigated using two approaches, i.e., immunobiology studies and immunogenome studies, which primarily examine immunological phenotypes and genotypes of A-bomb survivors, respectively. The studies are assessing immunobiological responses and genomic variations related to disease development in the Adult Health Study (AHS). The study outcomes are expected to contribute, not only to mechanistic understandings, but also to risk estimation of radiation-related diseases among the AHS subjects.

Immunobiology studies

Immunological changes we have previously observed in associations with radiation exposure among the AHS population were summarized in an earlier issue of *RERF Update* (2010, issue 1).[1] Most immunological phenotypes, including peripheral blood lymphocyte counts and functions, were altered by radiation in a manner substantially similar to immune changes associated with aging (Figure 1). Research currently underway aims to understand the mechanisms of radiation-induced alterations in the immune system and to determine the associations of immunological changes with diseases among A-bomb survivors.

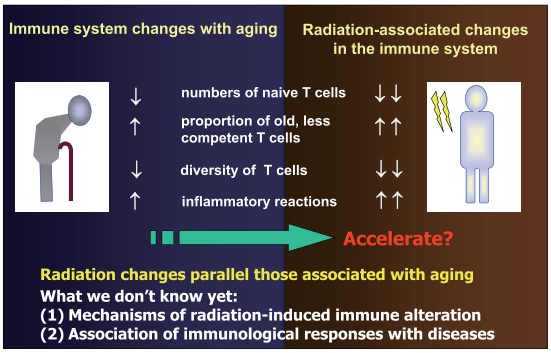


Figure 1. Immune system changes with aging and radiation

Radiation-induced immunosenescence

Based on accumulating evidence for immunosenescence phenotypes among the AHS population, we have hypothesized that A-bomb radiation has accelerated immunological aging. To accumulate a wealth of fundamental biological information on the impact of radiation on aging of the immune system, and to provide evidence of the impact of radiation on immune aging and related health effects, we initiated a five-year collaborative study with four Japanese and five U.S. institutions under a research contract with the National Institute of Allergy and Infectious Diseases (NIAID).[1] The research program consists of five focused projects with explicit goals of assessing mechanisms and effects of radiation on immune aging and dysfunction: 1) the role of HSCs and their microenvironment in T-cell development; 2) the role of DCs in T-cell responsiveness and function; 3) immune responsiveness to influenza vaccination; 4) a multivariable assessment of immune patterns to create an integrated scoring system of immunity; and 5) effects of A-bomb radiation on thymus architecture and function. Those study projects rely on the use of biosamples from AHS and LSS subjects and are all conducted in close collaborations with the Departments of Clinical Studies, Epidemiology, Statistics, and Information Technology at RERF.

Project 1: Effects of ionizing radiation exposure and aging on HSCs

This study will examine the effects of radiation exposure and aging on HSC including the determination of numerical and functional changes in the circulating HSC pool in relation to age and radiation dose. We hypothesize that radiation exposure induces premature aging of HSCs, resulting in reduced numbers and impaired self-renewal ability, that in turn accelerates the loss of lymphoid potential. The working hypotheses in the studies on AHS participants are therefore that there are age- and radiation dose-related: 1) alterations in the number of circulating $CD34^+$ HSCs; 2) increases in frequencies of $CD34^+$ HSCs that carry DNA damage and senescent phenotypes; 3) decreases in telomere lengths of circulating granulocytes among A-bomb survivors, which support the hypothesis that A-bomb irradiation accelerates aging of the hematopoietic system; and 4) decreases in the frequency of T-cell progenitors in circulating $CD34^+$ HSCs, to support the hypothesis that A-bomb irradiation accelerates the age-associated decline of T lymphopoiesis. For assessments of biological endpoints involved in these working hypotheses, we have developed a number of methods. Because $CD34^+$ HSCs are a very small population in the blood cells and because the blood volume we can obtain from AHS participants is limited, establishment of sensitive and reliable assays has been required. Examples of such methods involve cell-sorting-based limiting-dilution assays to evaluate hematopoietic colony formation abilities of HSCs, which facilitated evaluation of age-dependent alterations in frequencies of T- and NK-cell progenitors in circulating $CD34^+$ HSCs of healthy volunteers (manuscript in preparation). Upon establishment of the assay methods, measurements in the AHS participants were initiated in July 2011 and are scheduled to be completed in 2013.

In order to obtain a better understanding of the mechanisms involved in radiation-induced aging acceleration in the hematopoietic system, we

undoubtedly need to complement our human studies with suitable animal model systems. To investigate the process of hematopoietic and immune reconstitution following radiation-induced damage, this project uses a number of mouse models. Questions to be addressed in those mouse models are how irradiation modulates hematopoietic functions, myeloid and lymphoid cell differentiation, inflammatory responses, and genomic stability. Effects of radiation and aging on bone marrow stromal and thymic epithelial cells are also being analyzed in mouse models. Analyses of the characteristics and functions of hematolymphoid cells reconstituted in irradiated hosts include investigations in SCID-hu mice containing human blood cells; this model is thought to be useful to evaluate effects of low-dose radiation to which a large number of A-bomb survivors were exposed.[2] The animal experiments are being conducted by collaborators outside RERF, i.e., researchers in Memorial Sloan-Kettering Cancer Center, University of Georgia, Japan National Institute of Health Sciences, and Chiba University, but with close discussion between RERF members and the collaborators.

Project 2: Effects of ionizing radiation exposure and aging on DCs and their precursors

This study will examine effects of ionizing radiation exposure and aging on DCs and their precursors. DCs are crucial in triggering primary immune responses against pathogens, but also in the control of adaptive immunity, and may therefore be involved in impaired T-cell responsiveness and impaired homeostasis with aging in irradiated individuals. We thus hypothesize that A-bomb irradiation affects innate and adaptive immunity, possibly by altering DC populations toward a T-cell suppressor phenotype. The working hypotheses in the studies on AHS participants are therefore that there are age- and radiation dose-related: 1) significant alterations in the number of circulating conventional DCs (cDCs) and plasmacytoid DCs (pDCs); 2) increases in the production of immunosuppressive cytokines from DCs; and 3) increases in expression levels of genes involved in DC-mediated immunosuppressive effects on T cells. In collaboration with Duke University, we established microassay systems using custom polymerase chain reaction (PCR) and cytokine arrays for evaluation of human DC functions. DC measurements in the AHS participants were also initiated in July 2011 and scheduled to complete in 2013. Experiments with mouse models are also being conducted by collaborators in Keio University and the University of Tokushima, to better understand the innate and adaptive immune systems following radiation-induced damage of the hematopoietic system.

Project 3: Effects of ionizing radiation exposure and aging on vaccination responses and investigation of methods to augment immune responses

The primary purpose of this study is to assess radiation effects on the immune system in terms of the immune response to influenza vaccine among AHS subjects. This study uses a prospective design for the recruitment of study subjects, vaccination by collaborating attending doctors, and collection of blood samples before and after vaccination. The primary endpoint is the change in anti-influenza virus antibody titer levels from before to three weeks after vaccination. Secondary endpoints include levels of supernatant cytokines and inflammation-related proteins and their mRNA expression in flu-vaccine-stimulated blood mononuclear cells and phenotypes of lymphocyte and DC subsets. Those parameters will be analyzed in relation to age and dose of prior radiation exposure. Mechanistic studies are also being conducted *in vivo* in mouse models by collaborators at the University of Arizona.

In 2010, we conducted a pilot study with recruitment of 50 AHS subjects and young in-house volunteers (controls) for evaluation of subject-participation rate and for the development and validation of assay methods. The pilot was successful and achieved a high subject-participation rate, with full cooperation from the Hiroshima City Medical Association (HCMA) and the attending physicians. The sera obtained before and three weeks after vaccination from 50 AHS subjects and 20 younger in-house volunteers (controls) were used to assess anti-influenza virus antibody levels. The hemagglutination inhibition titers to A/H1N1 and A/H3N2 antigens in post-vaccination were significantly higher in a majority of AHS subjects and young controls than those in pre-vaccination. Pre- and post-vaccination frequencies of lymphocyte and DC subsets were determined, and most of those were found to be substantially unchanged between pre- and post-vaccination. Of interest is, however, that the post-vaccination frequencies of Th1 and Tc1 (but not Th2 nor Tc2) increased after vaccination. The frequency of cDCs at post-vaccination was marginally lower than that at pre-vaccination in the AHS subjects. The frequency of cDCs in AHS subjects was substantially unchanged between pre- and post-vaccination, and this is also the case in cDC and pDC frequencies in young controls.

On the basis of this pilot study, we developed a plan for the full-scale vaccination study in FY2011 and FY2012 enrolling about 300 AHS subjects and 20 young in-house volunteers. A questionnaire for the first-year full-scale study was sent to 320 AHS participants, and finally a total of 157 subjects have completed all measurements planned for the full-

scale vaccination study. A total of 140 attending physicians of those study subjects played an important cooperative role in the influenza vaccination and collection of blood before vaccination. In FY2012, we expect to successfully conduct the second-year full-scale study in the same way.

Project 4: Development of an integrated scoring system for human immune competence as it relates to age and ionizing radiation

The objective of this study is to develop an integrated scoring system for evaluating the immunological and inflammatory status of individuals as a function of age and radiation dose, and predicting the effects of radiation on the immune system and somatic mutation in exposed subjects. Our immunology study, unique to RERF, consists of repeated observations of various immunological parameters in A-bomb survivors with long-term follow-up. This may demonstrate significant radiation-related alterations in the immune system among survivors, even 65 years after the exposure to A-bomb radiations. Thus, in this study, a cross-sectional analysis is proposed that will include about 3,600 Hiroshima AHS subjects. A longitudinal analysis is also proposed with repeated measurements of immunological and inflammation-related markers on a subset of 300 selected AHS subjects. Biomarkers will be measured using antibody chip arrays on two sets of plasma samples collected from the 300 AHS subjects ten years apart. Telomere length assays will also be conducted on DNA from the same 600 samples. The results will be utilized to construct an integrated scoring system.

We have measured plasma levels of reactive oxygen species (ROS) in 1,520 AHS subjects in Hiroshima. The intracellular ROS (H_2O_2 and O_2^{-}) levels in blood cells were also measured in 1,600 blood samples using an intracellular ROS assay system. Twenty seven different plasma cytokines have also been measured in 1,700 AHS subjects using the Bio-Plex Pro cytokine assay. Lymphocyte subset frequencies have been measured in 2,300 AHS subjects. Preliminary analyses indicated that plasma ROS levels increased with increased radiation dose.[3] The intracellular H_2O_2 levels in T-cell subsets did not increase with age or radiation. The intracellular O_2^{-} levels increased with increased age in any T-cell subsets examined, and this increasing response was also seen for increased radiation dose in CD3, CD4, CD8, and naïve CD8 T cells.

Members of the Statistics Department and an additional U.S. collaborator will apply sophisticated methods (e.g., Bayesian network) to develop and evaluate the scoring system. The collection of informed consent for this study was initiated. Namely, we have obtained written informed consent for the use of donated biosamples and past measurement data in this study from AHS subjects who visited RERF for regular health examinations. The collection of informed consent and measurements in obtained blood samples will be completed in 2013, and then the scoring system will be established in 2014.

Project 5: A feasibility study for future analyses of the effects of ionizing radiation and aging on thymus architecture and function

The goal of this study is to evaluate the numbers and quality of the thymus autopsy specimens stored at RERF as well as at Hiroshima University Hospital, in order to verify the feasibility of future analyses of thymus architecture and function in response to aging and exposure to ionizing radiation. Autopsied thymus specimens of A-bomb survivors (LSS subjects) represent a valuable and unique resource for the study of the impact of aging and radiation exposure on the thymus and on thymic involution. However, it has been unclear until now how many thymus tissues are available with sufficient quality for a future comprehensive study, which will employ histochemical and molecular analyses. In this study, therefore, the availability and quality of thymus specimens will be surveyed among the LSS autopsy cases recorded in the Hiroshima RERF Tissue Archives of A-bomb survivors together with the data on age at death, radiation dose, gender, and clinical information. Selected representative samples will be assessed for their utility in various histochemical and molecular assays, such as staining with CD1a antibody, TREC assay, and quantitative reverse transcript (RT)-PCR assays for selected cytokines, chemokines, and transcription factors involved in thymic epithelial cell differentiation.

Currently, a total of 241 autopsy cases having a thymus sample were identified (108 cases below 70 years old at death and 133 cases who died at 70 years old or older). Epidemiological and clinical information of the cases such as age, gender, radiation dose, time until autopsy (elapsed time since death), pathologic and clinical diagnosis, and treatment history were obtained from the RERF database and autopsy reports. Histochemical analyses (hematoxylin and eosin staining and immunohistochemical staining with antibodies to cytokeratin) were initiated using selected samples.

Immunological phenotypes and diseases

In collaboration with the Departments of Clinical Studies and Statistics, we investigated associations of immunological phenotypes with disease development and health status in the AHS. To deepen the understanding of lymphocyte phenotypes related to the course of hepatitis C virus

(HCV) infection and progression of liver fibrosis in A-bomb survivors, we recently compared percentages of lymphocyte subsets among three groups of AHS populations: 162 HCV persistently infected, 145 spontaneously cleared, and 3,511 uninfected individuals.[4] We observed increased percentages of peripheral blood Th1 and total CD8 T cells and decreased percentages of NK cells in the HCV persistence group compared with the other two groups after adjustment for age, gender, and radiation exposure dose. Subsequently, we determined that increased Th1 cell percentages in the HCV persistence group were significantly associated with an accelerated time-course reduction in platelet count-accelerated progression of liver fibrosis, whereas Tc1 and NK cell percentages were inversely associated with progression. This study suggests that Th1 immunity is enhanced by persistent HCV infection and that measuring the percentages of peripheral Th1, Tc1, and NK cells may help predict progression of liver fibrosis.

Inflammation and genomic instability

To investigate whether radiation-induced genomic instability persisted *in vivo*, we quantified the number of micronucleated reticulocytes in peripheral blood of whole-body X-irradiated mice.[5] Even one year after irradiation, mice irradiated with 2.5 Gy of X ray showed significantly increased frequencies of micronucleated reticulocytes. The results indicate that delayed genomic effects of irradiation on the murine hematopoietic system can persist *in vivo* for prolonged periods.

To test the hypothesis that persistent inflammation may cause radiation-induced genomic instability through elevation of inflammatory cytokine levels, we evaluated the relationship between inflammation and genomic instability in a murine hematopoietic system *in vivo*. Persistent inflammation was induced with graft-versus-host disease (GVHD) in F_1 mice that received hematopoietic cell transplantation from parent mice. Both the frequency of micronucleated reticulocytes and the level of circulating TNF-α significantly increased in the GVHD F_1 mice, suggesting that inflammation manifested with an increased TNF-α level may be involved in genomic instability.[6]

Immunogenome studies

Epidemiological studies have clearly demonstrated long-lasting impacts of A-bomb radiation on human health, including dose-dependent increases in the incidence/mortality of cancer and some types of noncancer diseases. The immunogenome studies evaluate the genetic basis for inter-individual differences in immune functions and the impact of the genetics on susceptibility to radiation-associated diseases. The phenotype-genotype association analyses utilize the A-bomb survivors' stored biological materials and cumulative immunology data. The results obtained from the studies will have the potential to contribute to the individualized prevention of radiation-associated diseases in A-bomb survivors and also other exposed populations.

Cancer development and polymorphisms of immune-related genes

Epidemiological studies have demonstrated long-lasting impacts of A-bomb radiation on the incidence/mortality of inflammation-related cancers. Although enhanced inflammation has been consistently observed among A-bomb survivors, roles of inflammatory responses in radiation carcinogenesis are not understood. In this study, therefore, relationships are investigated among risks of radiation-associated cancers, individuals' genetic backgrounds, and A-bomb radiation exposure, focusing on polymorphisms of genes encoding molecules that are possibly involved in immunological defenses against cancer development or in inflammatory responses that may modify cancer risk. Four working hypotheses are tested in this study. The first hypothesis concerns the involvement of polymorphisms of genes related to innate immunity, such as NK and toll-like receptor genes. The second hypothesis is that cancer risk is related to inter-individual variations in acquired immunity, associated with *HLA* class I, class II, and non-classical class I genes. Persistent inflammatory status may also be related to cancer, and the third hypothesis is that the inflammatory response of individuals in part depends on polymorphisms of inflammation-related genes encoding cytokines, chemokines, and their receptors. The fourth hypothesis is that polymorphic DNA repair genes and/or drug-metabolizing enzyme genes may also be involved in inter-individual differences in susceptibility to radiation-associated cancers. We will explore candidate chromosome loci over the entire genome with use of genome-wide single nucleotide polymorphism (SNP) arrays as well as confirm our findings from an "immunogene-targeting" approach, in collaboration with researchers at the University of Tokyo.

(1) CA repeat number of epidermal growth factor receptor (*EGFR*) gene and lung cancer[7]

We carried out a case–cohort study within the AHS cohort to evaluate a possible association of CA repeat polymorphism of *EGFR* gene with lung cancer risk in A-bomb survivors. By dividing study subjects into Short and Long genotypes, defined as the summed CA repeat number of two alleles ≤37 and ≥38, respectively, we found that prior radiation exposure significantly enhanced lung cancer risk of

survivors with the Long genotype, whereas the risk for the Short genotype did not show any significant increase with radiation dose, resulting in indistinguishable risks between these genotypes at a high radiation dose.

(2) Evaluation of case-cohort study design to analyze gene-environment interactions

In collaboration with the Department of Statistics, conventional methods of case-cohort study design and analysis were evaluated for the studies of gene-environment interactions.[8]

(3) *IL18* polymorphisms and colon cancer

We identified two major haplotype alleles (*IL18-AT, IL18-CG*), which comprise two htSNPs located in the promoter region of *IL18*. A case-control study within the AHS cohort was conducted to evaluate risks of colorectal cancer for both *IL18* haplotypes and radiation exposure, using 210 cases and 843 controls. We found the following: 1) radiation exposure significantly enhanced the risk of colon cancer but not rectum cancer; 2) individuals harboring homozygous *IL18-CG* alleles showed a significantly increased risk of colon cancer, compared with those harboring at least one *IL18-AT* allele; and 3) a significant gene-radiation interaction was found: Colon cancer risk for homozygote *IL18-CG* alleles drastically increased when exposed to the highest radiation dose category (>0.7 Gy).

(4) *IL10* polymorphisms and stomach cancer

We found that, although the overall risk of stomach cancer significantly increased with radiation dose, this risk in both radiation-exposed and non-exposed survivors was greatly modulated by *IL10* haplotypes. We further investigated whether genetic risk of stomach cancer was modified by radiation dose in different pathological types, i.e., the intestinal and diffuse types. A preliminary study showed that risk of the intestinal type stomach cancer was modulated by *IL10* haplotypes but not much by radiation exposure, while risk of the diffuse type was modulated by radiation exposure and also by *IL10* haplotypes only at a high radiation dose.

(5) *NKG2D* polymorphisms and HCV-related hepatitis and hepatocellular carcinoma

NKG2D is a primary activating receptor that triggers cell-mediated cytotoxicity in NK cells against tumor and virus-infected cells. We previously identified the NKG2D haplotypes in the natural killer gene complex region on chromosome 12p. Two major haplotype alleles, LNK1 and HNK1, were closely related to low and high natural cytotoxic activity phenotypes, respectively.[9] Furthermore, the haplotype of HNK1/HNK1 has revealed a decreased risk of cancer compared with LNK1/LNK1.[9] Further, using flow cytometry, we evaluated the functional effects of NKG2D haplotypes and five htSNPs in terms of the cell-surface expression of NKG2D protein on NK and CD8 T cells of peripheral blood among 732 atomic-bomb survivors.[10] NKG2D expression on NK cells showed significant increases, in the order of LNK1/LNK1, LNK1/HNK1, and HNK1/HNK1 haplotypes (Figure 2), or with major homozygous, heterozygous, and minor homozygous genotypes for individual htSNPs (p for trend = 0.02–0.003). The same trend was observed for NKG2D expression on CD8 T cells. Our findings indicate that the NKG2D haplotypes are associated with the expression levels of NKG2D protein on NK and CD8 T cells, resulting in inter-individual variations in human cytotoxic response.

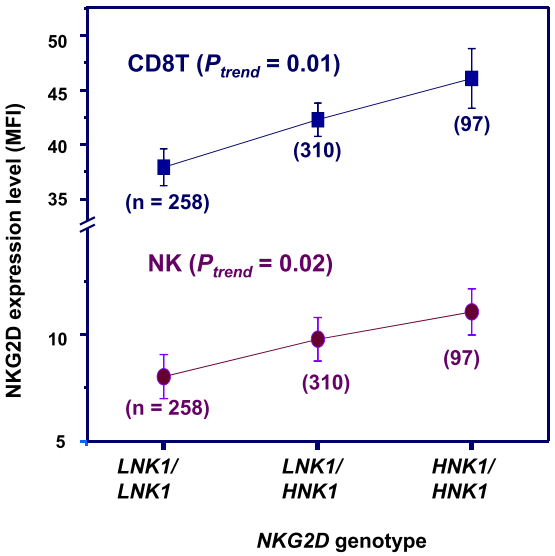


Figure 2. Genotypes associated with cell-surface NKG2D expression levels in human peripheral blood CD8 T and NK cell populations

We also conducted a case-control study of persistent HCV infection within the AHS cohort, selecting 134 cases with persistent HCV infection and 107 controls with HCV clearance. We observed significant effects of *NKG2D* haplotypes on persistent HCV infection only among female subjects, not male. The rate of HCV clearance is significantly lower (43%) in individuals having at least one particular *NKG2D* allele relating to low NK activity than the rate (60%) in those having high NK activity-relating *NKG2D* alleles (odds ratio [OR] 3.7, 95% confidence interval [CI]: 1.1–12.5). A case-cohort study of hepatocellular carcinoma comprised 115 cases and a sub-cohort of 2,132 within the AHS cohort. When dividing the study subjects into combinations of three exposure-dose categories (non-exposure, <0.7 Gy exposure, ≥0.7

Gy) and two *NKG2D* haplotype categories, risk of hepatocellular carcinoma was the highest for individuals who have at least one low NK activity-relating *NKG2D* allele and most heavily exposed to radiation (≥0.7 Gy) (relative risk 4.3, 95% CI: 1.0–18.0).

(6) Polymorphisms of DNA repair genes, somatic mutation, and cancer

It is known that the erythrocyte glycophorin A (*GPA*) mutation assay can detect erythrocyte mutant phenotypes that are derived from mutations that occurred by exposures to mutagens or gene replication errors in hematopoietic cells. Based on a prospective cohort study, we have shown that there are large individual variations as well as a radiation-dose-dependent increase in *GPA* mutant frequency among A-bomb survivors and that cancer incidence is higher in persons who have higher radiation-induced mutation frequencies.[11] In order to investigate genetic polymorphisms underlying individual variation in the sensitivity to radiation-induced genetic damage in the hematopoietic system, we are analyzing associations between genotypes of various DNA repair genes, *GPA* mutant frequency, and cancer development among AHS subjects. Regression analyses of the *p53BP1* haplotypes (*GGC* versus *TCA*) showed that although there was no association between *p53BP1* haplotyping and cancer incidence the dose-response curve of *GPA* Mf significantly differed by the haplotypes (manuscript in preparation).

Diabetes mellitus (DM) and polymorphisms of HLA-related genes

The purpose of this study is to assess the effect of radiation and various genetic factors on the risk of DM in the AHS cohort, and determine whether differences in frequencies of any particular genotypes between Hiroshima and Nagasaki survivors may account for why a significant association between risk of DM and radiation dose is observed in Hiroshima survivors but not in Nagasaki survivors. Although early studies of A-bomb survivors did not show associations between radiation exposure and a risk of DM, data on AHS subjects in 1992–1994 indicated a significant positive radiation-DM association in Hiroshima but not in Nagasaki, after adjusting for sex, age, and body mass index. This somewhat puzzling finding may reflect genetic differences between the Hiroshima and Nagasaki populations. Our preliminary results suggest that radiation may persistently impair immune responses, and that the radiation-DM association is especially relevant to a sub-group of A-bomb survivors who have a specific *HLA* class II haplotype. Those results suggest that the effects of radiation on the development of DM may vary according to genetic background.[12] We have identified *DQA1* and *DRB1* genotypes and analyzed the relationships between *HLA*-related genetic factors, risk of DM, and radiation dose in 711 DM patients (483 and 228 in Hiroshima and Nagasaki, respectively) and 1,878 controls (966 and 912 in Hiroshima and Nagasaki, respectively). In particular, heavily exposed Hiroshima subjects with *DQA1*01:02-DRB1*15:01:01, DQA1*01:02-DRB1*16:02:01, DQA1*03:01-DRB1*04:05 or DQA1*05:01-DRB1*14:03:01* haplotype revealed a significantly higher risk of DM than non-exposed subjects (OR = 1.73/Gy, 95% CI: 1.36 to 2.20). We have also genotyped 1,260 subjects based on the *TP53 Arg72Pro* polymorphism. A preliminary result suggests that the risk of DM in Hiroshima survivors with *TP53 Pro/Pro* genotype was significantly enhanced by radiation exposure.

2. Oncology Studies

Aiming at clarification of mechanistic relationships between radiation exposure and cancer development, we have been analyzing molecular alterations in archival cancer tissues obtained from the LSS subjects, especially focusing on thyroid, colorectal, and lung cancers. Our major hypothesis is that A-bomb radiation might affect various carcinogenic pathways in terms of particular genetic and/or epigenetic alterations found in radiation-exposed cancer cases. We also are evaluating epigenetic alterations in normal cells such as blood cells, to determine whether radiation exposure modulates aging-related epigenetic alterations that may lead to increased risks of aging-related diseases including cancers.

Development of assay methods for molecular analyses using long-term preserved tissue specimens

Archival tissue specimens are valuable resources of materials for molecular biological analyses in retrospective studies, especially for molecular oncology studies in cancer tissue specimens collected in the long term from A-bomb survivors. However, most surgical and autopsy tissue specimens have been preserved as unbuffered formalin-fixed and paraffin-embedded (FFPE) blocks for long periods of time: DNA or RNA is often found to degrade under such conditions. Although successful amplification with PCR is essential for analysis of DNA and RNA extracted from archival FFPE tissue specimens, we have often encountered problems with poor PCR amplification of target fragments. To overcome this, we improved a DNA extraction method to efficiently restore the PCR template activity of DNA.[13] Namely, the heat treatment of DNA samples in borate buffer resulted in successful PCR amplification of DNA fragments ranging from 91 to 152 bp. We also established an

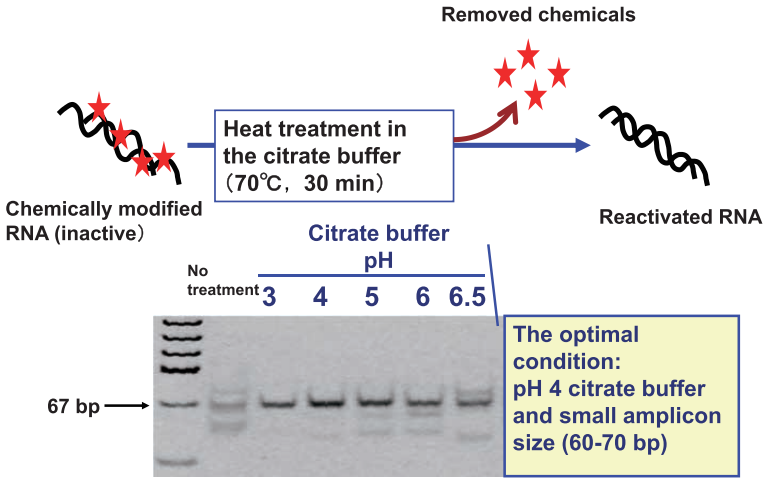


Figure 3. Improvement of the sensitivity of real-time RT-PCR

improved method for efficient RT-PCR amplification of RNA extracted from archival FFPE tissues by the elimination of RNA modification and the restoration of RNA template activity.[14] Namely, the preheating in citrate buffer (pH 4.0) of RNA extracted from long-term preserved tissue specimens resulted in significantly increased efficiency of RT-PCR. Improvement of the sensitivities of real-time PCR and real-time RT-PCR has been achieved by reducing amplicon sizes (Figure 3).[15]

Thyroid cancer studies

RERF epidemiology studies have found that an excess relative risk for PTC per Gy is remarkably high among survivors.[16,17] The data from the studies following the Chernobyl accident also indicate a strong relationship between thyroid cancer and radiation exposure.[18] Our molecular analysis on rearrangements of *RET*, *NTRK1*, and *BRAF* genes, and also point mutations of *BRAF* and *RAS* genes in adult-onset PTC cases from the LSS cohort, found that the relative frequency of PTC cases with *RET/PTC* or *NTRK1* rearrangements (mainly *RET/PTC*) in all available PTC cases significantly increased with increased radiation dose, while PTC cases with *BRAF* or *RAS* point mutations (mainly $BRAF^{V600E}$) significantly decreased.[19,20] Apart from those PTC cases with known gene alterations, we found that relative frequency of PTC cases with "non-detected gene alterations"—no alterations in *RET*, *NTRK1*, *BRAF*, or *RAS* gene—tended to increase with increased radiation dose, showing temporal change with a peak soon after exposure and then rapidly decreasing.[20] Those findings suggested that some PTC cases with non-detected gene alterations might be closely associated with radiation exposure, as are those with chromosomal rearrangements.

Among 25 PTC cases (19 exposed and 6 non-exposed) who carried no alterations in the *RET, NTRK1*, *BRAF*, and *RAS* genes, we recently found a new type of rearrangement, i.e., rearranged *ALK* gene in 10 of 19 exposed PTC cases but none of 6 non-exposed cases.[21] Trabecular/solid-like architectures, which are characterized by a solid and/or a trabecular appearance, were observed in 6 of 10 PTC cases with *ALK* rearrangements, while 11 of 80 PTC cases carrying one of the *RET*, *NTRK1*, *BRAF*, and *RAS* gene alterations had such architectures.[21] Forty-five PTC cases carrying one alteration in the *RET*, *NTRK1*, *BRAF*, or *RAS* gene have been thus far examined for rearranged *ALK*, and 43 cases were found to have no rearranged *ALK*, while this first screening suggested that 2 cases possibly carried rearranged *ALK* as well. The results suggest that chromosomal rearrangements, such as *RET* and *ALK* rearrangements, appear to play an important role in radiation-associated adult-onset thyroid carcinogenesis.

To understand why *RET* and *ALK* rearrangements preferentially exist in high-dose radiation-associated PTC, we formulated the following hypothesis: Ionizing radiation may confer a growth advantage to thyrocytes with *RET* or *ALK* rearrangement. Development of an expression vector transfection system using primary cultured mouse thyroid epithelial cells is indispensable for the future *in vitro* irradiation experiments to test this hypothesis. By improving the transfection system in primary cultures, evaluation of the tumorigenic potentials of rearranged *RET* and *ALK* is underway.

Colorectal cancer studies

Colorectal cancer has two main categories of genomic instability. The most common is chromosomal instability (CIN), in which the requisite genetic events occur due to the accumulation of chromosomal abnormalities. The other main type is

microsatellite instability (MSI), which is a consequence of impaired recognition and repair of mismatched bases. Furthermore, in the serrated polyp pathway with MSI, constitutive activation of the MAP kinase signaling pathway that is caused by gene alterations is recognized as an early event. More recently, the CpG island methylator phenotype (CIMP) pathway has been proposed as the principal underlying mechanism in MSI. In this study, therefore, we are focusing on molecular events related to those phenotypes, using archival surgical and autopsied colorectal cancer specimens which are preserved at RERF as well as archival specimens thus far obtained from the Department of Molecular Pathology, Hiroshima University Graduate School of Biomedical Sciences. MSI and CIN status and their related gene alterations are being assessed in terms of the association with pathoepidemiological factors including radiation dose. A pilot study identified five high-MSI colon cancer cases with the median radiation dose significantly higher than that in microsatellite stable and low-MSI cases, along with Ras-signaling gene alterations.[22] The data suggest that radiation exposure may influence MSI-related colorectal carcinogenesis through genetic and epigenetic alterations in *MLH1* and Ras-signaling genes.

Lung cancer studies

Based on the hypothesis that radiation exposure may affect the profile of genetic and/or epigenetic alterations related to lung carcinogenesis, we are analyzing: 1) molecular characteristics of lung cancer among A-bomb survivors; and 2) joint effects of smoking and radiation on lung cancer. In a pilot study, using DNA extracted from microdissected tissue specimens, we examined allelic imbalance of *p53*, *p16*, and *RASSF1A*, mutations of *p53*, *EGFR*, and *K-ras*, and methylation of *p16*, *RASSF1A*, and retrotransposone LINE1 (a marker of global methylation in genomic DNA), among 38 non-small cell lung cancer (NSCLC) cases (20 radiation-exposed cases and 18 non-exposed cases). Preliminary results indicated that *p53* mutation frequency and *RASSF1A* methylation levels in exposed NSCLC cases compared to non-exposed NSCLC are higher and lower, respectively, although these are not statistically significant differences. Further analyses with increased numbers of cases are being planned and a research protocol (RP) is under review.

Epigenetic studies

Our epigenetic studies are driven by a hypothesis that radiation may affect aging-associated epigenetic alterations potentially involved in cancer and noncancer diseases among A-bomb survivors. It is known that radiation affects DNA methylation status, e.g., gender- and tissue-specific global DNA hypo-methylation and *p16* promoter hyper-methylation, and that similar changes appear with aging. To test this hypothesis, therefore, we will take a three-step approach. Assessments will be conducted of: 1) aging effects in blood cells of non-AHS healthy volunteers; 2) radiation effects on aging-related epigenetic changes in the AHS; and 3) relationships between radiation-associated epigenetic alterations and biological risk markers of aging-related diseases among the AHS. Based on results from the first two steps (identification of aging- and radiation-associated changes in target genes among particular blood cell subpopulations), we will work closely with the Department of Clinical Studies to target disease and biomarker endpoints that appear most promising.

The first-step study is underway, using various blood cell subsets obtained from 48 non-AHS healthy female volunteers. We compared DNA methylation levels between a young group comprised of 26 women at ages <35 years and an elderly group of 22 women at ages >65 years. In naïve CD4 T cells, granulocytes, and peripheral blood mononuclear cells (PBMC), *MyoD, hTERT,* and *TUSC3* methylation levels were significantly higher in the elderly group than those in the younger group. These two groups revealed significant differences in methylation status of *HIC1*, *p16,* and *RARb2* in naïve CD4 T cells and PBMC, but not in granulocytes. LINE1 methylation levels in naïve CD4 T cells and PBMC, but not in granulocytes, were significantly lower in the elderly group than those in the younger group. The differences in methylation status of selected genes and whole genome between the elderly and young groups were found as potent DNA methylation markers related to aging effects, although such age effects may differ by blood cell types. Based on these preliminary results, we are preparing an RP for the second step.

3. Biosample Collection and Storage

The effects of A-bomb radiation have been studied using many different endpoints, and measurement techniques have continuously improved over time. In addition, studies unique to RERF involve repeated examinations of a series of biomarkers in a fixed cohort, i.e., the AHS cohort. Therefore, it is reasonable to expect that in the future it will be possible to study effects which we are not currently able to assess and that more robust risk assessments of radiation-related diseases will become possible on the basis of longitudinal data in the AHS cohort. For this reason, the Immunology Laboratory in our department has been engaging in blood sample collection and storage under three active RPs: 1) Cryopreservation of blood mononuclear cells; 2) Transformation of B cells by Epstein-Barr virus

and cryopreservation of the immortalized lymphocytes; and 3) storage of blood cells and plasma. Outlines of collections, assays, and storages of blood samples are illustrated in Figure 4.

Approximately 2–5 × 10^6 mononuclear cells/vial are cryopreserved for each donor, and 70,000 vials from a total 9,000 AHS subjects have been stored since 1992. We confirmed that the viability of cryopreserved cells was more than 80% and that thawed lymphocytes expressed normal surface antigens and immunological functions. Immortalization of lymphocytes from 2,700 subjects was set as the goal and has been completed. We have thus far cryopreserved 30,000 blood sample vials from AHS participants, including the expanded AHS participants who were exposed to radiation at the age of nine years or younger. The PCR amplification of DNA extracted from laboratory control blood stored at –80°C for 14 years on paper showed no recognizable degradation of DNA after the long-term storage.

Our molecular oncology studies largely rely on systematic collection and storage of tissue specimens from archival cancer cases among A-bomb survivors. Pathologists in local hospitals as well as RERF scientists contribute to collecting cancer tis-

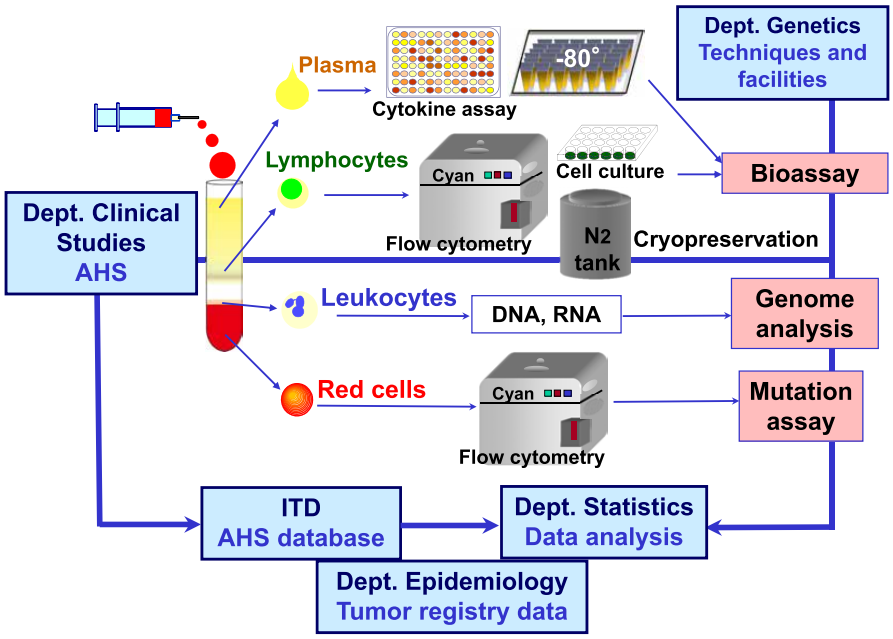


Figure 4. Analyses and storage of blood samples in the immunology studies

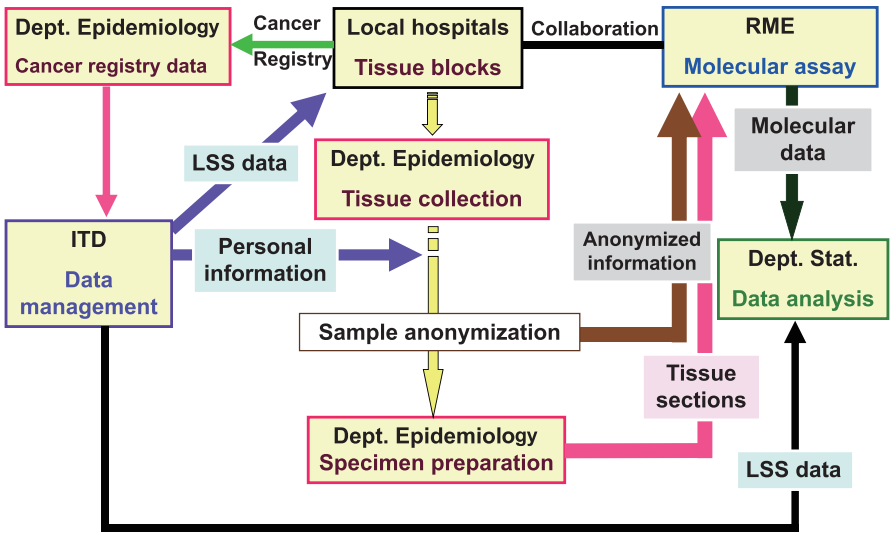


Figure 5. A flow of collection and analysis of cancer tissue specimens of A-bomb survivors (thyroid cancer)

sue specimens for these studies (the collaboration is shown in Figure 5), and development of a more comprehensive tissue specimen network is being planned by RERF.

Collaborations

The department collaborates with the Clinical Studies Department in sharing data on biomarker measurements and clinical information in the AHS. We are also collaborating with the Department of Epidemiology for the investigation of pathological specimens of colorectal, lung, and thyroid cancers, and with the Statistics Department in study-design and data-analysis efforts. The Information Technology Department offers strong support to the management of research data and samples in our department. The Departments of Radiobiology/Molecular Epidemiology and Genetics work cooperatively in experiments using animal and radioisotope facilities as well as high-tech facilities such as microarrays and cell sorters.

In addition to the NIAID collaborative immunosenescence project and pathological specimen collection in collaboration with Hiroshima and Nagasaki Universities and local hospitals, the department also has domestic and overseas collaborative studies with a number of researchers outside RERF. For example, immunogenome studies are conducted in collaboration with the University of Tokyo and Japanese National Cancer Center, and molecular oncology studies are performed in collaboration with Hiroshima and Nagasaki Universities and the U.S. National Cancer Institute (NCI).

Future Studies

The department will continue efforts to deepen mechanistic understanding of radiation health effects, which is needed as a foundation for more robust risk assessments of radiation-associated diseases along with improved prevention and treatment of these diseases.

Immunobiology studies

To further assess radiation-related immunosenescence, we will make efforts to extend the immunosenescence study project with a renewal of the NIAID contract and plan the following studies:

1) Molecular and histological alterations in LSS autopsied lymphoid organs, such as thymus, bone marrow, gut-associated lymphoid tissues and lymph nodes
2) Radiation impact on aging-associated immunological changes in longitudinal analyses of the AHS
3) Animal model studies to understand immunological mechanisms on radiation-induced aging-associated diseases

Immunogenome studies

The immunogenome studies will continue to seek associations between immune-related gene polymorphisms and various immunological phenotypes that have been determined in the immunobiology studies. With use of a case-cohort study design, factors responsible for relative risks of cancers and noncancer diseases for various radiation dose levels and genotypes will be assessed. The following approaches will be undertaken to advance the immunogenome studies:

1) SNP array analysis to search for the genes responsible for radiation-related diseases
2) Incorporation of genotype data into an immune scoring system developed in the immunosenescence study project

Oncology studies

The oncology studies will continue to investigate mechanisms of radiation-induced carcinogenesis with special reference to the role of inflammation in cancer development. In addition to analyses of molecular alterations in thyroid, colorectal, and lung cancer tissues in the LSS, the following approaches will be undertaken:

1) Molecular and histological alterations in precancerous tissues
2) *In vitro* and *in vivo* experiments using transgenic cells/animals
3) Evaluation of radiation effects on organ-specific stem/progenitor cells

Epigenome studies

To seek relationships between epigenetic changes and radiation-related diseases including cancers, oncologists and immunologists in the Departments of Radiobiology/Molecular Epidemiology and Clinical Studies will jointly undertake the following examinations in AHS subjects:

1) Radiation effects on aging-related epigenetic changes in blood cell populations
2) Relationships between radiation-associated epigenetic alterations and biological risk markers of aging-related diseases
3) Epigenome alterations in association with T-cell immunosenescence

References

1. Kusunoki Y, Hayashi T, Douple EB, Nakachi K. A special opportunity for RERF to expand studies of immunosenescence and other late effects of acute ionizing radiation exposure in A-bomb survivors. *RERF Update* 2010; 21(1):13-23.

2. Wang C, Nakamura S, Oshima M, Mochizuki-Kashio M, Nakajima-Takagi Y, Osawa M, Kusunoki Y, Kyoizumi S, Imai K, Nakachi K, Iwama A. Compromised hematopoiesis and increased DNA damage following non-lethal ionizing radiation of a human hematopoietic system reconstituted in immunodeficient mice. (submitted)

3. Hayashi T, Morishita Y, Khattree R, Misumi M, Sasaki K, Hayashi I, Yoshida K, Kajimura J, Kyoizumi S, Imai K, Kusunoki Y, Nakachi K. Evaluation of inflammation status in atomic-bomb survivors with special reference to radiation and age effects. (submitted)

4. Yoshida K, Ohishi W, Nakashima E, Fujiwara S, Akahoshi M, Kasagi F, Chayama K, Hakoda M, Kyoizumi S, Nakachi K, Hayashi T, Kusunoki Y. Lymphocyte subset characterization associated with persistent hepatitis C virus infection and subsequent progression of liver fibrosis. *Hum Immunol* 2011; 72:821-6.

5. Hamasaki K, Imai K, Hayashi T, Nakachi K, Kusunoki Y. Radiation sensitivity and genomic instability in the hematopoietic system: Frequencies of micronucleated reticulocytes in whole-body X-irradiated BALB/c and C57BL/6 mice. *Cancer Sci* 2007; 98:1840-4.

6. Kusunoki Y, Hamasaki K, Koyama K, Imai K, Hayashi T, Martin PJ, Nakachi K. Increased DNA damage in hematopoietic cells of mice with graft-versus-host disease. *Mutat Res* 2010; 689:59-64.

7. Yoshida K, Nakachi K, Imai K, Cologne JB, Niwa Y, Kusunoki Y, Hayashi T. Lung cancer susceptibility among atomic-bomb survivors in relation to CA repeat number polymorphism of *epidermal growth factor receptor* gene and radiation dose. *Carcinogenesis* 2009; 30:2037-41.

8. Cologne JB, Preston DL, Imai K, Misumi M, Yoshida K, Hayashi T, Nakachi K. Conventional methods of case-cohort study design and analysis with application to a study of cancer and gene-environment interaction. (submitted)

9. Hayashi T, Imai K, Morishita Y, Hayashi I, Kusunoki Y, Nakachi K. Identification of the *NKG2D* haplotypes associated with natural cytotoxic activity of peripheral-blood lymphocytes and cancer immunosurveillance. *Cancer Res* 2006; 66:563-70.

10. Imai K, Hayashi T, Yamaoka M, Kajimura J, Yoshida K, Kusunoki Y, Nakachi K. Effects of *NKG2D* haplotypes on the cell-surface expression of NKG2D protein on natural killer and CD8 T cells of peripheral blood among atomic-bomb survivors. *Hum Immunol* 2012; 73:686-91.

11. Kyoizumi S, Kusunoki Y, Hayashi T, Hakoda M, Cologne JB, Nakachi K. Individual variation of somatic gene mutability in relation to cancer susceptibility: Prospective study on erythrocyte glycophorin A gene mutations of atomic bomb survivors. *Cancer Res* 2005; 65:5462-9.

12. Hayashi T, Fujiwara S, Morishita Y, Kusunoki Y, Nakashima E, Nakanishi S, Suzuki G, Nakachi K, Kyoizumi S. HLA haplotype is associated with diabetes among atomic bomb survivors. *Hum Immunol* 2003; 64:910-6.

13. Taga M, Eguchi H, Shinohara T, Takahashi K, Ito R, Yasui W, Nakachi K, Kusunoki Y, Hamatani K. Improved PCR amplification for molecular analysis using DNA from long-term preserved formalin-fixed, paraffin-embedded tissue specimens. (submitted)

14. Hamatani K, Eguchi H, Takahashi K, Koyama K, Mukai M, et al. Improved RT-PCR amplification for molecular analyses with long-term preserved formalin-fixed, paraffin-embedded tissue specimens. *J Histochem Cytochem* 2006; 54:773-80.

15. Hamatani K, Eguchi H, Mukai M, Koyama K, Taga M, Ito R, Hayashi Y, Nakachi K. Improved method for analysis of RNA present in long-term preserved thyroid cancer tissue of atomic bomb survivors. *Thyroid* 2010; 20:43-9.

16. Thompson DE, Mabuchi K, Ron E, Soda M, Tokunaga M, Ochikubo S, Sugimoto S, Ikeda T, Terasaki M, Izumi S, Preston DL. Cancer incidence in atomic bomb survivors. Part II: Solid tumors, 1958–1987. *Radiat Res* 1994; 137:S17-67.

17. Preston DL, Ron E, Tokuoka S, Funamoto S, Nishi N, Soda M, Mabuchi K, Kodama K. Solid cancer incidence in atomic bomb survivors: 1958–1998. *Radiat Res* 2007; 168:1-64.

18. Astakhova LN, Anspaugh LR, Beebe GW, Bouville A, Drozdovitch VV, Garber V, Gavrilin YI, Khrouch VT, Kuvshinnikov AV, Kuzmenkov YN, Minenko VP, Moschik KV, Nalivko AS, Robbins J, Shemiakina EV,

Shinkarev S, Tochitskaya SI, Waclawiw MA. Chernobyl-related thyroid cancer in children of Belarus: A case-control study. *Radiat Res* 1998; 150:349-56.

19. Takahashi K, Eguchi H, Arihiro K, Ito R, Koyama K, Soda M, Cologne J, Hayashi Y, Nakata Y, Nakachi K, Hamatani K. The presence of *BRAF* point mutaion in adult papillary thyroid carcinomas from atomic bomb survivors correlates with radiation dose. *Mol Carcinogeensis* 2007; 46:242-8.

20. Hamatani K, Eguchi H, Ito R, Mukai M, Takahashi K, Taga M, Imai K, Cologne J, Soda M, Arihiro K, Fujihara M, Abe K, Hayashi T, Nakashima M, Sekine I, Yasui W, Hayashi Y, Nakachi K. *RET/PTC* rearrangements preferentially occurred in papillary thyroid cancer among atomic bomb survivors exposed to high radiation dose. *Cancer Res* 2008; 68:7176-82.

21. Hamatani K, Mukai M, Takahashi K, Hayashi Y, Nakachi K, Kusunoki Y. Rearranged anaplastic lymphoma kinase (*ALK*) gene in adult-onset papillary thyroid cancer among atomic-bomb survivors. *Thyroid* 2012. (in press)

22. Nakachi K, Hayashi T, Hamatani K, Eguchi H, Kusunoki Y. Sixty years of follow-up of Hiroshima and Nagasaki survivors: Current progress in molecular epidemiology studies. *Mutat Res* 2008; 659:109-17.

In Memoriam
Remembering Dr. Itsuzo Shigematsu
(25 November 1917–6 February 2012)

Toshiteru Okubo, Chairman

Dr. Itsuzo Shigematsu, giving a memorial lecture at the 25th anniversary of the establishment of RERF, November 2000

Although Dr. Itsuzo Shigematsu had always been in good health, he was hospitalized in Tokyo on April 8, 2011, for treatment of an aortic aneurysm and to undergo stent insertion. Although he was recovering well after the operation, from around November of last year, Dr. Shigematsu came down with pneumonia several times, which gradually weakened him physically. Dr. Shigematsu ultimately passed away on February 6, 2012.

Dr. Shigematsu was born in Osaka on November 25, 1917. After studying at Osaka High School (under the old Japanese education system) in Abeno, Osaka, he matriculated to the School of Medicine at Tokyo Imperial University. After graduating from the university in December 1931, he was soon recruited as a naval surgeon and experienced service on a cruiser named the "Kuma," as well as being stationed in military bases overseas. In 1942, when the tide of war was turning against Japan, he served in Rabaul, Papua New Guinea, the site of one of the bloodiest battles of the war and where he was when the global conflict ended.

After the war, Dr. Shigematsu worked for about 15 years in the Department of Epidemiology at the National Institute of Public Health, where he led Japan's epidemiological research efforts at a time when the field was still in its initial stages of development. During this period, he studied in the United States, acquiring a Master of Public Health (MPH) degree from Harvard University's graduate school. In 1962, he was hired as a professor in public health by the Kanazawa University School of Medicine. After working there for four years, he returned in 1966 to the National Institute of Public Health as chief of the Department of Epidemiology. For the subsequent 15 years until retirement in 1981, Dr. Shigematsu assumed important positions as an expert in public health and epidemiology, including as a member of various governmental councils, chief of research groups involving '*itai-itai*' disease and research on SMON (subacute myelo-optic neuropathy). He chaired various scientific societies, playing important roles not only in research but also in decision-making by the national government.

After retiring from the National Institute of Public Health, Dr. Shigematsu was appointed as the third chairman of the Radiation Effects Research Foundation (RERF) in July 1981. The long-term follow-up studies of fixed cohorts of A-bomb survivors established by the Atomic Bomb Casualty Commission (ABCC), the predecessor of RERF, are considered world-class models of epidemiological research. Therefore, the experience and network of connections acquired by Dr. Shigematsu, a leading expert in epidemiology, came to be fully utilized at RERF. Needless to say, the understanding of information on health and exposure to various factors from a study population is the key to good epidemiological research. Unless the quality of such information is high, reliable study results cannot be obtained. ABCC-RERF has actively tackled the work of estimating individual A-bomb radiation doses, and in accordance with his beliefs as an expert in epidemiology, Dr. Shigematsu exercised strong leadership in the establishment of a dose assessment system, succeeding at introducing the dosimetry system "DS86." As everyone knows,

Dr. Itsuzo Shigematsu (fifth from left), toasting ABCC-RERF's 50th anniversary with its former directors in June 1997

international radiation protection standards have been established on the basis of RERF's reports on radiation risks using DS86. Furthermore, since ABCC was founded in the chaotic period after World War II, there were a host of problems related to the management of the institution when ABCC was reorganized into RERF, in terms of its location and other aspects. Soon after arriving at RERF, Dr. Shigematsu established a group for reviewing the future vision of RERF, exercising effective leadership in resolving problems involving management of the institution.

The Chernobyl accident in 1986 allowed Dr. Shigematsu to also be active internationally in the field of radiation epidemiology. He assumed various important positions, including as a member of WHO's Senior Advisory Committee, a member of the Main Commission and Committee 1 of the International Commission on Radiological Protection (ICRP), and chairman of the International Advisory Committee on Chernobyl of the International Atomic Energy Agency (IAEA). His work in these posts was recognized internationally.

Dr. Shigematsu's impressive achievements are reflected in the numerous awards and honors he received. In Japan, for example, he received the Second Order of the Sacred Treasure from the Emperor of Japan and the Achievement Award for Prevention of Tuberculosis. Awards granted from institutions overseas include the Sievert Award from the International Radiation Protection Association, the Gold Medal from the Royal Swedish Academy of Sciences, and the Timofeev Medal from the Russian Academy of Sciences. Furthermore, Dr. Shigematsu was recommended to serve as a fellow of the Royal College of Physicians in London, an honorary member of the Moscow Academy of Advanced Education and Sciences, and an honorary member of the International Epidemiology Association. When receiving such awards and honors, Dr. Shigematsu always said that he had been honored in that way on behalf of everyone involved. Dr. Shigematsu truly was always considerate of other people's feelings.

Much to the regret of those concerned, Dr. Shigematsu retired from RERF in June 1997 after serving as chairman for four terms over a period of 16 years. He was already 80 years old at that time. However, even after retirement, he always made great efforts on RERF's behalf in his position as consultant emeritus. Furthermore, he willingly delivered lectures and accepted the post of chair at scientific meetings. He also authored and published a number of books, maintaining his drive as an epidemiologist until the very end of his life.

When we lost Dr. Shigematsu, we lost a superstar in the area of public health in Japan. However, he was able to educate and train many scientists in the fields of epidemiology and public health, with his last wishes now being carried on by many scientists active in the field of public health in Japan.

As one of those involved in public health, I hereby offer my sincere prayers for the repose of Dr. Shigematsu's soul.

In Memoriam

Late Professor James Franklin Crow
University of Wisconsin

(16 January 1916–4 January 2012)

Akio Awa, former Associate Chief of Research
(Former Chief, Department of Genetics)

We recently received very sad news, which I deeply regret having to report here. Dr. James Crow, an authority on genetics, passed away early this year, just before his 96th birthday. From the days of the Atomic Bomb Casualty Commission (ABCC)—the predecessor of the Radiation Effects Research Foundation (RERF)—Dr. Crow was a devoted supporter of RERF's program and consistently provided encouragement to us. I would like to express our deepest condolences to his family.

Dr. Crow was a trailblazer in the new discipline of genetics called population genetics, with his former students now active all over the world. Japan in particular has many geneticists who had the pleasure of knowing Dr. Crow per-

Dr. James F. Crow (left) and Dr. Akio Awa on the occasion of the Crow Committee meeting in 1975

sonally, and many of the leaders in human genetics in Japan were at one time his students. One of his well-known publications is a booklet titled "*Genetic Notes*" (Burgess Publishing Company, Minneapolis, Minnesota, USA). This famous publication was written as a textbook for students, and a group of Dr. Crow's Japanese students translated it into Japanese under the title "*Idengaku-Gaisetsu* (*Outline of Genetics*)." The Japanese translators of the booklet were renowned Japanese geneticists, the majority of whom were researchers at the Japanese National Institute of Genetics.

Population genetics is based on concepts that dramatically advanced the ideas of Mendel's classical genetics, and belongs to a scientific field that, combining temporal and spatial factors, analyzes and uncovers mutual relationships among animals and plants, as well as genetic and evolutional phenomena. Although the importance of Mendel's laws has not been diminished, the efforts of Dr. Crow and his students greatly expanded the concepts of these laws and thereby broadened research possibilities.

In addition to his research, the multi-talented Dr. Crow was a professional viola player who served as a member of Madison, Wisconsin's symphony orchestra.

I was involved in the field of cytogenetics (research involving chromosomes) for years, and had been deeply involved in human genetics until I retired. My main subject was the study of genetic effects from exposure to atomic-bomb radiation, and throughout my career, Dr. Crow always provided me with support and encouragement.

On a personal note, I would like to relate the following story that for me is truly unforgettable:

In February 1975, a body known as the Crow Committee met to determine research themes for the new research institute after ABCC's reorganization (present-day RERF). I made a presentation to the committee, after which Dr. Crow commended me with the recommendation that, "the highly successful cytogenetics program be continued, with an increase in scientific personnel as needed (ABCC Annual Report 1975–1976)." I felt lucky, mainly because Dr. Crow and I were colleagues involved in the same field of human genetics.

Being somewhat careless, I had felt unsure if my work would meet Dr. Crow's expectations, but my intention was to make the utmost effort. A researcher can be a "star" one day, but a "has-been" the next. Scientists are beings that have to fight like there's no tomorrow for a future that is unforeseeable.

I would like to conclude my memorial address by dedicating the following words to Dr. Crow and his bereaved family (son Franklin C. Crow and daughters Laura J. Crow and Catherine Rasmussen):

"Thank you, Jim. Thank you indeed. Akio."

In Memoriam
Mr. Seymour Jablon
(2 June 1918–9 April 2012)

Evan B. Douple, Associate Chief of Research

We received the sad news that a legendary foundation and cornerstone of the ABCC-RERF, Seymour Jablon, died on 9 April 2012, at the age of 93. If you were to count on one hand the persons who have had the greatest impact on the science of the Foundation, Mr. Jablon would be among the most influential. As a research staff member of the Medical Follow-up Agency (MFUA) in the Division of Medical Sciences of the National Academy of Sciences-National Research Council (NAS-NRC), Mr. Jablon was first dispatched to Japan in October 1955 as one of three members of the "Ad Hoc Committee for Appraisal of ABCC Program" which became known as the "Francis Committee" after the committee's chairman, Dr. Thomas Francis. The com-

mittee's report, *ABCC Unified Study Program*, established the fundamental policy for the longitudinal long-term study on a fixed population under stable conditions to conduct research and investigate late radiation effects from the atomic bombs. The report contributed immensely to the promotion and success of the research program for more than 56 years and established the Life Span Study and Adult Health Study cohorts that are studied to this day. The selection of the study population and the rationale of its scale were epoch-making at the time.

It was Mr. Jablon who dedicated himself to planning and promoting the establishment of the Tumor Registry in Hiroshima and in Nagasaki in 1957 and 1958 respectively and the Tissue Registry initiated in 1973–1974, which were the first ever to be established in Japan. The registries, which were also part of the Unified Study Program, not only contributed to the study of late effects of atomic-bomb exposures, but also to the elevation of medical care measures in Hiroshima and Nagasaki.

After participating in the drafting of the Unified Study Program, Mr. Jablon served two terms in Japan as Chief of the ABCC Department of Statistics, which included epidemiology and statistics, first from 1960–1963, and later from 1968–1971. He undertook the role of not only implementing the program and directly providing guidance and supervision to the administrative and research activities of the department, but he also introduced the first computer system in ABCC in 1962 and obtained a number of utility programs developed in the U.S. for significantly augmenting the tabulating and analyzing of the research data. He continued his strong support and provided advice on the management and administration of the ABCC program, first from 1963–1968 and 1971–1977 as the Associate Director of the NAS-NRC's MFUA, and from 1977 as Director of the MFUA.

In April 1975, Mr. Jablon participated in the reorganization of ABCC into RERF as a member of the U.S. government negotiation team and performed an important role as a true parent of the present RERF. He was also a member of the six-member "Committee for the Scientific Review of ABCC" that was chaired by Dr. James Crow and which visited Japan in 1975 and drafted a detailed guideline on the research program for transition into RERF. He retired from the NAS-NRC on October 2, 1987 after 39 years of outstanding service and then continued to support RERF while an Expert in the Radiation Epidemiology Branch of the U.S. National Cancer Institute. He also served a five-year term as a member of RERF's Board of Directors from 1 July 1991 until 30 June 1996. Because of his many years of promoting the exchange of scientists and research between Japan and the U.S. on the subject of the delayed medical effects of atomic-bomb radiation, Seymour Jablon was awarded the Third Order of the Sacred Treasure by the Emperor of Japan on 29 April 1987.

Seymour Jablon will be remembered as a distinguished and productive statistician who was also known for his sociable and genial character. He possessed a strong will and positive executive ability, and his character and insight were highly respected in the U.S. where he not only exerted great efforts for the procurement of funds and materials necessary for the operation of RERF, but where he recruited from the U.S. excellent scientists as RERF directors and researchers for the quality enhancement of the research program. His career accomplished so much and he positively impacted the careers of many other people who were privileged to have known him.

Mr. Seymour Jablon (left) and Dr. Dale Preston, two generations of Statistics Department chiefs, taken at the Beebe Symposium 2002

On the Occasion of My Mandatory Retirement

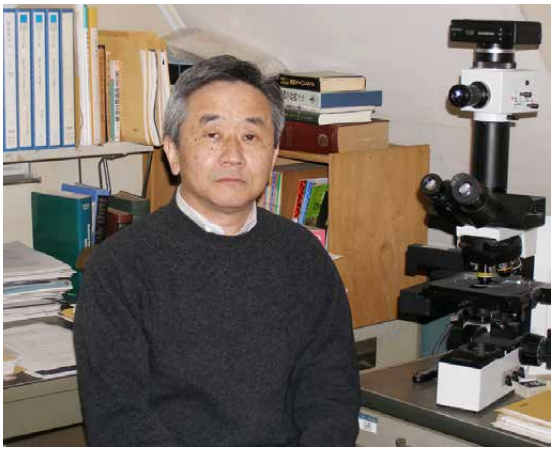
Yoshiaki Kodama, Chief
Department of Genetics

I first visited Hiroshima in 1979 to attend a scientific meeting. I was a research student at Hokkaido University's chromosome research laboratory (formerly known as the Makino study group) while also doing some part-time work on the side. At the meeting, I think I gave a presentation on a murine chromosome study or some similar topic. Back then I had never imagined that I would come to live in Hiroshima. At the end of that year, Professor Motomichi Sasaki of Hokkaido University's chromosome research laboratory told me that there was a job opportunity for me at RERF. After the start of the new year, I was asked to give a seminar at RERF, which was a sort of final test one had to go through before being officially hired, a tradition that is still followed at RERF. Although seminars were usually given in English at RERF, I presented my talk in Japanese because my English was poor. At the seminar I was pleasantly surprised and impressed by the presence of an interpreter (I believe the interpreter was Mr. Kenji Yorichika) providing simultaneous translation. It goes without saying that I had never had such an experience. That evening, Dr. Akio Awa took me to a Hiroshima Carp baseball game after dinner. We were seated in the infield stands. It was the first live baseball game I had ever attended in my life. I still clearly remember that the last pitcher in that game was the famous Yutaka Enatsu. As for my seminar, it seemed that I was given a passing score, because I was officially hired as a research scientist at the Cytogenetics Laboratory in July 1980.

When I joined RERF, I was the most junior research scientist at the laboratory, and I continued to hold that "junior" title for roughly the next 20 years. As new "junior" research scientists subsequently joined the laboratory, I started to be categorized as a senior research scientist. Looking back, the past 31 years and five months have flown by quickly. For those 30 years or so, I have been blessed with so many good people—outstanding teachers and mentors, highly capable junior research scientists, and technical and administrative staff who have provided excellent support. I truly consider myself a lucky man, and I cannot thank those people enough. Without mentioning individual names, I hereby express my sincerest appreciation to all of them.

The RERF Cytogenetics Laboratory has had eight research scientists who came from the same Hokkaido University chromosome research laboratory. Back then, that laboratory was the only one studying chromosomes in Japan and the only source of chromosome researchers. People called us the "Hokkaido mafia." Dr. Awa was the first to come to RERF and I was the last (the 8th person). In the latter 1960s, the pioneer days of the Cytogenetics Laboratory, Dr. Awa and others worked hard to establish the Giemsa staining-based detection method for identification of stable chromosome aberrations for estimation of exposure doses. In the 1990s this detection method was replaced by the FISH (fluorescence *in situ* hybridization) method, which is still used today. These research results have been very important in studying human health effects from atomic-bomb radiation, proving that the direction set by our predecessors was correct. As someone who has followed this course, I am truly grateful to such individuals.

Although I have completed one chapter of my career with my mandatory retirement, I will start another chapter at RERF as a fixed-term research scientist. I truly hope to be able to convey my knowledge and expertise gained at RERF to the next generation of research scientists.



Taking Early Retirement

Saeko Fujiwara, former Chief
Department of Clininical Studies, Hiroshima

After taking early retirement from the Radiation Effects Research Foundation (RERF) this past March, I assumed the position of Assistant Director at the Health Management and Promotion Center, Hiroshima Atomic Bomb Casualty Council, working on health examinations offered to A-bomb survivors and local citizens.

I joined RERF in 1979. I feel very fortunate to have worked with so many distinguished researchers and staff members there. Those who assisted me with my experiments late into the night when I had just joined the foundation (I was in charge of immunological experiments at that time) and those who gave me a helping hand regarding matters beyond the realm of work are still like older brothers and sisters to me even though they retired from RERF long ago. Two years after I entered the foundation, Dr. Itsuzo Shigematsu assumed the position of RERF Chairman. After a short time, Dr. Kazunori Kodama returned to RERF following his studies in the U.S. Furthermore, Dr. Yutaka Hosoda joined RERF as Chief of the Department of Clinical Studies, infusing the department with great energy. Before that time, the Department of Medicine (name of the department until March 31, 1985) performing only health examinations was considered enough, but the department transformed itself into a research department, where I participated in several research projects regarding the association of immunological markers and hyperparathyroidism with radiation exposure. Under the guidance of Dr. Shigematsu, epidemiological projects addressing osteoporosis and dementia were initiated as part of research into radiation exposure and aging. Dr. Shigematsu introduced me to researchers who played leading roles in the osteoporosis research communities in Japan and the U.S., providing me with opportunities to participate in, and present my research at meetings and scientific symposiums that were frankly too advanced for my young age and meager scientific achievements at that time. As a result, however, I was able to gradually convince the clinical researchers in the area of osteoporosis regarding the importance of epidemiology and the value of RERF's research activities, borrow expensive equipment, and receive various forms of support. In this way, epidemiological research of osteoporosis became my life's work.

RERF is said to be a treasure trove of radiation information, but the data accumulated thus far have come from the day-to-day efforts of past RERF research scientists and employees. I tell newly hired research scientists to sow seeds even though they might not be able to reap the fruits of such labor. I myself feel proud to have been involved in the launching of the clinical study of the children of A-bomb survivors. Progress in the preparation for the clinical study was very haphazard, but I feel that those who worked together for this purpose became like fellow soldiers rather than co-workers.

With my promotion to assistant department chief and department chief, I had to supervise the entire department and its future, instead of thinking of only my own research. For me, however, the presence of employees who enthusiastically dealt with the Adult Health Study (AHS) participants provided me with strong emotional support. I would therefore like to express my deep appreciation to fellow staff members for their sympathetic and courteous support and efforts to create a comfortable work environment.

I was born and raised in Hiroshima, and my mother and grandparents were A-bomb survivors. In my childhood, my grandfather, sister, and I never failed to attend the Hiroshima Peace Memorial Ceremony held every August 6. Thanks to the cooperation of AHS participants, I have been able to work at RERF on studies of interest to me over the years. In the hope that I can contribute to enhancement of the health of elderly A-bomb survivors, I thus decided to work for the Hiroshima Atomic Bomb Casualty Council.

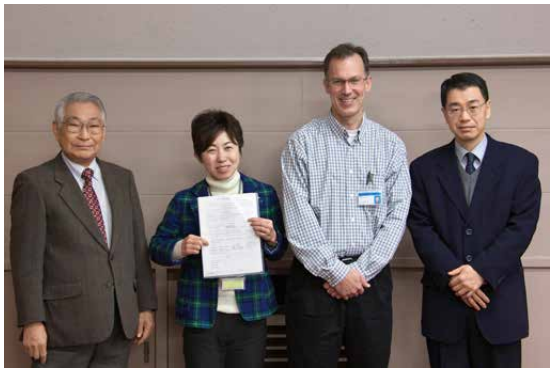
Since the Council and RERF share the same objective of working for the benefit of the A-bomb survivors, I hope that the relationship between the two organizations can be further enhanced in the future. I ask for everyone's continued cooperation toward meeting that goal.

I close by expressing my heartfelt appreciation to the entire RERF staff for their consistent and varied support over so many years.

Accession Number of Scientific Manuscripts Reaches 1,000

When an RERF research scientist has prepared a manuscript for publication, they are required to submit the manuscript to the RERF Scientific Reports Review Committee (SRRC). That committee assigns an "accession number" to the manuscript in chronological order and conducts a preliminary review that includes sending it internally to two RERF scientists and externally to one or two experts in the subject area in institutions outside of RERF and Japan. When the authors receive the resulting reviews, they respond to the concerns, questions, recommendations, and other issues. The process results in an improvement in the quality of the RERF manuscripts, provides review experience to the RERF scientists, and we assume that it results in an improved acceptance rate.

The ABCC records show that the review process originated with the establishment of the Scientific Manuscript Review Board in February of 1974. The first Board included Gilbert W. Beebe as one of the seven members and it was chaired by Isamu Nagai, Acting Associate Director of ABCC. When the ABCC became the RERF in 1975, the assignment of accession numbers continued. Early in 2012, the chair of the SRRC, Evan B. Douple, noticed that the accession numbers were approaching 1,000. He decided that he would provide a free dinner to the authors who submitted the manuscript with assigned accession number 1,000. He elected not to tell RERF's scientists in advance (and hoped that the number of authors would not be excessively large!). On February 2, the 1,000th accession number was assigned when the manuscript "Long-term follow-up of atomic bomb survivors" arrived from the Department of Epidemiology's Ritsu Sakata, Eric J. Grant, and Kotaro Ozasa. (see photograph). The published manuscript can be found in the journal *Maturitas* 72(2):99–103 (2012).



Dr. Ritsu Sakata (second from left), holding the RERF scientific report approval form with the accession number 1,000, joined by Chairman Toshiteru Okubo (far left), and her co-authors Drs. Eric J. Grant (second from right), and Kotaro Ozasa (far right)

Research Protocol Approved in November 2011–April 2012

RP 1-12 Development of an Archival System for Surgical Cancer Samples from Atomic-bomb Survivors

Ozasa K, Sugiyama H, Soda M, Yasui W, Arihiro K, Fujihara M, Arita K, Nishisaka T, Matsuura H, Nakashima M, Shigematsu K, Takahara O, Kusunoki Y, Katayama H, Suyama A

The latest paper regarding the number of solid cancer cases in the Life Span Study (LSS) cohort between 1958 and 1998 reported it as 17,448, of which the majority of cases requiring surgical procedures are believed to have undergone surgery at major hospitals in Hiroshima and Nagasaki. Such surgical cancer samples (in paraffin blocks) are maintained independently at such hospitals. Some hospitals, however, are forced to consider discarding the samples due to the lengthy storage they have committed to and the difficulty they have in securing storage space. In order to carry out pathological studies, such as in-depth molecular biological research on radiation carcinogenesis in the LSS cohort, long-term storage of such samples needs to be assured. The objective of this study is to develop an archival system for surgical cancer samples from atomic-bomb survivors in collaboration with pathologists of the major hospitals in Hiroshima and Nagasaki. For development of this system, surgical samples from the LSS cohort members stored at each hospital will be identified and reviewed for their storage status and usability in future studies. Upon identification of such samples, each hospital will be requested to store them over an extended period of time. When it becomes difficult for a hospital to continue maintaining the samples, they will be transferred to and stored at RERF. In addition to this research protocol (RP)'s collaborating investigators and their organizations, other major hospitals and their pathologists in Hiroshima and Nagasaki will be requested to participate in this RP. RERF will create and manage the database about all stored samples. This RP determines the procedures of collaborating research projects in which the storage samples will be used and the guidelines for the utilization, and the participants in this RP should respect them.

Recent Publications

(Japanese): the original article is in Japanese.

Adams MJ, Grant EJ, Kodama K, Shimizu Y, Kasagi F, Suyama A, Sakata R, Akahoshi M: Radiation dose associated with renal failure mortality: A potential pathway to partially explain increased cardiovascular disease mortality observed after whole-body irradiation. Radiat Res 2012 (February); 177(2):220-8. (RERF Report 9-11)

Chen J, Kerr GD, Cullings HM: A comparison of organ doses between mathematical and voxel phantoms with the DS02 photon fluences. Radiat Prot Dosimetry 2012 (January); 149(1):49-55. (RERF Report 22-11)

Cologne JB, Grant EJ, Nakashima E, Chen Y, Funamoto S, Katayama H: Protecting privacy of shared epidemiologic data without compromising analysis potential. J Environ Public Health 2012 (February); Vol. 2012, Article ID 421989, 9 pages, 2012. doi:10.1155/2012/421989. Epub only (RERF Report 2-06)

Cullings HM: Recommended improvements to the DS02 dosimetry system's calculation of organ doses and their potential advantages for the Radiation Effects Research Foundation. Radiat Prot Dosimetry 2012 (January); 149(1):2-14. (RERF Report 24-11)

Cullings HM, Kawamura H, Chen J: Body and organ dimensions of the 1945 Japanese population used in dosimetry system DS86 and data available for an expanded series of phantoms. Radiat Prot Dosimetry 2012 (January); 149(1):35-42. (RERF Report 23-11)

Fattibene P, Wieser A, Adolfsson E, Benevides LA, Brai M, Callens F, Chumak V, Ciesielski B, Della Monaca S, Emerich K, Gustafsson H, Hirai Y, et al.: The 4th international comparison on EPR dosimetry with tooth enamel. Part 1: Report on the results. Radiat Meas 2011 (May); 46:765-71.

Hida A, Akahoshi M, Takagi Y, Imaizumi M, Sera N, Soda M, Maeda R, Nakashima E, Ida H, Kawakami A, Nakamura T, Eguchi K: Lipid infiltration in the parotid glands: A clinical manifestation of metabolic syndrome. Exp Clin Endocrinol Diabetes 2012 (February); 120(2):110-5. (RERF Report 14-08)

Imaizumi M, Sera N, Ueki I, Horie I, Ando T, Usa T, Ichimaru S, Nakashima E, Hida A, Soda M,

Tominaga T, Ashizawa K, Maeda R, Nagataki S, Akahoshi M: Risk for progression to overt hypothyroidism in an elderly Japanese population with subclinical hypothyroidism. Thyroid 2011 (November); 21(11):1177-82. (RERF Report 10-10)

Kodama K, Ozasa K, Okubo T: Radiation and cancer risk in atomic-bomb survivors. J Radiol Prot 2012 (March); 32(1):N51-4. doi:10.1088/0952-4746/32/1/N51 (Special section: Selected articles from Proceedings of the International Expert Symposium in Fukushima: Radiation and Health Risks, 11–12 September 2011)

Masunari N, Fujiwara S, Kasagi F, Takahashi I, Yamada M, Nakamura T: Height loss starting in middle age predicts increased mortality in the elderly. J Bone Miner Res 2012 (January); 27(1):138-45. (RERF Report 2-11)

Nakamura N: Genetic effects of radiation. Nippon Rinsho [Jpn J Clin Med] 2012 (March); 70(3):457-60. (Japanese)

Nakamura N: Cancer risks following exposure to ionizing radiation. Surgery Frontier 2011 (December); 18(4):100-1. (Japanese)

Nakamura N, Hirai Y, Kodama Y: Gamma-ray and neutron dosimetry by EPR and AMS, using tooth enamel from atomic-bomb survivors: A mini review. Radiat Prot Dosimetry 2012 (January); 149(1):79-83. (RERF Report 26-11)

Neriishi K: Cataract: Based on A-bomb survivor studies. Nippon Rinsho [Jpn J Clin Med] 2012 (March); 70(3):441-5. (Japanese)

Neriishi K, Monzen Y, Okamoto N: Radiation effects on pregnant women, fetuses, and children. Nippon Rinsho [Jpn J Clin Med] 2012 (March); 70(3):451-6. (Japanese)

Ohishi W, Chayama K: Prevention of hepatitis B virus reactivation in immunosuppressive therapy or chemotherapy. Clin Exp Nephrol 2011 (October); 15(5):634-40.

Ohishi W, Fujiwara S, Cologne JB, Chayama K: Effects of radiation exposure and lifestyle on risk of non-B, non-C hepatocellular carcinoma. Shokaki Naika [Jpn J Gastroenterol] 2011 (October); 53(4):453-8. (Japanese)

Okubo T: Epidemiological studies: From experience in Hiroshima and Nagasaki to Fukushima. Igaku no Ayumi [J Clin Exp Med] 2011 (December); 239(10):995-1000. (Japanese)

Okubo T: Radiation risk studies and radiation protection limits. Sanfujinka no Jissai [Obstet Gynecol Prac] 2011 (December); 60(13):2055-61. (Japanese)

Ozasa K: Health effects of atomic-bomb radiation of Hiroshima and Nagasaki. Nogyo to Keizai [Agriculture & Economics] 2012 (January); 78(1):53-60. (Extra edition of January, 2012) (Japanese)

Ozasa K: Cohort studies of the atomic bomb survivors at the Radiation Effects Research Foundation. Nippon Rinsho [Jpn J Clin Med] 2012 (March); 70(3):399-404. (Japanese)

Ozasa K: Epidemiological studies of the atomic bomb survivors in Hiroshima and Nagasaki. Kyo-fu Idai Shi [J Kyoto Pref Univ Med] 2011 (December); 120(12):903-11. (Japanese)

Ozasa K, Shimizu Y, Suyama A, Kasagi F, Soda M, Grant EJ, Sakata R, Sugiyama H, Kodama K: Studies of the mortality of atomic bomb survivors, Report 14, 1950–2003: An overview of cancer and noncancer diseases. Radiat Res 2012 (March); 177(3):229-43. (RERF Report 4-11)

Sakata R, Shimizu Y, Soda M, Grant EJ, Sugiyama H, Pham TM, Hsu WL, Suyama A, Ozasa K. Recent RERF epidemiological studies of an atomic bomb (A-bomb) survivor cohort: Early onset of menopause observed among A-bomb survivor females. Nakashima M, et al., eds. A New Challenge of Radiation Health Risk Management. Proceedings of the 6th International Symposium of Nagasaki University Global COE Program "Global Strategic Center for Radiation Health Risk Control." Nagasaki: Nagasaki Newspaper Publish; 2012 (March). pp 145-9.

Sakata R, Shimizu Y, Soda M, Yamada M, Hsu WL, Hayashi M, Ozasa K: Effect of radiation on age at menopause among atomic bomb survivors. Radiat Res 2011 (December); 176(6):787-95. (RERF Report 7-11)

Satoh Y, Sasaki K, Shimoichi Y, Sugita K, Katayama H, Takahashi N: Copy-number variations observed in a Japanese population by BAC array CGH: Summary of relatively rare CNVs. J Biomed Biotechnol 2012 (January); Volume 2012, Article ID 789024, 10 pages, doi: 10.1155/2012/789024. Epub only (RERF Report 8-11)

Shore RE, Ozasa K, Suyama A, Fujiwara S, Akahoshi M, Cullings HM, Kodama Y, Kodaira M, Hayashi T, Hamatani K: Recent findings by the Radiation Effects Research Foundation on the health of atomic bomb survivors and their offspring. Nakashima M, et al., eds. A New Challenge of Radiation Health Risk Management. Proceedings of the 6th International Symposium of Nagasaki University Global COE Program "Global Strategic Center for Radiation Health Risk Control." Nagasaki: Nagasaki Newspaper Publish; 2012 (March). pp 27-33.

Takahashi I, Abbott RD, Ohshita T, Takahashi T, Ozasa K, Akahoshi M, Fujiwara S, Kodama K, Matsumoto M: 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). BMJ Open 2012 (February); 2:e000654. Epub only (RERF Report 13-11)