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Individual Variation of Somatic Gene Mutability in Relation to Cancer Susceptibility: Prospective Study on Erythrocyte Glycophorin A Gene Mutations of Atomic Bomb Survivors

Seishi Kyozumi, Yoichiro Kusunoki, Tomonori Hayashi, Masayuki Hakoda, John B. Cologne, and Kei Nakachi

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Abstract

It has previously been reported that hemizygous mutant fraction (MF) at the glycophorin A (GPA) locus in erythrocytes increased with radiation dose in heterozygotes among Hiroshima and Nagasaki atomic bomb survivors. In the present study, we analyzed the relationship between GPA MF and cancer risk using newly developed cancers among previously cancer-free subjects whose GPA MF had been measured between 1988 and 1996. Among 1,723 survivors (1,117 in Hiroshima and 606 in Nagasaki), we identified 186 subjects who developed a first cancer by the end of 2000. We compared the radiation dose responses of GPA MF between cancer and cancer-free groups using a linear-quadratic model fit by multiple regression analysis in combination with age, sex, and city. The slope of the GPA MF dose-response curve was significantly higher in the cancer group than in the cancer-free group among Hiroshima subjects. Moreover, no significant difference of GPA MF between cancer and cancer-free groups was found in unexposed controls in the two cities. The same conclusions were obtained using a linear-dose-response model and by further analysis using Cox regression of cancer incidence. These findings suggest that there might be interindividual variation in mutability of somatic genes and that Hiroshima survivors who have higher mutability in response to radiation exposure would be expected to have a higher probability of suffering radiation-related cancer. (Cancer Res 2005; 65(12): 5462-9)

Introduction

Interindividual variability in human responses to mutagen exposures, including ionizing radiation, is believed to be a critical element in determining individual risk of cancer as well as the incidence of cancer in a population. At least a part of such interindividual variability of cancer susceptibility may be attributed to capacity of responses to oxidative DNA damage generated by mutagens (1). Multistaged defense mechanisms may exist in the responses to oxidative DNA damage, involving the initial defense against reactive oxygen species by superoxide dismutase and catalase, inhibition of incorporating oxidized bases into DNA by hydrolase, and repair of DNA damage (i.e., base excision repair, transcription-coupled repair, global genome repair, mismatch repair, translesion synthesis, homologous recombination, and nonhomologous end joining). Among them, much attention has been paid to several DNA repair genes. There is increasing evidence that mild reductions in DNA repair capacity, assumed to be the consequence of common genetic variation, affect cancer predisposition (2, 3). Currently, molecular epidemiologic studies are being conducted in many laboratories to define the roles that polymorphisms in DNA repair genes play in individual cancer susceptibility (3–5). In contrast to such genetic markers, phenotypic markers of DNA repair capacity and cancer susceptibility comprise both genetically and environmentally determined fractions and express integrated effects of complicated processes where a number of gene products are involved. Thus, phenotypic markers have often played vital roles in cancer research, specifically in prospective cohort studies, assessing the exposure levels (biodosimetry) as well as cancer risk (2, 6).

Many phenotyping assays have been developed using blood cells and skin fibroblasts for quantifying in vivo somatic mutations and in vitro DNA repair capacity (6). The erythrocyte glycophorin A (GPA) mutation assay, which can enumerate hemizygous mutants at the GPA locus in long-lived hematopoietic stem cells of heterozygous donors, provides one useful phenotypical end point for the assessment of cancer risk. This was supported by the findings that highly elevated GPA mutant fractions (MF) were detected in patients with cancer-prone diseases, such as ataxia telangiectasia (7), Bloom's syndrome (8, 9), Fanconi's anemia (8, 10), and Werner syndrome (11, 12). These patients have defects in genes that are involved in several pathways of DNA repair mechanisms. It was also reported that GPA Ms can be used as an assessment marker for the development of secondary induced leukemia in patients treated for childhood acute lymphoblastic leukemia (13). These findings suggest that the GPA MF may, in some way, reflect individual repair capacity and cancer risk.

To clarify the association between radiation-induced mutation and cancer risk, prospective studies are critical to exclude the role of cancer itself in the association, such as through chemotherapy and radiation therapy. Because the atomic bomb (A-bomb) survivor population is an epidemiologically well-controlled cohort in terms of dose estimation (14) and cancer follow-up (15), such an analysis is feasible in this population. We previously measured hemizygous GPA MF in ~1,200 heterozygous A-bomb survivors in Hiroshima and Nagasaki between 1988 and 1993 and analyzed the dose response of GPA MF and the relationship between GPA MF and cancer risk (16). It was found that the doubling dose of GPA MF was similar to that of solid-cancer incidence in A-bomb survivors. Furthermore, the dose response was significantly higher in persons who had been diagnosed with cancer than in cancer-free controls.
individuals among Hiroshima survivors. This suggests an earlier onset of cancer due to enhanced mutagenesis or a higher radiation sensitivity in the cancer group. However, although we attempted to exclude all survivors who had undergone chemotherapy and radiotherapy, we may have missed some of them due to incompleteness of the medical records. Thus, because we could not completely exclude the possible effect of the therapies on GPA Mf, a prospective study was desired.

We have extended the GPA Mf measurements to ~1,900 survivors in total as of 1996 and followed them until 2000 to identify newly developed cancers among the previously cancer-free subjects. Based on these prospective data, we reanalyzed the relationship between GPA Mf and cancer development. In the present report, we show the reproducibility of the previous findings and discuss interindividual variation of susceptibility to radiation-induced mutagenesis, which may be associated with subsequent cancer risk.

Materials and Methods

Study subjects. Blood samples were obtained randomly from 1,902 survivors whose MN blood types were heterozygous by the hemagglutination test, who were participating in the Radiation Effects Research Foundation (RERF) Adult Health Study from June 1988 to August 1996. We excluded 179 survivors who were diagnosed with cancer before the GPA measurements and observed subsequent cancer development. Subject ages ranged from 43 to 100; mean ages were 65 for males and 67 for females.

Survivors in Hiroshima and Nagasaki, who have been diagnosed with malignant tumors (n = 186; 118 in Hiroshima; 68 in Nagasaki) through December 2000, were identified from the RERF tumor registry (17). Survivors in Hiroshima and Nagasaki, who have been diagnosed with malignant tumors (n = 186; 118 in Hiroshima; 68 in Nagasaki) through December 2000, were identified from the RERF tumor registry (17). Diagnoses and medical treatment histories for these survivors were also confirmed from the Adult Health Study medical charts. Identified malignant tumors included stomach (n = 32), colon (n = 31), lung (n = 19), liver (n = 18), breast (n = 10), rectum (n = 10), pancreas (n = 8), prostate (n = 7), gall bladder (n = 6), esophagus (n = 5), thyroid (n = 5), and other (n = 35) cancers.

The distribution of subjects by DS86 bone marrow doses (14), sex, and city are shown in Table 1. This distribution is similar to that of the total Adult Health Study population. The estimated dose includes both neutron and γ-ray components. The analyses described in the present report were based on weighted bone marrow doses computed as the γ dose plus 10 times the neutron dose and adjusted for the effect of imprecision on regression analyses (18). The weighting factor will be called the relative biological effectiveness of neutrons, and weighted doses are expressed in sievert (Sv). This study population consists of Hiroshima and Nagasaki survivors who were exposed to significant radiation doses of ≥0.004 Sv because of their location within 2 km of the hypocenter plus a second group whose exposures were at distances in excess of 3 km from the hypocenter and as a result led to them receiving radiation doses of <0.004 Sv (i.e., doses that are indistinguishable from background). The latter group of distally exposed survivors includes the most appropriate controls for all of our studies of the effects of A-bomb radiation exposures, including the present one.

Measurement of glycoporphin A mutation frequency. Using the GPA mutation assay, four types of mutant cells, Mφ, Nφ, MM, and NN cells, can be detected among the erythrocyte populations of MN heterozygous donors. Hemizygous Mφ and Nφ cells are caused by deactivation of N or M alleles of the GPA gene, respectively. Homozygous MM and NN cells may be induced by somatic recombination between the two chromosomes on which the M and N alleles reside. Among these four types of mutants, the reproducibility of NN cells was low, probably due to carbohydrate modification of the GPA molecules (19, 20). Also, MM mutant frequency is significantly affected by overlapping of Mφ mutants in the MM mutant window on the flow cytogram, particularly for the high-dose exposed who have high Mphi Mfs. Thus, in this report, statistical analysis was undertaken for the mean of Mφ and Nφ hemizygous GPA Mf (MF).

The detailed method for the flow cytometric measurement of mutant erythrocytes has been described previously (20). Briefly, using a single-cell sorter, FACStar (Becton Dickinson Immunocytometry System, San Jose, CA), four types of variants lacking the expression of one GPA allele were distinguished from normal MN heterozygous cells, by two-color staining with the GPA (M + N)–specific monoclonal antibody (mAb) 10F7 and the GPA (M)–specific mAb 6A7, two mutant cell types, hemizygous Nφ and homozygous NN cells, from MN heterozygous donors, can be detected simultaneously. By combining the GPA (M)–specific mAb 9A3 and the GPA (N)–specific mAb NN3, hemizygous Mφ and homozygous MM cells can be measured simultaneously. 10F7 and 9A3 mAbs were directly labeled with fluorescein and mAbs 6A7 and NN3 were conjugated with biotin followed by labeling with streptavidin-conjugated phycoerythrin (Biomeda, Foster City, CA). Mutant cells displaying a hemizygous or homozygous phenotype were sorted onto a glass slide. Cells showing typical erythrocyte morphology with fluorescein fluorescence matched for the mutant phenotype were assayed per sample.

<table>
<thead>
<tr>
<th>City</th>
<th>Sex</th>
<th>No. subjects</th>
<th>Survivor bone marrow dose (Sv, neutron RBE = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;0.004* 0.004-0.499 0.500-0.999 1.000-1.499 1.500+</td>
</tr>
<tr>
<td>Hiroshima</td>
<td>Male</td>
<td>360 (54)*</td>
<td>129 (22) 132 (13) 47 (12) 32 (3) 20 (4)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>757 (64)</td>
<td>311 (19) 292 (25) 93 (9) 29 (7) 32 (4)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1,117 (118)</td>
<td>480 (41) 424 (38) 140 (21) 61 (10) 52 (8)</td>
</tr>
<tr>
<td>Nagasaki</td>
<td>Male</td>
<td>221 (36)</td>
<td>84 (14) 53 (7) 45 (7) 25 (5) 14 (3)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>385 (32)</td>
<td>168 (8) 88 (12) 77 (8) 38 (2) 14 (4)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>606 (68)</td>
<td>252 (22) 141 (17) 122 (15) 63 (7) 28 (7)</td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>581 (90)</td>
<td>213 (36) 185 (20) 92 (19) 57 (8) 34 (7)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>1,142 (96)</td>
<td>478 (27) 380 (35) 170 (17) 67 (9) 46 (8)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1,723 (186)</td>
<td>692 (63) 565 (55) 262 (36) 124 (17) 80 (15)</td>
</tr>
</tbody>
</table>

Abbreviation: RBE, relative biological effectiveness.

*According to the DS86 dosimetry system, survivors whose dose estimation would result in a free-in-air kerma ≤5 mGy were automatically assigned doses of zero. However, most of the persons in this category were too far from the hypocenter to have received significant radiation exposure.

†Numbers of persons with cancer diagnosed subsequent to GPA Mf measurement are in parentheses.

Table 1. Distribution of subjects excluding persons who had malignant cancer before GPA Mf measurement
Statistical analysis. Dose responses for GPA Mf and incidence proportion of cancer were fit using ordinary (least squares) regression. Weighted, adjusted bone marrow dose was used as described above. Age at examination was centered at its mean (65 years). City, sex, and cancer status were treated as indicator variables. Application of least-squares regression to the GPA Mf and radiation dose-response data for purposes of statistical testing would necessitate logarithmic transformation of both the GPA Mf and radiation dose variables (16) to achieve approximate normality and constant variance of the response variable (log GPA Mf) and approximately uniform distribution of the predictor variable (log radiation dose), but we desired to mimic standard radiobiological practice and fit linear or linear-quadratic dose responses. Thus, we did not transform the variables for the purpose of estimating the radiation dose response and instead verified the fit of the least-squares regression using nonparametric curve-fitting methods. As a further check on adequacy of the dose-response fit, individual observations with large influence on the regression analysis were identified through single-deletion regression diagnostics and regression models were refit after excluding such points.

As a result, the small number of subjects with GPA Mf values >400 was not used in estimating the GPA Mf dose response. For fitting the cancer incidence proportion to radiation dose, least squares regression was applied to the binary indicator of cancer status. Approximately homogeneous variance and fit to the data were confirmed by comparing the fitted regression to a plot of binomial proportions grouped on radiation dose with approximately equal numbers of subjects (Fig. 1).

Follow-up for incident cancer subsequent to GPA measurement was analyzed using Cox regression with age as the time scale and adjustment for year of birth and age at examination. The effect of GPA Mf on cancer incidence was assessed using either the logarithm of continuous GPA Mf to reduce the influence of the small number of points with large values of GPA Mf, or the untransformed GPA Mf excluding the subjects with values larger than 400. For graphic presentation of the results of the Cox regression, summary plots of cumulative incidence (proportion of subjects who were cancer patients at examination) were fit using ordinary (least squares) regression analysis were identified through single-deletion regression diagnostics and regression models were refit after excluding such points. As a result, the small number of subjects with GPA Mf values >400 was not used in estimating the GPA Mf dose response. For fitting the cancer incidence proportion to radiation dose, least squares regression was applied to the binary indicator of cancer status. Approximately homogeneous variance and fit to the data were confirmed by comparing the fitted regression to a plot of binomial proportions grouped on radiation dose with approximately equal numbers of subjects (Fig. 1).

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Cancer prevalence in the study cohort. One hundred eighty-six subjects developed a first cancer between the GPA Mf measurement and the end of 2000. There was a statistically significant increase in cancer incidence proportion with dose after adjustment for city, sex, and age (Fig. 1). This suggests that the subjects of the present study developed cancers in a dose-dependent manner even >40 years after exposure.

Effects of cancer status and city difference on dose responses of glycophorin A mutant fraction. Figure 2 shows nonparametric curves for the GPA Mf (the mean of hemizygous MΦ and NΦ Mf) values according to bone marrow dose among all study subjects (n = 1,902), including cancer cases diagnosed before GPA Mf measurements. The plots suggest that the GPA radiation dose response is steeper among cancer patients in Hiroshima, particularly among those whose cancer was diagnosed subsequent to GPA Mf measurement (Fig. 2, left). Persons who had cancer diagnosed before the time of GPA measurement may not be representative, because some individuals with cancer might have been censored—debilitated or deceased—and unable to attend the Adult Health Study examination. In contrast, no apparent differences were observed between cancer and noncancer groups among Nagasaki survivors (Fig. 2, right).

To evaluate further the possible difference in cancer-related GPA dose response between the two cities, we did standard regression on GPA Mf using bone marrow dose, cancer status, sex, and age at examination as covariates, excluding subjects with values of GPA Mf >400 and those who had cancer diagnoses before GPA measurement (Table 2). Background GPA Mf (estimate for 0 Gy exposure) was lower in Nagasaki than in Hiroshima and lower in females than in males. An increase in GPA Mf with age of the subject at examination was only marginally significant, probably because age-dependent increase in GPA Mf reaches a plateau after about 50 years of age (21). There was an initial increase in the GPA response with bone marrow dose, followed by an attenuation in the slope (negative quadratic term). Although the quadratic term was statistically significant (P = 0.033), there was little quantitative difference in the results with or without the quadratic term except for a slightly lower dose-response slope without the quadratic term.

There was no significant difference in background GPA Mf between male subjects who subsequently developed cancer and those who did not, but among females the background GPA Mf was lower among those with cancer.

The fitted linear-quadratic regression models for each of the two cities, adjusted for sex and age, are shown in Fig. 3. The dose response of GPA Mf was significantly higher in Hiroshima subjects who subsequently developed cancer than in those who did not. Whether there was a difference in dose response by cancer status depended significantly on city (a three-way interaction between city, dose, and cancer status; P = .0081), with no difference in Nagasaki. Seven points were identified that had a high influence on the value of the city × cancer × dose three-way interaction term. Upon deleting these points, the value of the interaction term decreased somewhat but remained statistically significant. Among Nagasaki subjects, the initial slope of the GPA dose response in the linear-quadratic model adjusted for the average values of the other factors was 22.6 in cancer-free subjects and 22.3 in subjects with subsequent cancer (P = 0.29). In Hiroshima, the similarly adjusted initial slopes were 35.8 in cancer-free subjects and 51.3 in subjects with subsequent cancer (P = 0.0039). There was no evidence that the quadratic term of the dose response differed according to cancer status.

Figure 1. Dose response of cancer incidence proportion subsequent to GPA Mf measurement adjusted for city, sex, and age at examination. The total number of subjects is 1,723. Points, crude proportions in 10 dose groups with approximately equal numbers of subjects (n = 115) except for unexposed controls (n = 691); bars, SE of the estimated proportions and the quartiles of dose.

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Difference of glycoporphin A mutant fraction between cancer and noncancer groups among high dose–exposed subjects. Because the GPA Mf values are highly skewed, even when restricted to $\leq 400 \times 10^{-6}$, the results of statistical tests of parameters from the regression analysis may not be reliable. Therefore, we compared log-transformed GPA Mf values between high dose and unexposed persons, thereby avoiding assuming any particular dose-response model, using either a t test (Fig. 4) or regression analysis with adjustment for sex and age (Table 3). Among unexposed subjects, there was no difference in log GPA Mf between persons with and without cancer in either city. The log GPA Mf was significantly higher in subjects with subsequent cancer than without cancer among the heavily exposed (>1.5 Sv) subjects in Hiroshima ($t$ test $P = 0.012$, regression $P = 0.0057$) but was not significantly different between subjects with and without cancer in Nagasaki ($t$ test $P = 0.52$, regression $P = 0.21$).

The analysis of cancer onset rates during the follow-up period by Cox regression confirmed the finding that cancer risk was related to GPA Mf level among high-dose–exposed subjects in Hiroshima but not in Nagasaki (Fig. 5). Whereas there was no association between log GPA Mf level and cancer onset rate among unexposed persons or Nagasaki high-dose–exposed persons, there was significantly higher risk of cancer with higher GPA Mf value in Hiroshima high-dose–exposed persons ($P = 0.043$). The estimated relative risk of cancer for a 10-unit difference in GPA Mf (i.e., a 50% increase over the median) was 1.13 (95% confidence interval, 1.00-1.27; Table 4). Relative risks of cancer for GPA for all individual dose groups by city are shown in Table 4. There was no change in the significance of the results when log GPA was used.

The $t$ tests and Cox regression analyses were repeated after excluding persons whose follow-up was <1 year. There was no change in the pattern of results. The association among high dose and cancer on log GPA Mf was significant in Hiroshima ($P = 0.024$) but not in Nagasaki ($P = 0.41$). The effect of GPA Mf on cancer incidence was significant in high-dose persons in Hiroshima ($P = 0.041$) but not in Nagasaki ($P = 0.66$).

### Table 2. Regression analysis of GPA Mf using linear and linear-quadratic dose-response models

<table>
<thead>
<tr>
<th>Term</th>
<th>Estimated value ± SE [$\times 10^{-6}$] (P value)</th>
<th>Linear model</th>
<th>Linear-quadratic model</th>
</tr>
</thead>
<tbody>
<tr>
<td>City (Nagasaki)</td>
<td>$-7.1 \pm 3.0$ (0.018) $-7.5 \pm 3.0$ (0.013)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex (female)</td>
<td>$-6.5 \pm 2.1$ (0.0016) $-6.4 \pm 2.1$ (0.0020)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at exam (per year)</td>
<td>$0.13 \pm 0.076$ (0.078) $0.13 \pm 0.076$ (0.099)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose (initial slope, per SV)</td>
<td>$26 \pm 2.0$ (-0.0001) $32 \pm 3.5$ (-0.0001)</td>
<td>$-3.6 \pm 1.7$ (0.033)</td>
<td></td>
</tr>
<tr>
<td>Dose (quadratic term)</td>
<td>$5.7 \pm 4.6$ (0.21) $5.5 \pm 4.6$ (0.23)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer (present)</td>
<td>$-12 \pm 4.8$ (0.013) $-12 \pm 4.8$ (0.012)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex-cancer interaction</td>
<td>$-5.5 \pm 3.1$ (0.071) $-5.0 \pm 3.1$ (0.10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>City-dose interaction</td>
<td>$15 \pm 5.3$ (0.0049) $15 \pm 5.3$ (0.0038)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cancer-dose interaction</td>
<td>$-21 \pm 7.9$ (0.0068) $-21 \pm 7.9$ (0.0081)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE: Persons with GPA Mf $>400 \times 10^{-6}$ were excluded.
Discussion

It is increasingly accepted that the accumulation of multiple abnormalities in cancer-associated genes of a target cell is required for the development of cancer (22–24). Although the mechanism of radiation carcinogenesis is still unknown, some evidence has been presented that A-bomb radiation seems to reduce the number of gene changes needed for cancer induction, thereby inducing earlier onset of cancer in the exposed, compared with the unexposed (25). If GPA Mf reflects nonspecific mutability of all somatic cells in an individual, it can be presumed that GPA Mf may also reflect the prevalence of mutations at cancer-associated genes. Among survivors exposed to the same dose, those who have a higher GPA Mf would be expected to have a higher probability of suffering cancer at any given point in time. In fact, as shown in the present study, the dose response of GPA Mf in the cancer group among Hiroshima survivors was found to be significantly higher than that in the noncancer group. The dose response among persons with cancer diagnosis before GPA measurement was intermediate to that of the groups with no cancer or cancer after GPA measurement, which is further consistent with a higher cancer risk for high GPA Mf radiation–exposed subjects because persons at high risk of cancer would more likely be censored (unobserved due to death or debility) before GPA measurement. The individual difference of GPA Mf in the same dose group might be explained by individual variation in the capacity to repair radiation-induced DNA damage. We discuss below the validity and feasibility of these hypotheses regarding individual differences in DNA repair capacity among A-bomb survivors.

A potentially important source of interindividual variability in relation to cancer risk is DNA repair capacity, including the DNA repair–defective cancer-prone diseases, such as ataxia telangiectasia, Bloom’s syndrome, Fanconi’s anemia, and Werner syndrome. Apart from these rare and extreme familial cases, there is increasing evidence that a moderate reduction in DNA repair capacity contributes to the sporadic incidence of cancer in the general population (2, 3). Conventional phenotype assays have detected considerable interindividual variation in DNA repair capacity (2, 26, 27). These reduced repair capacity phenotypes have been associated with an increased risk of cancer (2, 28, 29). Evidence of the importance of moderate reduction in DNA repair capacity is also accumulating from mouse models, which have provided results regarding cancer risk increased by heterozygous knock-out in DNA repair genes (30, 31) and those regarding strain differences in cancer susceptibility (32, 33). Furthermore, a number of molecular epidemiologic studies have been initiated using the data from systematic screening of populations for common variants in DNA repair genes (3, 34). Associations of common variants in several repair genes with increased cancer risk have been reported in case-control studies (5). In general human populations, it has been suggested that individual differences in peripheral blood T cell chromosome aberration frequencies may be associated with individual differences in cancer susceptibility (35, 36). These accumulating data are consistent with the hypothesis that interindividual variation in DNA repair capacity has an impact on cancer risk.

Statistical analysis in this study showed that there was a city difference (i.e., significant interaction among radiation, cancer, and city in their association with GPA Mf; Table 2). This does not necessarily imply that there is a significant correlation between GPA Mf dose response and cancer in Hiroshima subjects but not in Nagasaki subjects. Small numbers of cases in Nagasaki make it difficult to clearly state the apparent negative finding. However, the observed city difference may be, at least in part, due to possible differences in ethnic background between Hiroshima and Nagasaki, which were suggested by the previous biochemical genetic study of

Figure 3. Dose response of GPA Mf among cancer (post-GPA Mf measurement) and noncancer groups based on the linear-quadratic regression model in Hiroshima and Nagasaki. The number of subjects in each group is listed in Table 1. Symbols indicate subjects who were diagnosed with cancer after GPA Mf measurement (▲) and subjects without cancer (○). Lines denote fitted dose-response curves for cancer and noncancer groups. P values represent the statistical significance of difference of dose response (quadratic term) between cancer post-GPA Mf measurement and noncancer groups.

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A-bomb survivors and their children (37). We might presume that the proportion of individuals who have higher mutability of somatic genes is somewhat larger in Hiroshima than in Nagasaki. Interestingly, the background GPA Mf of Hiroshima is significantly higher than that of Nagasaki, as shown in the present study. Such a city difference was also reported for the background solid-tumor mortality (38) and chronic myelogenous leukemia (CML) of survivors (39). City differences of dose response (lower in Nagasaki) were also suggested for the solid tumor mortality (15), the chromosome aberration frequencies in lymphocytes (40), and the incidence of CML (39). These city differences have been attributed to dose estimation errors and/or to a qualitative difference in the radiation produced by the bombs (i.e., the difference in the amount of neutron and γ-ray components), assuming that there is no city difference in radiation sensitivity of A-bomb survivors (15, 40). Because recent genomic analyses have shown extensive interindividual—including ethnic—variations in gene polymorphisms, as mentioned above, this assumption should be reassessed.

The following possible caveats of the present study should be kept in mind. We conducted a prospective study, which is critical to exclude possible effects of chemotherapy and/or radiotherapy on in vivo somatic mutations. Nevertheless, because the period between GPA Mf measurement and cancer diagnosis is rather short (average: about 3.7 years; range: 22 days-9.4 years; n = 187), it is possible that tumors of preclinical size had already developed before GPA Mf measurement. Tumor burden, even with a very small lesion, might increase somatic mutations through high metabolic rate and excessive endogenously generated oxidative stress. However, this may not be the case because GPA Mf values were nearly constant in the cancer subjects (n = 29) whose Mf were measured more than twice during the 8-year examination period before cancer diagnosis (data not shown). Further, the radiation dose responses of GPA Mf between cancer and cancer-free groups did not change after excluding all persons whose follow-up ended within 1 year of GPA measurement (data not shown). Another factor complicating the interpretation is uncertainty in dose estimation, because persons with radiation-related outcomes, such as cancer, are more likely to have underestimated dose than persons without. However, our comparison between high-dose–exposed

Table 3. Relationship between log GPA Mf and cancer status in high dose–exposed survivors

<table>
<thead>
<tr>
<th>City</th>
<th>Term</th>
<th>Estimated difference in log GPA Mf</th>
<th>SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hiroshima</td>
<td>Exposed</td>
<td>0.503</td>
<td>0.0477</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
<td>0.0568</td>
<td>0.0495</td>
<td>0.46</td>
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<td></td>
<td>Exposure-cancer interaction</td>
<td>0.342</td>
<td>0.123</td>
<td>0.0057</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
<td>−0.0622</td>
<td>0.0634</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Exposure-cancer interaction</td>
<td>0.177</td>
<td>0.141</td>
<td>0.21</td>
</tr>
<tr>
<td>Nagasaki</td>
<td>Exposed</td>
<td>0.365</td>
<td>0.0670</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Cancer</td>
<td>−0.0622</td>
<td>0.0634</td>
<td>0.33</td>
</tr>
<tr>
<td></td>
<td>Exposure-cancer interaction</td>
<td>0.177</td>
<td>0.141</td>
<td>0.21</td>
</tr>
</tbody>
</table>

NOTE: Results of fitting log GPA Mf, comparing high dose (>1.5 Sv) with nonexposed with adjustment for sex and age at examination.
and nonexposed persons, which is unaffected by dose uncertainties, did not change the conclusions.

Although our hypothesis is valid, it is far from proved based on the present study alone. We believe that the evolving ability to study polymorphisms in DNA repair genes can contribute to understanding about the relationship between DNA repair capacity and cancer risk in A-bomb survivors. Because the difference in GPA Mf between cancer and cancer-free groups is significant only for high dose-exposed survivors, a candidate polymorphic gene affecting interindividual variability could be one involved in repair of DNA double-strand breaks induced by high-dose irradiation. Double-strand breaks are potentially cytotoxic to cells and mutagenic. At least two molecular mechanisms are involved in the pathway of double-strand break repair: homologous recombination repair and nonhomologous end joining (3, 5, 41, 42). Homologous recombination repair occurs predominantly in the S or G2 phase of cell division and exchanges DNA strands between the damaged chromatid and the intact sister chromatid. Nonhomologous end joining repair involves

**Figure 5.** Cox regression analyses of cancer incidence following GPA Mf measurement within unexposed and high dose exposed (>1.5 Sv) in Hiroshima and Nagasaki. Dashed line, low GPA Mf; solid line, high GPA Mf; low and high are defined as below or above the median GPA Mf level among cancer cases. Numbers of cases of cancer as are shown in Fig. 4. P values represent the statistical significance of difference of cancer incidence between high and low GPA Mf groups within each dose group.

**Table 4.** Relative risk of cancer for GPA (relative risk for 10-unit difference in GPA Mf)

<table>
<thead>
<tr>
<th>Exposure category</th>
<th>Hiroshima</th>
<th>Nagasaki</th>
<th>Both cities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero dose (&lt;0.004 Sv)</td>
<td>0.96 (0.80, 1.16)</td>
<td>0.82 (0.57, 1.18)</td>
<td>0.91 (0.77, 1.07)</td>
</tr>
<tr>
<td>All exposed (≥0.004 Sv; no dose adjustment)</td>
<td>1.03 (0.99, 1.08)</td>
<td>1.00 (0.93, 1.09)</td>
<td>1.02 (0.99, 1.06)</td>
</tr>
<tr>
<td>All exposed (≥0.004 Sv; with dose adjustment)</td>
<td>1.03 (0.98, 1.08)</td>
<td>0.98 (0.89, 1.08)</td>
<td>1.02 (0.98, 1.06)</td>
</tr>
<tr>
<td>Low dose (≤1.5 Sv)</td>
<td>1.01 (0.95, 1.07)</td>
<td>0.99 (0.89, 1.10)</td>
<td>1.00 (0.96, 1.05)</td>
</tr>
<tr>
<td>High dose (&gt;1.5 Sv)</td>
<td>1.13 (1.00, 1.27)</td>
<td>0.98 (0.82, 1.17)</td>
<td>1.06 (0.98, 1.14)</td>
</tr>
</tbody>
</table>

P = 0.043

P = 0.08

P = 0.043

P = 0.08

P = 0.08

P = 0.14

NOTE: Relative risk of cancer for GPA is based on Cox regression analysis of cancer risk by age, with adjustment for sex and year of birth, and relative risk for 10-unit difference in GPA Mf is restricted to GPA values of 400 × 10^{-6} and below.
direct ligation of the two double-strand break ends. These two pathways are thought to involve numerous molecules. Although extensive polymorphic variation in double-strand breaks repair genes has been reported, only a few common polymorphisms of these genes have been examined in epidemiologic studies for their association with cancer risk (43–48). We are planning to study these genes has been reported, only a few common polymorphisms of pathways are thought to involve numerous molecules. Although direct ligation of the two double-strand break ends. These two

References


Acknowledgments


Grant support: Radiation Effects Research Foundation (RE(R), Hiroshima and Nagasaki, Japan. RE(R) is a private nonprofit foundation funded equally by the Japanese Ministry of Health, Labour, and Welfare and the U.S. Department of Energy, the latter through the National Academy of Sciences. This publication was based on RE(R) Research Protocol 3-87 and supported in part by grants-in-aid for Scientific Research from the Ministry of Health, Labour, and Welfare.

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We thank Kazunori Kodama, Masazumi Akahoshi, and other clinical staff at RER(R) for providing blood samples; Mika Yonezawa for manuscript preparation; and Kazumi Tanabe and Yoshiko Kudo for excellent technical help.

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