Increased T-cell Receptor Mutation Frequency in Radiation-Exposed Residents Living Near the Semipalatinsk Nuclear Test Site

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From 1949 to 1989, 488 nuclear explosions were carried out in Semipalatinsk, and the cancer risk is increased in this region. Measuring somatic-cell mutation frequencies may be a useful tool for evaluating cancer risk within radiation-exposed populations. Here, we report the first evidence of increased T-cell receptor (TCR) mutations in peripheral blood from radiation-exposed residents of Semipalatinsk. The TCR mutation frequency in the highly exposed residents (Dolon and Sarzhal) was significantly higher than in the control group (Kokpekti). There was no statistically significant difference between the control group and the weakly exposed group (Kaynar and Semipalatinsk-city). The TCR mutation assay appeared to be a useful biological dosimeter even after a period of 40 years since radiation exposure. This may be the result of specific conditions, such as the presence of internal exposure.

INTRODUCTION

At the former USSR nuclear test site in Semipalatinsk, Kazakhstan, 488 nuclear explosions were carried out from 1949 to 1989, including 118 atmospheric explosions between 1949 and 1963.¹ During the period, several hundred thousand people were exposed to radioactive fallout. An increased cancer risk has been reported over the region.² ³ As somatic-cell mutation is a major cause of cancer formation, evaluation of somatic-cell mutation is an important tool to predict cancer risk for radiation exposed-populations. Although the conventional assay of chromosome aberration scoring is considered to be the best method for biological dosimetry, it has a number of limitations, including the amount of time required to perform the assay and the cost of analyzing samples from large numbers of patients. Recently, Kyoizumi et al⁴ reported a new method called the T-cell receptor (TCR) mutation assay that required only a small volume of blood, took only several hours to complete, and was shown to be a sensitive indicator of ionizing radiation. One disadvantage of this assay was that the half-life of the majority of mutated cells was roughly 2–3 years, with the number of mutant cells declining gradually to near baseline 10 years after exposure⁵ ⁶. Thus, it was thought that the TCR mutation assay might not be a suitable dosimetry method to assess radiation exposure from several decades ago. Testa et al⁷ recently reported that unstable-type chromosomal aberrations, such as dicentric and rings, were not only good markers for recent exposure, but were also increased in the Semipalatinsk population. Therefore, we hypothesized that the TCR mutation assay might be a useful tool to predict cancer risk for Semipalatinsk residents. We examined the rate of TCR mutation in peripheral blood lymphocytes from exposed residents compared to a control group to determine the somatic mutation frequency. For the TCR to be expressed on the T-cell surface, the complete TCR/CD3 complex is required so that a defect in any of the components of the TCR results in the loss of CD3 expression on the T-cell surface.⁸ Therefore, cell surface expression of CD3 can be used as a marker of TCR mutation rate.

MATERIAL AND METHODS

Subjects and samples

After gaining informed consent and ethics committee approval, peripheral blood samples were obtained from 96 residents living near Semipalatinsk nuclear test sites in August 2003. The population included highly exposed residents (> 1.0 Gy) of the Dolon and Sarzhal villages (n = 40,
4 male and 36 female, 57.3 ± 7.6 years), weakly exposed residents (less than 0.5 Gy) of Kaynar village and Semipalatinsk-city (n = 31, 2 male and 29 female, 55.9 ± 8.1 years), and cancer patients who had received radiation therapy within 5 years and had lived near the nuclear test sites (n = 8, 2 male and 6 female, 56.3 ± 18.5 years). All cancer patients had lived in Semipalatinsk-city, and they were 3 lung cancer patients, 3 metastatic brain tumor patients, and 2 with metastatic bone tumor. This cancer patients group was the positive control group in this study, and the TCR mutations could be attributed to both therapy-radiation exposure and radiation exposure from several decades ago.

The control group consisted of residents who were not exposed or exposed to extremely weak radiation from Kokpekti village (n = 17, 2 male and 15 female, 48.0 ± 12.4 years), because radiation-contaminated clouds in atmospheric explosion did not pass through the village. Age distribution among these populations used for TCR mutation assay showed no statistically significant difference.

**T-cell receptor mutation assay**

Peripheral blood mononuclear cells were isolated by density separation using Ficoll-Hypaque. TCR mutation assay was carried out as previously described. Briefly, 10^6 cells were stained with fluorescein isothiocyanate (FITC)-labeled CD4 and phycoerythrin (PE)-labeled CD3 antibodies (Becton Dickinson, San Jose, CA, USA). After washing, cell suspensions were analyzed by FACScan (Becton Dickinson) with gates set for FITC (FL1) and PE (FL2). Fluorescence data were acquired for 2.0 × 10^5 lymphocytes and the proportion of CD3-negative cells in the CD4-positive lymphocytes subpopulation was measured as the TCR mutant frequency.

**Statistics**

Results were expressed as means ± SD, and statistical significance among groups was determined by one-way analysis of variance followed by Scheffes’s F test.

**RESULTS AND DISCUSSION**

Figure 1 shows the frequencies of TCR mutation among the groups. TCR mutation frequencies were 5.49 ± 1.78 (x 10^-4) in the highly exposed group, 2.88 ± 1.14 (x 10^-4) in the weakly exposed group, 2.90 ± 1.27 (x 10^-4) in controls, and 6.85 ± 1.44 (x 10^-4) in the cancer patient group who had received radiation therapy. The TCR mutation frequency in the highly exposed residents and cancer patients who had received radiation therapy were significantly higher than in the control group (p = 0.00035 and p = 0.00016, respectively). There was no statistically significant difference between the control group and the weakly exposed group (p = 0.72207). Furthermore, the TCR mutation frequency of patients who had received radiation therapy were higher than that of the highly exposed group (p = 0.02044).

Contrary to our findings, Kyoizumi et al. reported no significant increase in TCR mutation frequency in Hiroshima atomic bomb survivors who had received radiation exposure several decades ago. Although we cannot rule out the other possibility that higher aberration rates were caused
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from long-lived lymphocytes and chromosomal instability, we speculate this discrepancy could be due to the different styles of radiation exposure between Hiroshima and Semipalatinsk, with acute exposure in Hiroshima compared to chronic irradiation over a 40-year period in Semipalatinsk. Furthermore in Semipalatinsk, residents were exposed to both internal and external radiation, with significant internal exposure caused by $^{239}$Pu, $^{240}$Pu, $^{137}$Cs, and $^{90}$Sr, which were incorporated into their organs with long half lives.\(^1,2,3\) Therefore, it is not surprising that some mutated T-cells remained even after 40 years. The observation was rather comparable with the increased level of TCR mutation in patients who had an internal deposit of Thorotrast.\(^{11}\)

To our knowledge, this is the first report to show an increased TCR mutation frequency associated with radiation exposure in Semipalatinsk residents. Further studies concerning TCR mutation frequencies, including the relationship between estimated individual absorbed dose and TCR mutation frequency in cancer patients, are needed to clarify the effectiveness of this assay in Semipalatinsk. Estimations of individual doses are important taking into account the essential heterogeneity of radioactive fallout following nuclear tests in the SNTS.\(^{12}\)

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REFERENCES


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