Monitoring Exposure to Atomic Bomb Radiation by Somatic Mutation

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Atomic bomb survivors are a population suitable for studying the relationship between somatic mutation and cancer risk because their exposure doses are relatively well known and their dose responses in terms of cancer risk have also been thoroughly studied. An analysis has been made of erythrocyte glycophorin A (GPA) gene mutations in 1,226 atomic bomb survivors in Hiroshima and Nagasaki. The GPA mutation frequency (Mf) increased slightly but significantly with age at the time of measurement and with the number of cigarettes smoked. After adjustment for the effect of smoking, the Mf was significantly higher in males than in females and higher in Hiroshima than in Nagasaki. All of these characteristics of the background GPA Mf were in accord with those of solid tumor incidence obtained from an earlier epidemiological study of A-bomb survivors. Analysis of the dose effect on Mf revealed the doubling dose to be about 1.20 Sv and the minimum dose for detection of a significant increase to be about 0.24 Sv. No significant dose effect for difference in sex, city, or age at the time of bombing was observed. Interestingly, the doubling dose for the GPA Mf approximated that for solid cancer incidence (1.59 Sv). And the minimum dose for detection was not inconsistent with the data for solid cancer incidence. The dose effect was significantly higher in those diagnosed with cancer before or after measurement than in those without a history of cancer. These findings are consistent with the hypothesis that somatic mutations are the main cause of excess cancer risk from radiation exposure. — Environ Health Perspect 104(Suppl 3):493-496 (1996)

Key words: somatic cell mutation, atomic bomb survivors, GPA gene mutation, cancer risk evaluation, radiation exposure

Introduction

As knowledge in the molecular biology of cancer accumulates, the field of cancer risk evaluation is gaining attention. Analysis of oncogenes and tumor suppressor genes has demonstrated that mutations such as base substitutions, deletions, recombinations, and amplification of DNA are crucial for carcinogenesis (1). The monitoring of somatic mutations in vivo can be a biological marker useful in evaluating the cancer risk from exposure to environmental mutagens (2). Several somatic mutation assays have been established for the glycophorin A (GPA) genes in erythrocytes (3,4) and for the hypoxanthine phosphoribosyltransferase (hprt) (5,6), HLA-A (7,8), and TcRα and β genes (9,10) in T lymphocytes. Although these assays do not detect or quantify variations in genes directly involved in carcinogenesis, they can be considered to indirectly reflect them. Among these assays, the GPA and TcR assays are characteristically different; the GPA assay is suitable for use as a lifelong biological marker of exposure to mutagens because it detects mutations that accumulate mainly in long-lived bone marrow stem cells (4,11,12), and the TcR assay has potential value as a biological dosimeter for those experiencing recent or chronic radiation exposure (13,14).

Populations such as atomic bomb (A-bomb) survivors and persons who were administered the radioactive medical contrast agent Thorotrast are suitable for studying the relationship between somatic mutations and cancer risk. Their radiation doses are relatively well known and epidemiological monitoring has been extensive (15,16). In this report, we will summarize the results among A-bomb survivors using the erythrocyte GPA mutation assay for biologically substantiating estimated radiation doses and epidemiologically derived risk estimates.

Materials and Methods

Subjects

Peripheral blood samples were obtained from 713 A-bomb survivors from Hiroshima and 513 survivors from Nagasaki, including 427 age- and sex-matched controls who were exposed distally and received less than 0.005 Gy. Doses range from 0.005 to 5.0 Sv. Ages range from 43 to 96 years with an average age of 64.5 years. Donors having the MN heterozygous blood type were examined for GPA mutations.

For statistical analysis, subjects were restricted to those who had not undergone radiotherapy or chemotherapy or blood transfusion before mutant frequency (Mf) measurement. Current cigarette-smoking behavior was ascertained simultaneously with blood drawing. From the Radiation Effects Research Foundation (RERF) tumor registry database, 81 Hiroshima A-bomb survivors were identified who had malignant tumors, and their diagnoses and medical treatment were confirmed from medical charts (17).

Erythrocyte GPA Mutation Assay

GPA is one of the major glycophorin complexes abundantly expressed on the surface of mature erythrocytes and is the antigenic determinant of the MN blood type. A pair of monoclonal antibodies and a flow
cytometer can be used to detect mutant erythrocytes lacking either the M or N products of GPA alleles among normal erythrocytes from MN heterozygous people. The flow cytometric measurement of mutants has been described previously in detail (3,4,18).

Four types of mutants, NO, MO, NN and MM, can be detected using flow cytometry. Hemizygous NO and MO mutant cells are caused by deactivation of M or N alleles of the GPA gene, respectively. Homozygous NN and MM mutants may be induced by somatic recombination of chromosome 4 on which the N and M alleles reside.

Among these four types of mutants, we will report here the precise analysis of hemizygous mutant frequencies (MF).

The analysis used here was based on weighted bone marrow doses (19) computed as the y-ray dose plus 10 times the neutron dose. The weighting factor will be referred to as the relative biological effectiveness (RBE) of neutrons, and weighted doses are expressed in sieverts (Sv). The detailed method for dose–response analysis of MF will be discussed elsewhere (Kyoizumi et al.; Radiat Res, in press).

Results and Discussion

Background Mutant Frequency

The background GPA MF (data not shown) can be characterized as follows. The frequency of GPA-NO and -MO deletion type (D-type) mutants in the control population was significantly lower in Nagasaki than in Hiroshima. MFs in both cities were significantly higher in males than in females. The reproducibility of the mutation assay suggests that interindividual variability is low. Cigarette smoking effects GPA MF slightly but significantly; a current smoker’s MF is higher by approximately 0.61% per cigarette smoked daily. With age, the MF slightly increases (approximately 0.7%/year); however, the degree of increase is small compared with the effect of radiation.

These characteristics of the background GPA MF correspond with those of background solid-cancer incidence based on analysis of 29 years of epidemiological data on A-bomb survivors. For example, both solid-tumor incidence and mortality of A-bomb survivors increased with advancing age and were higher in males (15). Furthermore, mortality was higher in smokers than in nonsmokers and higher in Hiroshima survivors than in Nagasaki survivors (20).

The GPA MF was fairly stable for individuals during the 5-year study period, but a cross-sectional analysis showed an age-related increase of the MF in persons ranging from 40 to 90 years old. This may represent a long-term accumulation of mutations in bone marrow stem cells, which is similar to the age-related differences in somatic MFs also reported for other gene loci in T cells (8,9). The induction and decay rates of in vivo mutant cells must be taken into account when considering the magnitude of the age-dependent increase of mutations (21).

Dose Response of D-type Mutant Frequency

GPA-NO and GPA-MO MFs were significantly correlated (R = 0.34; p < 0.001).

Figure 1 shows the dose–response curve of the GPA D-type MF for about 1,200 survivors using the estimated dose to bone marrow (BM), based on the Dosimetry System 1986 (DS86) and assuming a neutron relative biological effectiveness of 10. Figure 1A shows a linear dose–response curve, excluding outliers whose MF and dose were more than 400 × 10^-6 and 5 Sv, respectively. Figure 1B shows the cubic analysis of log–log transformed data without excluding the outliers. We used a log transformation for both GPA MF and BM dose because the skewness of both variables gave unduly high influence to a small number of points at the upper extreme of the distribution of values, even after removing outliers. In addition, log transformation of dose can equalize the effect of dose-estimation error on the dose response throughout the range of doses. In both statistical analyses, MF increases significantly with increase of BM dose; however, a purely cubic model best fit these data. This implies that the initial slope of the dose–response curve on the log–log scale is 0. On the untransformed scale (Figure 1A), the log–log cubic dose response flattens out considerably, resembling a linear dose–response model. Interestingly, the ratio of the GPA MF in persons exposed to 1 Sv to the controls is 1.87, which is close to the relative risk of solid-tumor incidence at 1 Sv (1.63) (15).

Although the data are not shown here, the dose responses of the GPA-MO and GPA-NO MFs did not differ significantly (p = 0.41) by asymptotic Wald χ² test. No significant effects of sex, city, smoking, or age at the time of exposure on the dose response of GPA MF were detected. GPA D-type MF exhibits a significantly positive correlation to the frequency of lymphocytes bearing stable-type chromosome aberrations (number of subjects = 132, R = 0.384, p < 0.001).

The doubling dose for the GPA D-type MF is calculated to be about 1.20 Sv using the cubic model [95% CI, 0.95–1.56], whereas the minimum dose for detecting a significant increase in MF is about 0.24 Sv using a threshold model [95% CI, 0.045–0.51 Sv]. Interestingly, the doubling

Figure 1. Radiation dose response of the frequency of GPA deletion type mutations. A, Linear dose–response curve; B, cubic analysis of log–log transformed data.
dose of the GPA Mf was also close to that of solid-cancer incidence in A-bomb survivors (1.59 Sv) (15), and the minimum detection dose for the GPA Mf was not inconsistent with the data for solid-cancer risk in the low-dose groups of survivors, i.e., the point at which the relative risk for those exposed in the 0.200 to 0.499 Sv range first becomes significantly higher than 1 (22, 23).

These observations of dose response are consistent with the hypothesis that radiation-induced somatic cell mutations are the major cause of excess cancer risks after radiation exposure.

In this context, we analyzed the GPA Mf of patients with cancers. Figure 2 shows among Hiroshima survivors the dose responses of the GPA D-type Mf for 81 subjects previously or subsequently diagnosed with cancer and for 548 subjects with neither malignant nor benign tumors. Statistical analysis using the log–log cubic model revealed that the dose effect of D-type Mf was significantly higher in those diagnosed with cancer before or after measurement than in those without a history of cancer (p < 0.009). The ratio of these dose responses is about 2.3 at 5 Sv.

Thus, a higher frequency of mutations may contribute to the development of cancer, or the cancer group may be more sensitive to radiation. In either case, the possibility of dosimetry error must be considered, too. Reports in the literature of elevated Mf in cancer-prone persons are numerous. Highly elevated GPA Mfs have been detected in patients with ataxia telangiectasia (14,24), Bloom’s syndrome (14,25–27), and Fanconi’s anemia (25). In addition, among many Thorotrast patients who are at high risk of developing malignant tumors and leukemia (16), the Mf is significantly higher (13,14). Thus, we have proposed that the GPA Mf can be used as a biomarker for the assessment of cancer risk among radiation-exposed people.

Finally, understanding the interaction between ionizing radiation and other suspected causes of cancer is critical to human cancer-risk assessment. The possible interactive effects of radiation exposure and tobacco smoking on GPA Mf in those with cancer versus persons without cancer should be examined in the future. Lower GPA Mf in females suggests the possible effect of sex hormones on either hematopoietic stem-cell proliferation rates or survival of GPA mutant cells. Continuing somatic mutation studies of the A-bomb survivors and clinical and epidemiological follow-up of survivors whose Mf is high may one day clarify whether the GPA mutation assay is a suitable end point for predicting cancer risk.

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