RERF update RERF

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LSS Cancer-incidence Data Now Available on Disk

Earlier this year RERF researchers and their collaborators published a series of general comprehensive reports on cancer incidence in the Life Span Study (LSS) cohort (Thompson et al, *Radiat Res* 137:S17–S67, 1994; Preston et al, *Radiat Res* 137:S68–S97, 1994).

The data used in these analyses are now available to persons outside RERF on two high-density disks that contain two primary data files. One file summarizes site-specific data on solid-cancer incidence, 1958–1987. The second file contains data on the incidence of leukemia (including various subtypes), lymphoma, and multiple myeloma, 1950–1987. Also included is a file of factors that can be used to convert the mean colon dose values of the solid-cancer data set to doses for other organs of interest. Documentation of file formats and disease-category definitions are provided, as well as command scripts that can be used to fit some of the models presented in the recently published cancer-incidence reports.

The solid-cancer and leukemia data sets are detailed tabulations of person-years, case counts, and summary data constructed from data for individual survivors. The solid-tumor data covers 80,206 survivors; the leukemia data 86,594 survivors. These totals include survivors with Dosimetry System 1986 (DS86) shielded kerma estimates greater than 4 Gy. Data sets are structured to facilitate exclusion of the survivors having total shielded kerma estimates above 4 Gy, as was done in the published reports.

The solid-tumor data set is based on data obtained from the Hiroshima and Nagasaki tumor registries together with LSS mortality follow-up data. Because the tumor registries did not start operation until 1958, follow-up is limited to 1958–1987. The ABCC/RERF leukemia registry was the main source of data on the incidence of leukemia, lymphoma, and myeloma; however, data were also obtained from the

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COURTESY OF THE CHUGOKU SHIMBUN

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Annual Commemorative Ceremonies Held in Near Drought Conditions

At events held in Hiroshima and Nagasaki on 6 and 9 August, respectively, about 75,000 persons paid tribute to those who perished in the atomic bombings 49 years ago.

In the fiercely hot temperatures parching reservoirs and rivers throughout most of western Japan, Prime Minister **Tomiichi Murayama** attended both official ceremonies, which occur at the times of the detonations of the atomic bombs—08:15 in Hiroshima and 11:02 in Nagasaki. He is the first prime minister in 3 years to attend the memorial ceremonies.

RERF Chairman **Itsuzo Shigematsu** and Permanent Directors **Mortimer Mendelsohn** and **Donald Harkness** represented RERF at the solemn proceedings in Hiroshima. Permanent Director **Yutaka Hasegawa** and **Tadashi Nakaoka**, Nagasaki Laboratory chief of administration, attended the ceremony in that city. ❑

At left, on 6 August crowds stream out of Hiroshima's Peace Park after placing flowers in front of the memorial cenotaph. In the background is the Atomic-bomb Dome.

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Perspectives

A Glimpse into the Future

by Donald Harkness, **RERF Update** *Editor in Chief*

It is easy to lose sight of RERF's research goals when seemingly every month or two a new crisis presents itself with respect to either RERF's budget or US funding to support National Academy of Sciences employees at RERF. Faced with no news on those issues—which should not be construed as good news, I will say nothing more on those topics for now.

This summer following a talk I gave about RERF research at the University of Wisconsin's Department of Medicine (my former work place), three persons posed the same question: "How much longer will the studies in Hiroshima and Nagasaki continue?" That question has helped me refocus my perspective on RERF's mission and the work yet to be completed.

RERF's objective as set forth in the Act of Endowment is ". . . to conduct research and studies, for peaceful purposes, on the medical effects of radiation in man and on diseases which may be affected by radiation." At this juncture, examining the status of RERF's survivor cohorts—for among these populations the medical effects on humans will be revealed—may partly answer my colleagues in Wisconsin.

As shown in the accompanying table, more than 50% of the Life Span Study and its Adult Health Study subsample are alive today, and, not surprisingly, much higher percentages are alive in the in-utero and F_1 cohorts. Perhaps the most remarkable figure is that in 1993 nearly 85% of LSS participants who were under 30 years of age at the time of the bombings (ATB) were living. Because radiation exposure at younger ages ATB (ie, <10 y) results in a greater risk of several malignancies, continued monitoring of these cohorts as they reach the cancer-prone ages may reveal critical information. With more than half of the AHS sample still alive (participation rate: 74.5%), adding to that 30-year aggregate of physiological and biochemical measurements may yet uncover nonmalignant effects of radiation exposure.

So what is the answer to the question "How much longer?" In a sense, the answer is elusive—a moving target. But it is clear that much work remains. After 10 more years, we may better discern the specific benefits of continuing the major cohort studies. In the meantime, I would answer: "Until 2005 or 2010!" ❑

Table. Status of major RERF study cohorts

Cohort	No. of persons	No. having DS86 dose	Alive in 1993 (%)
LSS (all ages)	120,321	86,572	53.1
Age ATB < 30 y		46,263	84.9
Age ATB 30–49 y		25,759	25.7
Age ATB ≥ 50 y		14,550	0.2
AHS (subsample of LSS)	17,397	14,171	54.1
In utero (mortality study)	2 813	1 791	85.7
F_1 (mortality study)	76,817	67,586	93.7

Note: DS86 = Dosimetry System 1986; LSS = Life Span Study; ATB = at the time of the bombings; AHS = Adult Health Study; F_1 = first generation born to the atomic-bomb survivors.

News Briefs

✓ Administrative Responsibilities Reassigned

After the resignation of permanent directors **Seymour Abrahamson** and **Tomoyuki Kono** on 30 June as part of cost-cutting measures, the supervision of departmental operations was reassigned as follows:

Mortimer Mendelsohn: Department of Statistics and Research Information Center. (He is also RERF vice chairman.)

Yutaka Hasegawa: Departments of Epidemiology and Epidemiologic Pathology at the Hiroshima Laboratory. (He is also director of the Nagasaki Laboratory.)

Donald Harkness: Department of Clinical Studies, Department of Genetics, Department of Radiobiology, Publication and Documentation Center, and Radioisotope Facility. (He is also RERF chief of research.)

Yasukiyo Hirano is chief of the Secretariat.

✓ Collaboration with Russian Scientists Continues

In early September, RERF Department of Statistics Chief **Dale Preston** visited the Ural Research Center for Radiation Medicine, Chelyabinsk, to collaborate with **M Degteva** and **M Kossenko** on comparisons of the local Techa River population with RERF's Life Span Study cohort—the former having received prolonged, low-dose-rate exposures. At Ozersk (the former secret city of Chelyabinsk-65), Preston conferred with **N Koshurnikova**, **V Khokhrayakov**, and **N Shilnikova** about issues related to the Mayak plutonium-production facilities and the potential for dosimetry and epidemiological studies of children residing near the plant. During his stay in Ozersk, a small release of radioactive materials reportedly occurred.

✓ Research Staff News

Hiroshima

Department of Genetics: On 1 September, research scientist **Sadayuki Ban** was transferred from the Laboratory of Cytogenetics to the Laboratory of Cell Biology, Department of Radiobiology.

Department of Statistics: Research assistant **Shizue Izumi** returned to the Hiroshima Laboratory after receiving a master's degree in biostatistics from the School of Public Health, University of Washington, Seattle. At the American Statistical Association meeting held in Toronto in August, she delivered a presentation based on her master's thesis.

Nagasaki

Department of Clinical Studies: On 1 September, **Masako Tsuruta**, chief, Division of Clinical Laboratories, was appointed industrial health physician. On 30 June, **Hiroaki Nonaka**, acting chief, Division of Clinical Laboratories, resigned. Division of Medicine research scientist **Kiyosumi Oishi** resigned on 31 August. These two clinicians will be replaced by two part-time physicians. ❑

Progress in the RERF Biochemical Genetics Study

The ongoing hunt for heritable mutations among the children of the atomic-bomb survivors will involve surveying large numbers of nucleotides for various types of mutation.

by Chiyoko Satoh and Mieko Kodaira, Department of Genetics, RERF

To study at the DNA/RNA level the possible genetic effects on the children of atomic-bomb (A-bomb) survivors, in the RERF Laboratory of Biochemical Genetics we are developing technologies and establishing cell lines that will be used in future screening projects.

Establishing cell-line repositories

Our goal is to establish cell lines from B lymphocytes from 1000 families using Epstein–Barr virus transformation. Resorting to cell lines was deemed necessary because in mutation studies, both parents of a child must be available, and by now—almost 50 years after the bombings—the parents of many first-generation children are unavailable due to death or emigration. Among 500 of these families, one or two parents were exposed to A-bomb radiation and the combined parental dose is more than 0.01 Sv; the average combined parental dose for these families is 0.5 Sv. The other 500 families are controls. Each family comprises a mother, a father, and all available children.

Each established cell line is proliferated to between 5×10^8 and 1×10^9 cells, from which an adequate amount of DNA can be extracted for various analyses, and each cell line is preserved in liquid nitrogen. Aliquots of intact lymphocytes and granulocytes are also preserved in liquid nitrogen as reference materials to determine if mutations encountered during screening occurred during cell-line establishment.

To date, cell lines have been established for more than 500 families in Hiroshima and for more than 440 families in Nagasaki, where the project started 1 year later. Because samples were selected on the basis of the revised tentative 1965 dosimetry, some parents do not have Dosimetry System 1986 (DS86) dose estimates. Thus, we are planning to add some new families that have parental DS86 doses.

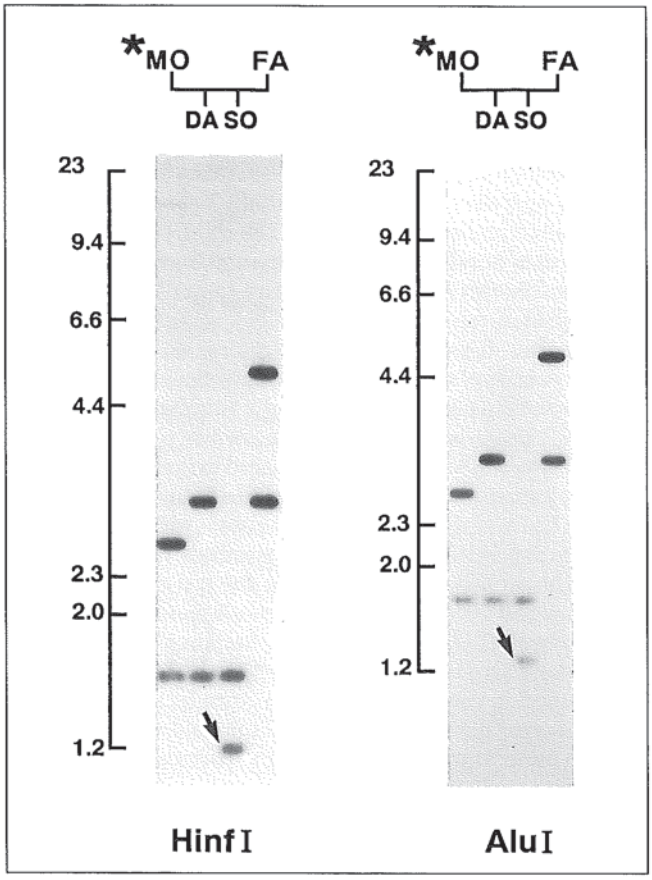

Figure. A new length mutation at the CEB-1 locus detected in a child of Family 0716. Arrows indicate mutant bands in the digests of HinfI and AluI, respectively. One of the son's (SO) bands is identical to one of the mother's (MO) bands, but the other band is identical to neither of the father's (FA) bands. DA = daughter. Asterisk indicates that the mother was exposed to atomic-bomb radiation. Positions and lengths (in kilobase pairs) of DNA markers are at the left of the photographs.

Determining potential DNA targets

As reported in *RERF Update* 3(4):1, 1991, at a workshop on human germline mutagenesis held at the Hiroshima Laboratory in November 1991, workshop attendees recommended a pilot study to compare various types of DNA as potential targets for detecting germinal mutations.

In the middle of 1992, we began to examine 100 families—a subset of the 1000 families used in establishing our cell lines. Various genes or DNA sequences have been examined using three techniques: (1) denaturing gradient gel electrophoresis of the products of polymerase chain reaction (PCR) for detecting single nucleotide substitutions and small deletions and insertions in DNA or RNA fragments of 2–3 kilobase pairs (kb), (2) Southern blotting for detecting rather long sequence differences made from insertions/deletions/rearrangements (I/D/R) in unique sequences and for detecting changes in the number of repeats in minisatellites or variable number of tandem repeats (VNTRs), and (3) high-resolution electrophoresis of PCR-amplified microsatellites for detecting the difference in the numbers of unit sequences in microsatellites. In this article, we will report on our examination of minisatellites and microsatellites.

Exploring the mini- and microsatellites

Minisatellites or VNTRs are dispersed throughout the human genome but show a tendency to cluster in telomeric regions. They consist of short-sequence units (~5–40 base pair [bp] in length) iterated in tandem to form arrays of approximately 1–10 kb and show allelic variation in the number of repeat units. At several minisatellite loci that exhibit high heterozygosity, high germline mutation rates for new length alleles were observed. We examined six minisatellite loci from 50 exposed families including 64

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Progress in Biochemical Genetics

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children and 50 control families with 60 children. Among the 64 children from the exposed families, only one child has parents who were both exposed. Thus, by examining 124 children of the 100 families, we could examine 65 gametes (sperms and oocytes) produced in exposed parents and 183 gametes derived from unexposed parents (63 gametes from the unexposed parents in the exposed families plus 120 from the control parents). The average parental gonad dose for the 65 gametes was 1.9 Sv.

DNA samples extracted from the cell lines were digested by *Hinf*I restriction enzyme, which does not cleave within the minisatellite tandem-repeat array, and conventional agarose gel electrophoresis was carried out. Southern filters produced from the agarose gels were sequentially hybridized with six minisatellite probes. We compared the children's bands with their parents' bands and identified mutant bands when bands of identical length were absent in both parents. When mutants were detected, we examined the DNA samples from these families after digestion by a second restriction enzyme, *Alu*I, that cut outside of the repeat array at positions different from *Hinf*I digestion sites. An example of a mutation detected by the CEB-1 probe is shown in the Figure (see p 3). A decrease in mobility of the son's band as compared to that of his father's band by approximately 2 kb in the *Hinf*I digest was reproduced in the *Alu*I digest, which is consistent with the idea that the mutation involves the loss of numbers of repeat units within the minisatellite. To exclude the possibility that the mutations had been generated during cell-line establishment, we examined DNA from intact lymphocytes from the members of those families in whom mutations had been detected. In all mutation cases, we observed mutant bands identical to those observed in the cell-line DNA of the children, confirming that the mutations had occurred in the parents' germ cells.

We detected six mutations after examining 65 gametes at three minisatellite loci (*λMS1, CEB-1, pλg3*); no mutations were detected at the remaining three loci. Interestingly, 22 mutations were detected at the same three minisatellites loci in the 183 unexposed gametes. The average mutation rates (mutation/loci/gamete) for the exposed and the unexposed gametes were 0.015 [6 ÷ (65 × 6)] and 0.02 [22 ÷ (183 × 6)], respectively. The average mutation rates at the six minisatellite loci of the gametes derived from the exposed parents and the unexposed parents did not show a statistically significant difference. Moreover, at each of the three minisatellite loci where mutations had been detected, the mutation rate did not differ significantly between the exposed and unexposed gametes.

We have estimated the number of germ cells needed to demonstrate a significant difference between the mutation rates of the exposed and the unexposed germ cells. For a locus with a spontaneous mutation rate of 0.02 identical to that of the mean mutation rate of the six loci examined in our study, it would require two samples of 1188 germ cells (Kodaira et al., in preparation). This means we have to examine 19 loci from each of these 64 children from the 50 exposed families, ie, 13 additional loci with a mutation rate of 0.02/gamete/loci are necessary. We think this is a practical number. We now are looking for new minisatellite loci with spontaneous mutation rates greater than 0.02 for additional screening. Multilocus-minisatellite probes that detect many hypervariable minisatellite loci simultaneously and that also are being used for DNA fingerprinting may be suitable probes for our purpose.

Microsatellites consist of around 10–50 copies of motifs from 2 bp to 6 bp. They are highly polymorphic in the copy number of motifs, are randomly distributed in human DNAs, and occur frequently. The aberrant expansion of exonic trinucleotide repeats has recently been found to result in genetic diseases such as fragile X syndrome, myotonic dystrophy, spinobulbar muscular atrophy (SBMA), and Huntington's disease and represents a new form of mammalian mutagenesis. In the FMR-1 gene responsible for the fragile X syndrome, a CGG-repeat in the 5′-untranslated region is normally polymorphic, displaying alleles ranging from 6–54 repeats, but affected individuals have more than 200 copies. In addition, high spontaneous mutation rates (~0.013–0.015) at the tetranucleotide repeats in some of the genes in Caucasian families and instability of microsatellites in hereditary nonpolyposis colorectal cancer have been reported.

We examined the numbers of trinucleotide repeats in the *FMR-1, AR* (the gene responsible for SBMA), and *DM* genes of 124 children, the former two genes being on the X chromosome. Sequences including microsatellites were amplified by PCR and products were electrophoresed on a sequence gel. Bands from these children were compared with those of their parents. A total of 177 alleles from the children with triplet repeats derived from the exposed parents and 443 alleles derived from the unexposed parents were screened for mutations. No mutations were detected among 620 alleles in the children. We now are examining several tetranucleotide repeats and soon can determine whether these microsatellites are suitable targets.

Two-dimensional gel electrophoresis of DNA

The fourth technique that will be introduced into the pilot study is known as two-dimensional gel electrophoresis (2-DE). Using 2-DE, approximately 2000 restriction fragments of DNA from an individual can be separated on a gel. The beauty of this method is that no probes are necessary, and it can detect deletions and insertions, as well as base-pair substitutions at the restriction sites. Because these events generally occur only in one of the two chromosomes—the remaining chromosome being normal, spots of DNA fragments from affected individuals will be detected at the normal position with an intensity 50% less than the intensity of spots from unaffected individuals. Spots of DNA fragments resulting from deletions, insertions, or base-pair substitutions will disappear, increase their intensities, or move to other positions, respectively. Thus, to detect these events occurring in heterozygotes for an affected allele and a normal allele, accurate quantitation—ie, the ability to detect a 50% decrease or increase in the intensity of the spots— is necessary. The gel quality has reached a level such that the electrophoresis patterns derived from a single DNA sample exhibit distribution patterns of spots that can be superimposed.

Two-dimensional gel electrophoresis of DNA samples from three mother/father/child trios has been done in our laboratory by research scientist Jun-ichi Asakawa, and autoradiograms were analyzed using a computer-assisted image analyzer at the University of Michigan Medical School, where software developed for SM Hanash and James V Neel has proved to be effective in quantitating the intensity of the spots.

We hope to report on 2-DE analysis in an upcoming issue of *RERF Update*. ❑

A Message from the Reticulocytes

Using reticulocytes, the immature red blood cells, RERF researchers have validated earlier findings that radiation exposure induces a dose-dependent increase in somatic gene mutations in humans.

by Seishi Kyoizumi and Mitoshi Akiyama, Department of Radiobiology, RERF

In late January of 1986, RERF's Radiobiology Department was filled with the excitement of five scientists—Ron Jensen, then of Lawrence Livermore National Laboratory; Mike Bean, then a visiting scientist from Virginia Mason Research Center; RERF research scientist Nori Nakamura, and ourselves. We were plotting the glycophorin A (GPA) mutation data of atomic-bomb (A-bomb) survivors against tentative 1965 dosimetry (T65D) doses. Five month before—without providing information on radiation exposure, we had sent blood samples from 30 A-bomb survivors to Jensen and his coworkers, Rich Langlois and Bill Bigbee, who measured the GPA mutation frequencies of the survivors. Surprisingly, the plot in front of our eyes was showing a dose-dependent increase in mutations. Everybody in the room realized that this was the first evidence demonstrating radiation-induced somatic gene mutations in humans. In particular, Jensen was excited, because the data indicated that erythrocyte variants detected using his assay were induced by genotoxic agents such as radiation. A year later, these results were published in *Science* (236:445–8, 1987).

Since then, the GPA assay has become widely accepted as a reliable way of measuring past radiation exposure. At RERF, we established an improved GPA assay for use in our large-scale studies of A-bomb survivors and other radiation-exposed persons (*Cancer Res* 49:581–8, 1989).

Immature red blood cells provide RNA clues

However, one problem remained: we were not able to analyze mutated genes because erythrocytes lack nuclei. Therefore, the possibility that GPA-negative erythrocytes had been artifactually generated by antibody-staining errors lingered. During the intervening 7 years, biotechnology had advanced considerably, and polymerase chain reaction (PCR) made it possible to amplify DNA or RNA from a small amount of blood. Finally, we struck upon the idea of using reticulocytes, which are known to circulate in peripheral blood at a frequency of about 1% of total red cells and to contain various amounts of messenger RNA (mRNA). We suspected that a faint GPA "message" left in the reticulocytes could be amplified using PCR.

Because erythrocytes are replenished every 120 days, the origin of the radiation-induced GPA mutations in A-bomb survivors has to be the pluripotent stem cells from the bone marrow. Mature mutant erythrocytes differentiate from mutant CD34+ erythroid-committed progenitor cells (BFU-E) during the erythroblast and reticulocyte stages. Because expression of GPA molecules on the cell surface begins during the earlier erythroblast stage, we theorized that detection of later developing mutant reticulocytes would be possible.

To analyze GPA mutations in reticulocytes, we enriched the reticulocytes found in the peripheral blood using Percoll density gradient centrifugation. Afterwards, we confirmed by flow cytometry that 60%–70% of the cells in the low-density fractions consisted of reticulocytes expressing various amounts of mRNA.

As presented at the RERF Scientific Council meeting this year, we have developed a reverse transcription–polymerase chain reaction (RT-PCR) to detect GPA mRNA in reticulocytes. By using a two-step RT-PCR method, we can detect GPA mRNA extracted from as few as 100 reticulocytes from adult peripheral blood.

For preliminary analysis using this system, we analyzed Percoll-enriched reticulocytes from an A-bomb survivor who has an extremely high Nϕ mutant frequency (about 3000×10^{-6}). We sorted normal and Nϕ mutant reticulocytes from this survivor. Using RT-PCR, normal reticulocytes were shown to express both N- and M-type GPA mRNA by restriction-fragment-length-polymorphism (RFLP) analysis. In contrast, Nϕ mutant reticulocytes express only N-type GPA mRNA and lack M-type GPA mRNA.

Thus, we demonstrated for the first time that GPA mutants detected by a cell sorter are really genetic mutants and not antibody-staining artifacts. Also, these data suggest that A-bomb radiation induced nonproductive-type mutations in the GPA gene—most probably gene deletion. To confirm this assumption, we will expand this reticulocyte study to other survivors having high mutant frequencies.

In-vitro irradiation of erythroid progenitor cells

In a different approach, we are trying to induce GPA mutations in erythroblasts by means of in-vitro irradiation of erythroid progenitor cells (BFU-E). Preliminary data suggest that mutant erythroblasts can be induced by x-irradiation. Because the two-step RT-PCR method can detect GPA transcripts produced in a single erythroblast, we can analyze the GPA message in radiation-induced mutant erythroblasts at the single-cell level. We will report the results of molecular analyses of mutant erythroblasts in the near future.

On the basis of the results of this experimental system, we might be able to compare the in-vitro dose response with the survivors' dose response, as well as characterize in greater detail the molecular nature of GPA mutations in erythroblasts using reticulocytes from the A-bomb survivors. These systematic approaches may clarify the properties of radiation-induced GPA gene mutations at the molecular level. ❑

Investigating the Link between Liver Cancer and Hepatitis

Because hepatitis B and C infection are closely associated with hepatocellular carcinoma in Japan, RERF researchers are scrutinizing these viruses at the DNA level in cancerous and noncancerous liver tissue from atomic-bomb survivors.

by Terumi Mizuno, Toshio Seyama, and Mitoshi Akiyama, Department of Radiobiology, RERF

Several kinds of cancer occur with greater frequency among atomic-bomb (A-bomb) survivors than in the general Japanese population. The complexities of cancer development involve multiple stages, and researchers at RERF are investigating how radiation enters into this complicated picture. One way to tackle this perplexing problem is through a detailed analysis of various gene alterations that likely exist in tumors as characteristic DNA lesions left by ionizing radiation.

Why investigate the link between liver cancer and the hepatitis viruses?

Recently, Thompson et al reported a significant radiation dose response for primary liver cancer (PLC) among members of RERF's Life Span Study (*Radiat Res* 137:S17–S67, 1994). Etiological factors in liver-cancer development include the hepatitis B virus (HBV) and hepatitis C virus (HCV), alcohol consumption, and aflatoxin B1 exposure (Harris, *Can Cells* 2:146–148, 1990). In Japan, epidemiological studies suggest that more than 70% of PLC is caused by HBV and HCV infection. Thus, the frequency of viral infection cannot be ignored when investigating the role of A-bomb radiation in hepatocellular carcinoma (HCC). The analysis of these viruses in liver cancers among A-bomb survivors may give some insight concerning the role of radiation in the increased risk of liver cancers in the A-bomb survivors.

RERF researchers have long had an interest in the relationship between radiation exposure and liver cancer. Reporting here on only one aspect of this work, we analyzed the frequency of HBV infection in liver tissue using molecular biology techniques. Furthermore, we investigated how HBV participates in liver-cancer development and what relationship exists between HBV infection and other gene changes, such as oncogene activation and tumor-suppressor gene inactivation—the two types of events that are believed to be central to carcinogenesis.

Recently, it was shown that one of the HBV products, the HBX protein, can interact with the *p53* gene product, suppressing its function (Wang, *PNAS* 91:2230–4, 1994). A similar action occurs in cervical cancer in which human papillary viruses inactivate the *p53* gene function (Dyson, *Science* 243:934–7, 1989). Thus, the role of HBV in hepatocarcinogenesis may be via *p53* inactivation. The analytical method we used is the polymerase chain reaction (PCR) using DNA extracted from paraffin-embedded cancerous and noncancerous liver tissues. HBV is a 3.2-kilobase-pair DNA virus and the functional three domains—the S, pre-C, and X regions—were amplified by PCR. We examined 237 PLC tissue samples and 396 control nontumor liver-tissue samples. Amplification was successful in about 80% of the extracted DNA.

HBV found in genomic DNA more often among liver-cancer tissues

When PLC tissues and control tissues were compared, the HBV infection frequency was higher in the PLC group (16%) than in the control group (3%)—a statistically significant difference (see Table below). These findings suggest that HBV infection in liver cells is etiologically involved in human HCC and that one or more genetic events is needed to accomplish liver cell transformation to malignancy.

Among PLC patients, the frequency of HBV infections differed little between tumors and nearby normal liver tissue; ie, when tumor tissue was HBV-infected, the sur-

Table. Frequency of hepatitis B virus infection in cases of hepatocellular carcinoma (HCC) and in control cases by city and sex.

		HCC cases			Nontumor cases (controls)		
City	Sex	Total	Negative	Positive	Total	Negative	Positive
Both	Total	188	158	30 (16.0%)	396	358	11 (2.8%)
	Male	115	94	21 (18.3%)	266	261	5 (1.9%)
	Female	73	64	9 (12.3%)	130	124	6 (4.6%)
Hiroshima	Total	122	88	21 (17.2%)	289	281	8 (2.8%)
	Male	72	57	15 (20.8%)	186	183	3 (1.6%)
	Female	50	44	6 (12.0%)	103	98	5 (4.9%)
Nagasaki	Total	66	57	9 (13.6%)	107	104	3 (2.8%)
	Male	43	37	6 (14.0%)	80	78	2 (2.5%)
	Female	23	20	3 (13.0%)	27	26	1 (3.7%)

rounding normal tissue was also infected, and vice versa. Similarly, we found no clear differences by city of residence or sex.

Previously, Asano et al reported that hepatitis B surface (HBs) antigen positivity was twice as high in Nagasaki as in Hiroshima among city- and age-ATB–matched patients who did not have overt liver disease (*J Natl Cancer Inst* 69:1221–7, 1982).

The discrepancy between our findings and those of Asano et al might be attributed somehow to the fact that we examined the frequency of actual integration of viral genome in the genomic DNA of the liver cells rather than the existence of antibody in the serum.

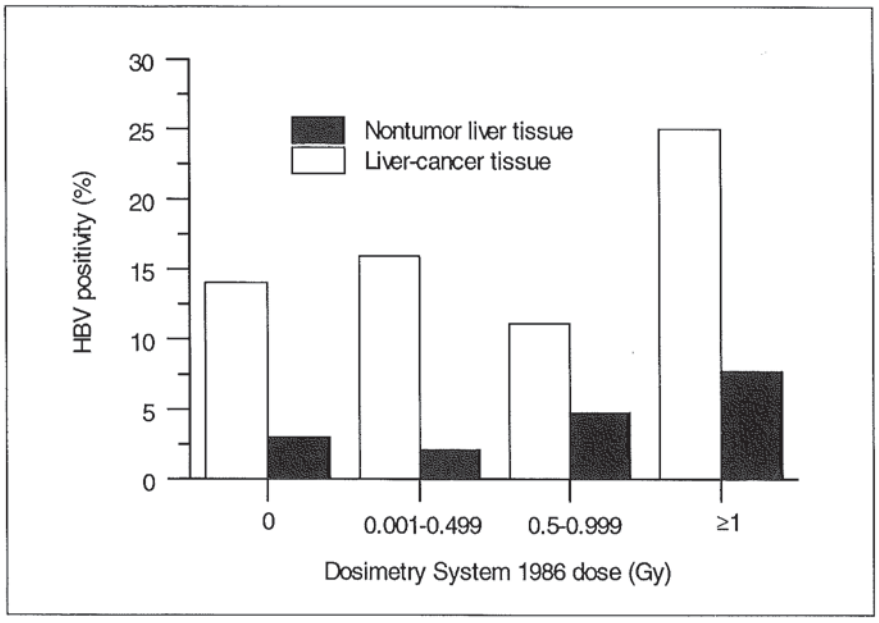

Figure. Frequency of hepatitis B virus (HBV) infection by Dosimetry System 1986 dose estimates.

Radiation exposure and HBV infection

What role, if any, radiation dose plays in susceptibility to infection by HBV remains unclear. Though there appears to be a trend, the correlation between HBV infection and A-bomb radiation does not reach statistical significance. Analysis of more cases from among the highly exposed survivors might strengthen this observed correlation.

Akiyama et al have observed a dose-dependent higher titer of Epstein–Barr virus (EBV) antibody among survivors (*Radiat Res* 133:297–302, 1993), possibly indicating that more frequent viral infections among survivors may result from reduced immunological competence caused by A-bomb radiation.

HCV genome detected in preserved tissue for the first time

Recently, using reverse transcription–PCR (RT-PCR) in paraffin-embedded liver tissues, we succeeded in detecting the HCV genome, whose molecular analysis in preserved tissues has long eluded researchers.

In the Radiobiology Department, during the next phase of our liver-cancer work, we will try to discern the frequency of HCV genomic material detectable in normal and cancerous hepatic tissues from A-bomb survivors.

Currently, in RERF's Department of Clinical Studies, a serological study of HCV among Adult Health Study participants is also being conducted.

From the standpoint of molecular epidemiology and molecular oncology, understanding the interactions of viral infection, oncogenes, and tumor-suppressor genes may bring into better focus the full picture of radiation-induced hepatocarcinogenesis. ❑

Cancer-incidence Data on Disk

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Hiroshima and Nagasaki tumor registries. Because of the availability of the leukemia-registry data, follow-up for the leukemia data set begins on 1 October 1950. The different number of survivors in the two data sets is due to the different starting dates for the follow-up periods. In our recently published principal analyses, case counts are limited to first primary cancers diagnosed in the registry catchment area.

To produce these data sets, data for individual survivors were stratified on city, sex, age at exposure (5-year intervals), calendar time, and dose. Dose categories in the leukemia data set are defined in terms of total bone-marrow dose, whereas dose categories in the solid-cancer table are defined using total dose to the colon. The leukemia data set is identical to that used in the analyses of Preston et al. The solid-cancer data set is identical to that used in the pooled analysis of all solid tumors and in the analyses of colon-cancer risks. However, other site-specific analyses in Thompson et al were based on data sets of the same basic form in which the data were grouped by the appropriate organ dose. It is impractical to distribute all of these data sets. However, as noted above, the disk contains city and age-at-exposure specific conversion factors that can be used to compute estimates of doses to other organs along with a command script that provides an explicit illustration of how one can use these factors to compute doses for other organs.

Each record in the main data files includes indicators of sex, city, organ dose category, age-at-exposure category, calendar period, and other factors. Doses are summarized by the mean values of the gamma and neutron organ doses and the mean RBE 10–weighted total organ dose, ie, $D_\gamma + 10(D_n)$.

The solid-tumor data set has 3249 records; the leukemia data set has 4896 records. The files are ASCII text files in which the records have a fixed format, and fields are separated by blanks so that they can easily be read into spreadsheets or other data-analysis programs.

Person interested in obtaining a copy of these data should contact the RERF Publication and Documentation Center, 5-2 Hijiyama Park, Minami-ku, Hiroshima, 732 Japan. Facsimile: 81-82-263-7279. E-mail (Internet): maruyama@rerf.or.jp. The cost of the disks is US$50. All files are included on two 3.5-inch 1.4-MB MS-DOS–formatted floppy disks. However, 5.25-inch disks can be made upon request. ❑

Dosimetry System 1986 Dose: Distance and Shielding

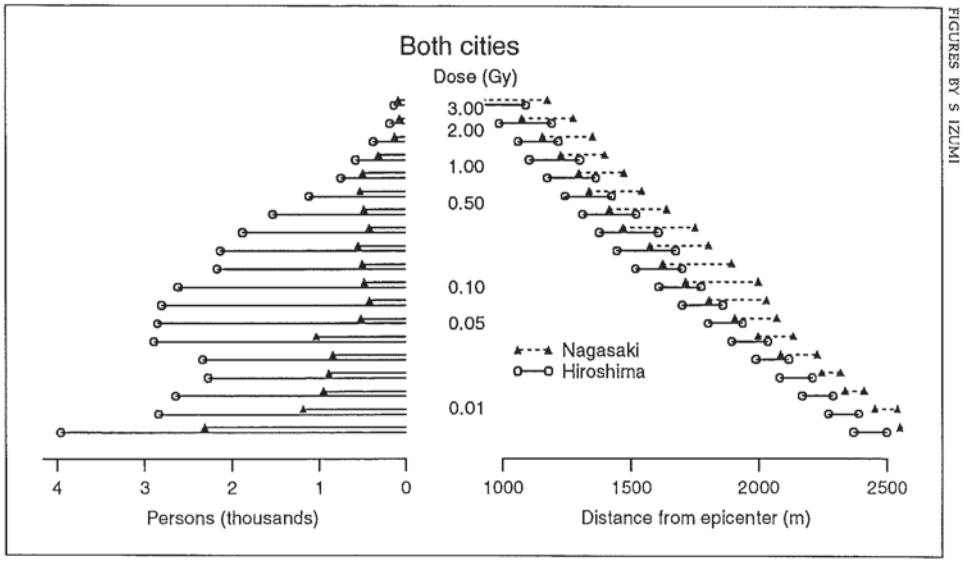
by S Izumi, D Pierce, and D Preston, Department of Statistics, RERF

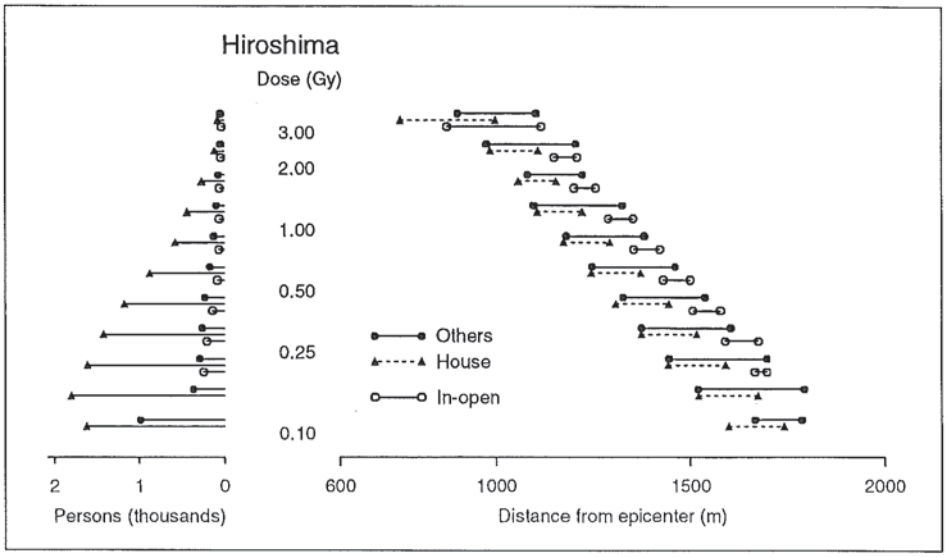
The distribution of slant distance from the epicenter for survivors at specified levels of shielded radiation dose is shown here. Descriptions are given by city and shielding category for colon doses. The top figure presents the data by city, whereas the bottom two figures show the data by shielding category for each city. Data on all Life Span Study survivors with doses greater than 5 mGy are used for the top figure, but only those survivors with doses greater than 0.1 Gy are used for the bottom two figures.

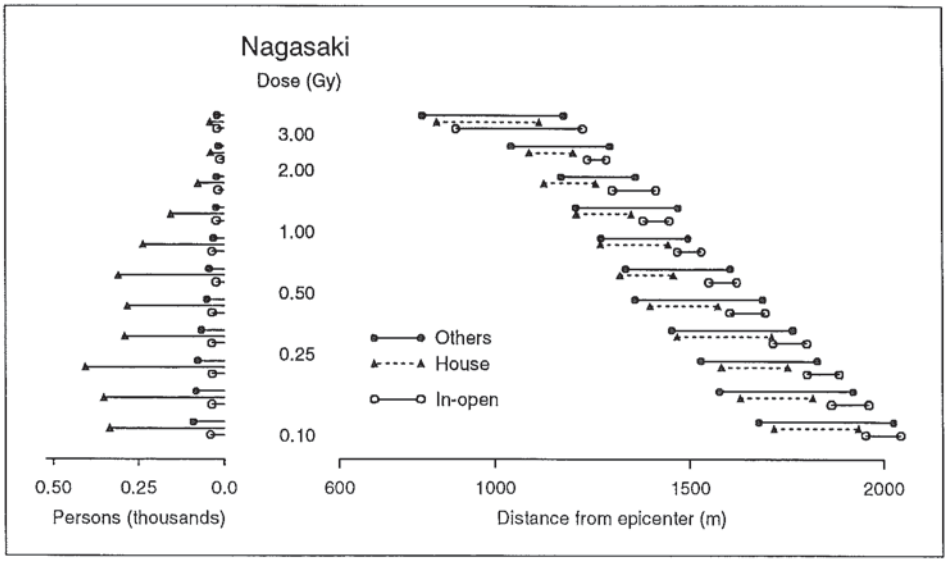
Dose categories were defined so the upper bound of each category equals the square root of two times its lower bound, corresponding to an increase of about 41%. The left panels indicate the number of survivors in each of the dose categories for each city. Line segments in the right panels represent the middle 80% (10th percentile to 90th percentile) of the distribution of slant distances.

Dose decreases more rapidly with distance in Hiroshima than Nagasaki, and the range of distances associated with a given dose category is somewhat greater in Nagasaki than Hiroshima. The plots indicate that Hiroshima survivors who received a dose ranging from 0.8 to 1.1 Gy were generally exposed at distances of between 1100 and 1300 m in Hiroshima and between 1250 and 1400 m in Nagasaki.

In the bottom two plots, we further stratify the data by shielding category within city. The data for each dose group are subdivided as follows: in the open and unshielded; inside a house or tenement; and any other shielding conditions. ❑







FIGURES BY S IZUMI

Recent Scientific Publications

Editor's note: As announced in the Summer 1993 issue of RERF Update, *the RERF Technical Report Series, begun in 1959, will be terminated after the processing of 1992 manuscripts is complete. Henceforth, summaries of journal articles based on approved RERF manuscripts will accompany the complete journal citation. Other selected summaries of interest will also be published occasionally. Variation in title or text styles reflects different journal styles. Reprints, when available, can be obtained from the RERF Publication and Documentation Center, 5-2 Hijiyama Park, Minami-ku, Hiroshima, 732 Japan. Facsimile: 81-82-263-7279. Internet address: maruyama@rerf.or.jp*

Publications in the Open Literature

Incidence of female breast cancer among atomic bomb survivors, 1950–1985. M Tokunaga, CE Land, S Tokuoka, I Nishimori, M Soda, S Akiba. *Radiat Res* 138:209–23, 1994.

An incidence survey among atomic bomb survivors identified 807 breast cancer cases and 20 second breast cancers. As in earlier surveys of the Life Span Study population, a strongly linear radiation dose response was found, with the highest dose-specific excess relative risk (ERR) among survivors under 20 years old at the time of the bombings. Sixty-eight of the cases were under 10 years old at exposure, strengthening earlier reports of a marked excess risk associated with exposure during infancy and childhood. A much lower but marginally significant dose response was seen among women exposed at 40 years and older. It was not possible, however, to discriminate statistically between age at exposure and age at observation for risk as the more important determinant of ERR per unit dose. A 13-fold ERR at 1 Sv was found for breast cancer occurring before age 35, compared to a 2-fold excess after age 35, among survivors exposed before age 20. This *a posteriori* finding, based on 27 exposed, known-dose, early-onset cases, suggests the possible existence of a susceptible genetic subgroup. Further studies, involving family histories of cancer and investigations at the molecular level, are suggested to determine whether such a subgroup exists.

A case-control interview study of breast cancer among Japanese A-bomb survivors. I. Main effects. CE Land, N Hayakawa, SG Machado, Y Yamada, MC Pike, S Akiba, M Tokunaga. *Cancer Causes and Control* 5:157–65, 1994.

Women with breast cancer (cases = 196) and without the disease (controls = 566), selected from the Life Span Study sample of A-bomb survivors and nonexposed residents of Hiroshima and Nagasaki, Japan, and matched on age at the time of the bombings, city, and estimated radiation dose, were interviewed about reproductive and medical history. A primary purpose of the study was to identify strong breast cancer risk factors that could be investigated further for possible interactions with radiation dose. As expected, age at first full-term pregnancy was strongly and positively related to risk. Inverse associations were observed with number of births and total, cumulative period of breast feeding, even after adjustment for age at first full-term pregnancy. Histories of treatment for dysmenorrhea and for uterine or ovarian surgery were associated positively and significantly with risk at ages 55 or older, a finding that requires additional study. Other factors related to risk at older ages were the Quetelet index (weight [kg]/height [cm]^2) at age 50, history of thyroid disease, and hypertension. Neither age at menarche nor age at menopause was assocated significantly with risk. Subjects appeared to be poorly informed about history of breast cancer or other cancer in themselves or in their close relatives; this finding suggests that innovative strategies may be required when studying familial cancer patterns in Japanese populations.

A case-control interview study of breast cancer among Japanese A-bomb survivors. II. Interactions with radiation dose. CE Land, N Hayakawa, SG Machado, Y Yamada, MC Pike, S Akiba, M Tokunaga. *Cancer Causes and Control* 5:167–76, 1994.

Three breast cancer risk factors were evaluated in terms of their interactions with radiation dose in a case-control interview study of Japanese A-bomb survivors. Cases and controls were matched on age at the time of the bombings and radiation dose, and dose-related risk was estimated from cohort rather than case-control data. Each factor—age at first full-term pregnancy, number of deliveries, and cumulative lactation period summed over births—conformed reasonably well to a multiplicative interaction model with radiation dose (the additive interactive model, in which the absolute excess risk associated with a factor is assumed to be independent of radiation dose, was rejected). An important implication of the finding is that early age at first full-term pregnancy, multiple births, and lengthy cumulative lactation are all protective against radiation-related, as well as baseline, breast cancer. Analyses by age at exposure to radiation suggest that, among women exposed to radiation in childhood or adolescence, a first full-term pregnancy at an early age *following* exposure may be protective against radiation-related risk.

Relationship of five anthropometric measurements at age 18 to radiation dose among atomic bomb survivors exposed *in utero*. E Nakashima. *Radiat Res* 138:121–6, 1994.

Five body measurements—standing height, body weight, sitting height, chest circumference and intercristal diameter—of 18-year-old atomic bomb survivors exposed *in utero* in Hiroshima and Nagasaki were analyzed in relation to DS86 uterine dose. Age *in utero* was divided into four periods: 0–7, 8–15, 16–25 and ≥26 weeks. This categorization is based upon the study of radiation-induced brain damage. The linear regression analyses for these five variables showed significant decreases with increasing dose. The regression coefficients were –2.65 cm/Gy for standing height, –2.46 kg/Gy for body weight, –0.92 cm/Gy for sitting height, –1.37 cm/Gy for chest circumference and –0.32 cm/Gy for intercristal diameter. The multivariate test statistic for the overall dose effect on five body measurements was significant, but the interaction between dose and gestational period was not significant. Principal-component analysis was applied to the five variables. For the first-component scores, the dose effect was significant, but the interaction between dose and gestational period was not significant. For the second-component scores, the dose effect was significant specifically at 0–7 weeks. The radiation dose effect on the second principal component found at 0–7 weeks of gestation suggests that malformations occur in this period.

Aneuploidy in somatic cells of in utero exposed A-bomb survivors in Hiroshima. K Ohtaki, R Sposto, Y Kodama, M Nakano, AA Awa. *Mutat Res* 316:49–58, 1994.

Cytogenetic data on cultured lymphocytes of the in utero exposed A-bomb survivors in the RERF Adult Health Study cohort have been analyzed using the G-banding technique to determine the frequency of aneuploid cells. The data consist of blood samples collected between 1985 and 1987 from 264 Hiroshima individuals for whom DS86 maternal uterine dose estimates are available: 124 proximally exposed (74 males and 50 females) with an estimated dose of 0.005 Sv or more, and 140 distally exposed (76 males and 64 females) with a dose estimate of 0 Sv, assuming the neutron relative biological effectiveness (RBE) of 10.

continued on next page

Recent Scientific Publications

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A main feature of aneuploidy was that aneuploid frequency in autosomes depended generally on chromosome length; aneuploidies were significantly more frequent in shorter chromosomes than in longer chromosomes. The frequency of aneuploidies also depended on type, with chromosome loss approximately five times more frequent than chromosome gain. However, chromosome 21, as well as the sex chromosomes, were notable in that aneuploidy was much more frequent for these chromosomes than would be predicted from a simple relationship with length. X chromosome aneuploidies were significantly more frequent in females than in males. There was no dependence of aneuploid frequencies on dose when measured 40 years after the exposure.

p53 mutations in lung cancers from non-smoking atomic-bomb survivors. Y Takeshima, T Seyama, WP Bennett, M Akiyama, S Tokuoka, K Inai, K Mabuchi, CE Land, CC Harris. *Lancet* 342:1520–1, 1993.

Tobacco smoke contains many carcinogens and has been linked with the development of lung cancer. We sequenced the conserved regions of the p53 tumour suppressor gene in lung cancers from 17 nonsmokers from Hiroshima, Japan; 9 were atomic-bomb survivors. The mutations were predominantly transitions (all G:C to A:T); there were no G:C to T:A transversions. By contrast, lung cancers from 77 Japanese smokers have a predominance of G:C to T:A transversions in which the guanine residues occur on the non-transcribed DNA strand. These findings further implicate tobacco smoke carcinogens in the molecular pathogenesis of lung cancer.

Establishment of two human thyroid carcinoma cell lines (8305C, 8505C) bearing p53 gene mutations. T Ito, T Seyama, Y Hayashi, T Hayashi, K Dohi, T Mizuno, KS Iwamoto, N Tsuyama, N Nakamura, M Akiyama. *Int J Oncology* 4:583–6, 1994.

New cell lines, designated 8305C and 8505C, were established from undifferentiated thyroid carcinomas of a 67-year-old female patient and a 78-year-old female patient, respectively. Pathologically both of these primary undifferentiated carcinoma tissues contained residual well differentiated components, suggesting well differentiated to undifferentiated carcinoma progression. Cell kinetic analysis indicates that the cell population doubling time is 43 h for 8305C and 36 h for 8505C. The saturation density at confluency is 5.7×10^4 cells/cm^2 for 8305C and 1.1×10^5 cells/cm^2 for 8505C. To identify genetic changes that may have occurred in these two cell lines, tumor suppressor genes p53, Rb, APC and MCC were analyzed. Sequence analysis confirmed a C:G to T:A transition at the first base of p53 gene codon 273 in 8305C and a C:G to G:C transversion at the first base of p53 codon 248 in 8505C. Polymerase chain reaction–loss of heterozygosity assays confirmed allelic deletion of the p53 gene from the 8505C cell line. Loss of heterozygosity of other tumor suppressor genes was not observed. Given that p53 mutations associate with undifferentiated carcinoma but not with well differentiated carcinoma during multistep carcinogenesis of the thyroid, these cell lines should prove useful for research into the role of p53 gene mutations in malignant transformation.

Immune responses to Epstein–Barr virus in atomic bomb survivors: study of precursor frequency of cytotoxic lymphocytes and titer levels of anti-Epstein–Barr virus-related antibodies. Y Kusunoki, S Kyoizumi, Y Fukuda, H Huang, M Saito, K Ozaki, Y Hirai, M Akiyama. *Radiat Res* 138:127–32, 1994.

Precursor frequencies of cytotoxic lymphocytes to autologous Epstein–Barr virus-transformed B cells and serum titers of anti-Epstein–Barr virus-related antibodies were measured in 68 atomic bomb survivors to clarify the immune mechanism controlling Epstein–Barr virus infection. The precursor frequency was negatively correlated with the titer of anti-early antigen IgG, which is probably produced at the stage of viral reactivation. A positive correlation between the precursor frequency and titer of anti-Epstein–Barr virus-associated nuclear antigen antibody was also observed, indicating that the precursor frequency reflects the degree of *in vivo* destruction by T cells of the virus-infected cells. These results suggest that T-cell memory specific to Epstein–Barr virus keeps the virus under control and that the precursor frequency assay is useful for the evaluation of immune responses to Epstein–Barr virus. However, no significant effect of atomic bomb radiation on the precursor frequency was observed in the present study, probably due to the limited number of participants.

***Hae*III polymorphism in intron 1 of the human *p53* gene.** T Ito, T Seyama, T Hayashi, T Mizuno, KS Iwamoto, N Tsuyama, K Dohi, N Nakamura. *Hum Genet* 93:222, 1994.

A new *Hae*III polymorphism, which is found in the first intron of the human *p53* gene, provides a genetic marker for tumor suppressor *p53* gene alterations.

Early-onset breast cancer in A-bomb survivors. CE Land, M Tokunaga, S Tokuoka, N Nakamura. *Lancet* 342:237, 1993 (letter to the editor).

Meeting report: A nomenclature for classifying aberrations detected by chromosome painting. AA Awa. *J Radiat Res* (Tokyo) 34:303–4, 1993 (letter to the editor).

In-utero exposed atomic bomb survivors: cancer risk update. Y Yoshimoto, RR Delongchamp, K Mabuchi. *Lancet* 344:345–6, 1994 (letter to the editor). ❑

RERF update RERF

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