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DNA repair capacity measured by high throughput alkaline comet assays in EBV-transformed cell lines and peripheral blood cells from cancer patients and healthy volunteers

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Abstract

We collected peripheral blood (PB) from 556 patients with various types of cancer who had undergone radiotherapy and from 81 healthy volunteers. We exposed whole PB and Epstein-Barr virus-transformed lymphoblastoid cell lines (EBLs) derived from the PB mononucleocytes to X-irradiation (5 Gy). Using the alkaline comet assay, we measured the immediate DNA damage and, at 15 min, the % residual damage. In PB, the immediate damage was similar in patients and healthy volunteers while the % residual damage (mean ± S.D.) was significantly higher in patients with breast (54.3 ± A23.9), cervical (54.7 ± A23.9), head/neck (56.8 ± A24.4), lung (60.1 ± 23.5), or esophageal cancers (59.5 ± A33.7) than in healthy donors (42.9 ± 19.6) ($P < 0.05$). We did not observe such differences in the EBV-transformed cell lines. Thus, radiation sensitivity of fresh PB cells measured by the alkaline comet assay was related to cancer status.

Keywords: Alkaline comet assay; Cancer patients; Radiotherapy; Automated analysis

1. Introduction

The International Programme on Chemical Safety has proposed guidelines for the use of the comet assay in human biomonitoring [1], and its clinical applications

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have expanded [2–10]. Automated image analysis allows rapid evaluation of the comet assay results and enables routine clinical analysis of fresh blood. A problem with the use of fresh blood, however, is that quite often, only one sample can be obtained, and that precludes repeated testing and the use of additional techniques. This problem can be overcome, and a large pool of cells generated, by transforming mononuclear cells from fresh samples to a lymphoblastoid cell line using Epstein-Barr virus (EBV). Moreover, the enhanced chromosomal radiosensitivity that is sometimes found in fresh blood samples [11–13] is not reflected in EBV-transformed cell lines (EBLs) derived from them [14].

In the present study, we X-irradiated whole PB and EBLs derived from the PB mononucelocytes, and investigated how each responded in the alkaline comet assay. We determined whether the PB and EBLs from patients with breast cancer and those from healthy donors responded similarly and, based on data from healthy donors and patients with various types of cancer (breast, cervical, head/neck, lung, esophageal, and prostate), whether the assay detected a correlation between DNA repair capability and cancer susceptibility.

2. Materials and methods

2.1. Patients and healthy donors

We recruited 556 patients with cancer who had undergone radiotherapy at collaborating Japanese institutions. All the patients, and 81 healthy donors, provided written informed consent to participate in the study, which was approved by the Ethical Committee of the National Institute of Radiological Sciences in June 2001.

2.2. Peripheral blood sample collection

We collected 5 ml blood from an arm vein of each donor into a tube containing anticoagulant (Na heparin). For untransformed cell samples, we used 1 ml of whole blood. We stored the samples in plastic tubes at 4 °C until use.

2.3. EBV transformation

We separated mononuclear cells from the PB samples by centrifugation with Ficoll-Paque at $400 \times g$ for 30 min at room temperature. We added $(10–30) \times 10^6$ cells to 1 ml B95-8 supernatant for EBV infection, incubated the mixture for 2 h at 37 °C, and then added 2 ml RPMI medium containing 20% FCS. We incubated the cells in a 5% CO_2 atmosphere for 2 weeks, suspended them in RPMI 1640 containing 20% FCS and 5% DMSO, and stored them in liquid nitrogen following MEXT guidelines [15]. Before performing the assay, we thawed the frozen cells rapidly, and cultured them for 1 week.

2.4. Alkaline comet assay

We conducted the alkaline comet assay as described by Mayer et al. [16], with minor modifications, and followed the guidelines of Tice et al. [3]. We treated cell suspensions (3×10^5 cells/ml) or whole blood samples (50 μl) with 5 Gy of X-rays using a PANTAC 320S (Shimadzu, Japan). To determine initial damage (ID) or (after culturing the cells at 37 °C for 15 min) residual damage (RD), we embedded cell suspensions in 0.7% low melting point agarose at ∼42 °C and spread 50 μl of the suspension on CometSlides (Trevigen, Gaithersburg, MD). After the agarose layer solidified, we immersed the slides in lysis buffer (100 mM EDTA, 10 mM Tris, 2.5 M NaCl, 1% sodium lauryl sarcosinate, 1% Triton X-100 and 10% DMSO; pH 10) for 1 h at −4 °C, then in alkaline solution (pH 13.2; 0.3 M NaOH, 1 mM EDTA) for 20 min at 4 °C, and then transferred them to a horizontal electrophoresis apparatus containing alkaline electrophoresis buffer (1 mM EDTA, 0.3 M NaOH). Electrophoretic separation proceeded at 1.1 V/cm for 20 min at 4 °C. We neutralized the slides with 0.4 M Tris–HCl (pH 7.4) and stained them with 50 μl SYBR Green diluted 1:10,000 in TE buffer (10 mM Tris–HCl, 1 mM EDTA; pH 7.5). We evaluated 201 cells per slide using a fluorescence microscope (Axioplan 2 Imaging, Carl Zeiss, Tokyo) and image analysis software (Metafar CometScan and Comet Imager, MetaSystems, Altlussheim, Germany). We quantified the DNA damage as mean tail moment (MTM), defined as the product of the mean fraction of DNA in the tail (total tail intensity/total comet intensity) and the mean tail migration (distance from the tail center of gravity to the head center of gravity) [17,18]. We calculated % RD as 100 × (MTM 15 min after irradiation minus MTM of untreated cells)/(MTM immediately after irradiation minus MTM of untreated cells).

2.5. High-throughput system

We routinely scanned eight samples simultaneously using a Metafer4/Cometscan scanning system [19], comprising the MetaCyte software package, a Zeiss Axioplan 2 Imaging MOT microscope, a Marzhauser motorized scanning stage for eight slides, a CCD camera, a trackball, and a PC central unit with monitor and printer. The system was fully automated, including the scanning procedure. Scoring a slide took an average of 1–2 min for non-irradiated cells and 15–20 min for irradiated cells.

2.6. Statistical analysis

We analyzed the comet assay parameters as continuous variables based only on internal comparisons of study patients. We used the nonparametric Kruskal–Wallis test to determine statistical differences in mean tail moment (ID or RD values) between healthy donors and cancer patients and the