Meeting Report

Japan–US Cooperative Cancer Research Seminar on molecular epidemiological characteristics of lung and colon cancer development among atomic-bomb survivors, Bethesda, USA, February 23–24, 2006

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This seminar was held as an activity of the Japan–US Cooperative Cancer Research Program for a research project titled Molecular Epidemiological Characteristics of Lung and Colon Cancer Development among Atomic-bomb Survivors (Japanese Principal Investigator, Kei Nakachi and US Principal Investigator, Curtis C. Harris; duration, April 1, 2004 –March 31, 2006), supported by both the Japan Society for Promotion of Science and the US National Cancer Institute (NCI).

Mechanistic understanding of radiation-associated carcinogenesis is indispensable for the establishment of cancer prevention for those exposed to excess radiation, including atomic-bomb survivors. This Japan–US Cooperative Cancer Research Seminar was held to investigate future directions of molecular epidemiology studies on radiation-associated cancer, on the basis of the data obtained thus far from studies in progress. In the opening address, Dr Curtis Harris (NCI, USA) welcomed and thanked all the participants on behalf of the Japan–US Cooperative Cancer Research Seminar. Dr Harris then provided a brief explanation of the purpose of the seminar, specifically emphasizing the promotion of cancer research based on cooperation from scientists in the USA and Japan. Dr Eiichi Tahara (Radiation Effects Research Foundation [RERF], Japan) also thanked the participants and extended his gratitude to Dr Harris and the staff of his laboratory for organizing and hosting the seminar.

Overview of molecular epidemiology studies among atomic-bomb survivors

Dr Kazue Imai (RERF, Japan) gave an overview of the occurrence of cancer and other diseases among atomic-bomb survivors in relation to their past exposure to atomic radiation. Using data collected by the RERF Epidemiology Department from over 50 years of follow-up studies on 120 000 subjects, Dr Imai indicated that increased risk of cancer is to date the most important late effect of radiation exposure. Following excess leukemia seen in the early years after atomic-bomb exposure, significant excess risk of selected solid cancers, including cancers of the stomach, colon, liver, bladder, lung, breast, ovary, thyroid, and skin, have also been observed. Although organ-specific risk due to radiation exposure differs by sex and age at exposure, excess relative risk per sievert (Sv) for death from all solid cancers combined is estimated to be about 0.5 for those who were 30 years old at exposure. Importantly, there is still excess risk for many solid cancers among radiation-exposed survivors even 60 years after the atomic bombings. Unfortunately, however, the mechanisms underlying these epidemiological findings remain unclear.

Based on the cancer data of atomic-bomb survivors explained by Dr Imai, Dr Kei Nakachi (RERF, Japan) outlined the molecular epidemiology studies currently being carried out at RERF, emphasizing the need for a mechanistic understanding of health effects of radiation exposure, not only for disease prevention among atomic-bomb survivors but also for establishing prevention strategies for radiation-associated cancers and other diseases in general. The prevention of radiation-associated diseases, cancers in particular, is an important issue for many people, such as patients undergoing radiation therapy, those occupationally or accidentally exposed to excess radiation, and even to people in the general population exposed to diagnostic X-rays. In order to better understand the mechanisms and prevention of radiation-related diseases, RERF Radiobiology/Molecular Epidemiology Department is currently using three investigative approaches: immunology, immunogenomics, and molecular oncology.

Evidence has recently accumulated supporting the idea that many lifestyle-associated diseases such as coronary heart disease, diabetes mellitus, and even cancer are associated with immunological alterations of the host (e.g. chronic inflammatory responses and disturbances of immunosurveillance systems). This association has been investigated on the basis of the altered immunity found among atomic-bomb survivors, which can be observed even now, 60 years after the bombings. Immunology and immunogenome studies include: (i) T-cell repertoire and clonal expansions; (ii) T-cell functions and inflammatory response; and (iii) association between cancer development and immune-related gene polymorphisms.

Molecular oncology studies using thyroid, lung, and colorectal cancer tissue of atomic-bomb survivors have recently begun at RERF with the intent of discovering critical molecular events that may be observed in these cancers of atomic-bomb survivors, but that are also found in sporadic (not radiation-associated) cancers. This study may therefore underpin the molecular characteristics of radiation-associated carcinogenesis.

Dr Nakachi then gave a brief report on two studies selected from current RERF molecular epidemiology research: acceleration of immunological aging by past atomic-radiation exposure; and genome analysis on cancer immunosurveillance. In general, T-cell immune functions attenuate with aging; however, persistent

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inflammation enhances with aging. These two opposite processes are believed to be in part responsible for the occurrence of various aging-related diseases. Past radiation exposure appears to further enhance this aging-associated attenuation of T-cell functions among atomic-bomb survivors as well as aging-associated increases of subclinical persistent inflammation levels as measured by various inflammatory markers. The effect of past atomic-bomb radiation exposure has been assessed as a function of aging; the relative increase of inflammatory markers per Gy of exposure has been estimated to be roughly equivalent to an increase in immunologic age of 9 years. Another study Dr Nakachi described was a genome approach undertaken in a cohort study among the general population to identify genetic factors involved in cancer immunosurveillance. Strong associations were found that linked natural cytotoxic activity of peripheral lymphocytes, a marker for cancer immunosurveillance capacity, to the haplotypes of the *NKG2D* gene, which encodes an activating homodimeric C-type lectin receptor that triggers cell-mediated cytotoxicity in natural killer cells. The *NKG2D* haplotypes will be investigated among atomic-bomb survivors in relation to innate immunity and development of radiation-associated cancer.

Lung cancer – radiation-associated and sporadic

Dr Michael Alavanja (NCI, USA) explained that tobacco smoke and ionizing radiation might share a common genotoxic mechanism, inducing oxidative stress by transmitting or generating reactive oxygen species (ROS). Dr Alavanja hypothesized that glutathione-S-transferase M1 (*GSTM1*) null homozygotes would have decreased ability to neutralize ROS, which might increase susceptibility to lung cancer. A case-only design was used with 271 archival tissue samples and radon concentration and secondhand smoke (SHS) exposure data among never-smokers from lung cancer cases pooled from three previously completed case-control studies. Radon concentrations of more than 120.62 Bq/m^3 were associated with a greater than threefold risk of lung adenocarcinoma: odds ratio (OR) = 3.4 (95% confidence interval [CI], 1.1–10.6) for *GSTM1* null homozygotes compared with *GSTM1* carriers; the linear trend was significant (*P* for trend = 0.03). SHS exposure among never-smokers revealed OR = 2.2 (95% CI, 1.1–4.3). Dr Alavanja emphasized that genetic predisposition factors are probably more important in low-dose carcinogenesis such as with SHS and radon exposure in home settings. Additionally, these findings supported the hypothesis that radon and SHS promoted neoplasia through shared elements of a common pathway.

To investigate the mechanisms of radiation-associated lung cancer, we need to understand the mechanisms of sporadic (not radiation-associated) lung cancer among the general population. Dr Leah Mechanic (NCI, USA) examined associations between sporadic lung cancer and 14 *TP53* polymorphisms, including haplotype tagging and coding single nucleotide polymorphisms (SNPs), in an ongoing NCI–Maryland Lung Cancer case-control study, and subjects in a case-only study from the greater Baltimore area were genotyped. Of these polymorphisms, *TP53_01* (Arg72Pro) and *TP53_11* (T>G, IVS7+92) were associated with increased risk of lung cancer in African Americans. Individuals with combined *TP53_01* (Arg/Pro or Pro/Pro) and *TP53_11* (T/G or G/G) genotypes had elevated risk of lung cancer, with OR = 3.4 (95% CI, 1.4–8.0). Moreover, individuals with Pro-T-A-G haplotypes of the combined *TP53* polymorphisms *TP53_01*, *TP53_65*, *TP53_66* and *TP53_16* had increased risk of lung cancer, with OR = 1.8 (95% CI, 1.0–3.2), compared to those with Arg-T-A-G haplotypes. In contrast, haplotypes of the combined *TP53* polymorphisms *TP53_01*, *TP53_34*, *TP53_66*, and *TP53_16* were found in Caucasians; there was some evidence of interaction of *TP53* SNPs with smoking. The *TP53* Arg72Pro polymorphism was shown to modulate p53 transcription-independent apoptosis, through more effective mitochondrial localization. In this case-only study, *TP53* haplotypes were associated with increased *TP53* somatic mutation frequency in lung cancer in Caucasians. None of the individual polymorphisms, or *TP53* haplotypes, was associated with lung cancer survival. The association between *TP53* haplotypes and lung cancer will be further studied in radiation-associated lung cancer.

The RERF epidemiology studies have indicated that excess relative risk of lung cancer among atomic-bomb survivors remained high even 60 years after the atomic bombings. Dr Masataka Taga (research associate, NCI, USA, and RERF, Japan) reported on the current progress of a molecular oncology study on lung cancer among atomic-bomb survivors, which has been carried out in collaboration between the Department of Radiobiology/Molecular Epidemiology, RERF, and the Laboratory of Human Carcinogenesis, NCI, since 2004. *P53* (exons 5–8), *EGFR* (exons 18, 19 and 21), and *K-ras* (codons 12, 13 and 61) gene mutations were examined in archival cancer and adjacent non-cancer tissue samples from 44 nonexposed patients and 28 exposed patients among atomic-bomb survivors. The mutations were further analyzed in relation to clinicopathological factors including atomic-bomb radiation dose. Radiation-exposed patients showed a higher frequency of *p53* mutation than nonexposed patients, although the difference was not statistically significant. Median radiation dose among lung cancer patients with *p53* mutation of either G:C > T:A transversion or deletion was significantly higher than that among patients with other types of *p53* mutations or that among patients without *p53* mutations. Of 15 exposed patients with lung adenocarcinoma or squamous cell carcinoma, two patients were found to have *p53* double mutations. In contrast, *EGFR* (or *K-ras*) mutations did not show association with radiation exposure. The results from this preliminary analysis support the necessity of extended study with an increased number of patients, specifically those exposed to high doses of radiation, along with analysis of other molecular events.

Dr Naoko Sueoka (Saga University, Japan) reported on *EGFR* mutations within sporadic lung cancer of Japanese patients. *EGFR* mutations in lung cancer have been recently reported to be a useful predictive marker for gefitinib (an EGFR tyrosine kinase inhibitor) response, because of their involvement in lung carcinogenesis, as shown by a transgenic mouse model with *EGFR* mutations. *EGFR* mutations were observed in 34 of 97 patients (surgical specimens from 81 patients and pleural effusions from 16 non-resectable lung cancer patients). *EGFR* mutations were comprised of one mutation in exon 18, 18 mutations in exon 19, and 15 mutations in exon 21. Thirteen of the 15 patients with exon 21 mutations were female non-smokers whose pathological type was adenocarcinoma with bronchioloalveolar carcinoma (BAC). The 18 patients with exon 19 mutations included eight male current or former smokers. BAC was observed in 11 of these patients, which was less frequent than for the exon 21 mutations. These results of *EGFR* mutations in exon 21 and exon 19 suggest different mutagenesis and carcinogenesis pathways involving *EGFR*. Seven patients with *EGFR* mutations were treated with 250 mg gefitinib per day, and all responded to the treatment. Next, genetic host factors involved in occurrence of *EGFR* mutations were reported with specific reference to relationships between *EGFR* mutations and the length of a CA-repeat in intron 1. Allelic distribution of *EGFR* CA-repeat in lung cancer patients showed that 16 and 20 CA-repeats were frequently observed. *EGFR* mutations were more frequently observed in female non-smokers with fewer CA-repeats. In addition, on dividing the study patients into three groups by number of CA-repeats (i.e. 16 or fewer, 17–19, 20 and more), *EGFR* mRNA levels in normal adjacent tissue with 16 or fewer CA-repeats were found to be higher than those of the other groups.

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Dr Yun-Ling Zheng (Georgetown University, USA) reported on relationships of γ-radiation-induced G2/M arrest and DNA repair capability as assessed in an ongoing case-control study on lung cancer, measured by quantifying bleomycin-induced chromosome breaks in cultured peripheral lymphocytes of study subjects. The mean percentage of γ-radiation (1 Gy)-induced G2/M arrest was significantly lower in cases than in both the general population group and the hospital control group. When dichotomized at the 50th percentile value in combined control groups, a lower level of γ-radiation-induced G2/M arrest was associated with increased risk of lung cancer among African-Americans with adjusted OR = 2.25 (95% CI, 0.97–5.20). In addition, the mean number of bleomycin-induced breaks per cell was significantly higher in the case group than the control groups. These results imply that both bleomycin-induced chromosome breaks and less-efficient G2/M arrest induced by γ-radiation are associated with increased risk of lung cancer among African-Americans, although the association was weaker in Caucasians.

Dr Peter Shields (Georgetown University, USA) talked about nicotine addiction and tobacco-related health effects. Nicotine is a powerful drug influencing the functions of various neurohormones such as dopamine, norepinephrine, acetylcholine, vasopressin, serotonin, and beta-endorphin. He rightfully pointed out that overcoming nicotine addiction may well hold the key to smoking cessation; current treatments of nicotine addiction include counseling, nicotine replacement therapy, bupropion (zyban), and other methods. Several studies on cigarette smoking and genetics were outlined, focusing on polymorphisms in the human dopamine transporter (*DAT1*) gene and the dopamine receptor (*DRD2*) gene. A case(smokers)-control(non-smokers) study revealed that smoking status was greatly dependent on *DRD2* genotype; a cohort of high school students also showed that the risk of progressing from "puffing" to habitual smoking involved this genotype. A clinical trial using bupropion to promote smoking cessation also showed greater OR for the combined genotype of *DAT1* and *DRD2* than that for use of bupropion versus a placebo. These findings suggest that future antismoking intervention will need to consider genetic factors of individual smokers.

Colorectal, thyroid, and stomach cancers found in atomic-bomb survivors

Epidemiological findings from the RERF cohort study of atomic-bomb survivors have revealed that colon cancer showed significant risk elevation, while rectum cancer did not. Microsatellite instability (MSI) reflects major genomic instability; MSI is associated with proximal sites of the sporadic colon carcinogenesis. Dr Hidetaka Eguchi (RERF, Japan) reported on the current progress being made in a molecular oncology study on colorectal cancer among atomic-bomb survivors, focusing on MSI in relation to atomic-radiation exposure. This study uses archival cancer and adjacent non-cancer tissue samples collected from 10 nonexposed and 27 exposed patients. MSI-positive colorectal cancer was frequently observed at proximal sites in female patients, and this was also the case in sporadic colorectal cancer. The median radiation dose in MSI-positive colorectal cancer patients was significantly higher than that in MSI-negative patients; the median age at the time of atomic bombing or diagnosis in MSI-positive patients was significantly lower than that in MSI-negative patients. The latter finding seems to be in accordance with the previous finding that colorectal cancer among atomic-bomb survivors showed higher excess relative risk among those exposed to atomic-bomb radiation at younger ages. The results from this preliminary study suggest a possible association between radiation exposure and MSI status in colorectal cancer occurring among atomic-bomb survivors. However, further analyses with larger numbers of patients will be needed.

Dr Kiyohiro Hamatani (RERF, Japan) reported on current progress in a study of thyroid papillary cancer (PTC) among atomic-bomb survivors, in view of epidemiological data that this cancer was strongly associated with radiation exposure. Archival cancer and adjacent non-cancer tissue samples from 56 radiation-exposed (≥ 5 mGy) survivor patients and 29 nonexposed patients with PTC were analyzed, in terms of the initial events in thyroid papillary carcinogenesis, *RET* rearrangements (*RET*/*PTC*) and $BRAF^{V600E}$ mutation. The median radiation dose among PTC patients with *RET*/*PTC* was significantly higher than that among patients without *RET*/*PTC*. However, the median radiation dose among the patients with $BRAF^{V600E}$ mutation was significantly lower than that among patients without this mutation. Furthermore, the median latency period (time period elapsed from atomic bombing to diagnosis) and the age at diagnosis in exposed patients with $BRAF^{V600E}$ mutation were significantly longer and higher, respectively, than those without the mutation; no association was found between age at the time of atomic bombing and $BRAF^{V600E}$ mutation. *RET*/*PTC* rearrangements and $BRAF^{V600E}$ mutation were mutually exclusive. These results suggest that the initial development of either *RET*/*PTC* rearrangements or *BRAF* mutation may be influenced by atomic-radiation exposure, although each of them activates the RET/RAS/BRAF MAPK signaling pathway.

Dr Tomonori Hayashi (RERF, Japan) described recent findings on stomach cancer development among atomic-bomb survivors, in terms of interaction between immunological host factors and radiation exposure. Immunological host protection is considered to play an important role not only in the development of virus-related cancer, but also in carcinogenesis in general. However, exposure to radiation affects the immune system of the host in a dose-dependent manner. Case-control studies within a subcohort of the RERF Adult Health Study were conducted on the basis of immune-related gene polymorphisms. *HLA-A*2601* genotype was associated with a low risk of stomach cancer among those who were not exposed to atomic radiation, although atomic-bomb survivors with this genotype had a markedly enhanced risk of stomach cancer with radiation dose. Another case-control study conducted in parallel with this *HLA* study revealed an association between inflammation-related gene polymorphisms and stomach cancer development. Although overall risk of stomach cancer significantly increased with increased radiation dose, this risk was greatly modulated in both nonexposed and radiation-exposed survivors by interleukin 10 (*IL-10*) haplotypes, which makes it possible to identify people at high risk of radiation-associated stomach cancer. This study also demonstrated that plasma levels of IL-10 among individuals, which increased with past radiation dose and varied by *IL-10* haplotype, indicated stomach cancer risk of individual survivors as a promising surrogate marker of this cancer.

Mechanisms of radiation effects – *in vitro* studies

Dr Simon Powell (Washington University, USA) focused on the role of p53 as a central regulator of homologous recombination (HR) and replication, independent of its transactivation activity. By employing a plasmid-based HR assay in p53-null H1299 lung carcinoma cells, the HR-suppressing properties of a panel of p53 mutants, which varied in ability to interact with human replication protein A (RPA), were studied. Both wild-type p53 and a transactivation-deficient p53 mutant suppressed HR and prevented RPA from binding to single-stranded DNA both *in vitro* and *in vivo*. Conversely, p53 mutations that specifically disrupt the RPA binding domain without compromising the p53 transactivation function did not affect HR or RPA binding to single-stranded DNA. Suppression of HR was also lost with missense mutations in the p53 core domain, which retained the ability to interact with RPA, suggesting that additional binding

interactions of p53, for example with Rad51 or recombination intermediates, can also impact HR.

After substituting endogenous RPA2 either with an intact myc-tagged analog (RPA2Wt), or with a phosphomimetic or a phosphorylation-deficient (RPA2D or RPA2A) myc-tagged mutant, UV treatment led to dissociation of the p53-RPA complex and an increase of p53 transcriptional activity in the cells expressing RPA2Wt or RPA2D. RPA2A expression, in contrast, stabilized the p53-RPA complex and attenuated transcriptional activation of wild-type p53, although RPA2A failed to abolish transcriptional activation of p53 mutated within the RPA binding domain. These findings confirmed that the p53–RPA interaction was critical for this regulation, and that the interaction was regulated by RPA phosphorylation; RPA phosphorylation regulated p53 transcriptional activity only in S-phase. Finally, the magnitude of suppression of HR by p53 was greater for replication-associated HR, relative to double-strand break (DSB)-induced HR. These data suggest that p53 sequestering by RPA regulates p53 transcriptional activity during S-phase, and that p53 binding to RPA regulates HR in S-phase. The role of p53 in maintaining genome stability might be significantly mediated by the p53–RPA interaction in S-phase.

Dr Tom Hei (Columbia University, USA) reported on recent *in vitro* studies on low-dose radiation and the "bystander" response. Heritable biological effects had been thought to require direct damage to DNA, typically demonstrated by increased frequency of mutations (e.g. *CD59* mutations) with increased number of cell-traversing α-particles. The bystander effect involves induction of biological effects in cells not directly traversed by a charged particle but in close proximity to cells that are; observation of this effect represents a paradigm shift in understanding of radiation biology and target theory. The bystander effect was demonstrated by chromatid damage within primary human bronchial epithelial cells not directly hit by traversing α-particles generated by a microbeam irradiation, but that simply neighbor these cells that are directly hit. Multiple pathways were involved in the bystander phenomenon, and different cell types responded differently to bystander signaling. In confluent monolayers, gap junction communication played a crucial role in mediating the bystander effect. Cells without connexin gap junction protein showed no bystander response, and p53 thus may not be essential. However, reactive oxygen and reactive nitrogen species have been implicated as mediating molecules in subconfluent cultures. A recent study suggests that the cyclooxygenase-2 (COX-2) signaling cascade plays an essential role in the bystander process. Treatment of bystander cells with NS-398, a COX-2 inhibitor, significantly reduced the bystander effect including hypoxanthine phosphoribosyl transferase (HPRT) mutation. Furthermore, up-regulation of the extracellular signal-related kinases (ligands tumor necrosis factor-α, transforming growth factor-β, insulin-like growth factor, IL-1, and IL-8) and mitogen-activated protein kinases (specifically Erk and p38) appear to represent important intercellular signaling events of the bystander phenomenon. The implication of bystander observations is that the relevant target for various radiobiological endpoints is far larger than the individual cell, suggesting a need to reconsider the validity of linear extrapolation in calculating risk estimates for low-dose radiation exposure.

Concluding remarks

All participants at this seminar agreed with the necessity of promoting further collaboration between Japanese and US researchers, to understand the effects of radiation and the mechanisms of cancer induction for the benefit of all mankind. The atomic bombings have taken many lives and ineffably marked the bodies and minds of survivors. Our responsibility is to learn from this tragedy and to contribute to the future prevention and control of radiation-associated cancer.

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