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Markers of the Individual Radiosensitivity of Human Extraembryonic Cells in vitro

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Abstract—The genotoxic effects of exposure to ionizing radiation during the early stages of human embryonic development can be fatal. Despite this, the radiosensitivity of human embryonic and differentiated extraembryonic cells is poorly studied. In this work, the efficiency of a DNA double-strand break repair in human extraembryonic fibroblasts was investigated. It was shown that the repair of radiation-induced DNA damage in human extraembryonic fibroblasts is likely to reflect the ability of these cells to repair spontaneous DNA double-strand breaks.

Keywords: γH2AX foci, micronuclei, DNA double-strand breaks, DNA repair, extraembryonic fibroblasts, individual radiosensitivity

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INTRODUCTION

The proficiency of DNA repair is dependent on the cell type and determines the individual sensitivity of cells to various mutagens, including ionising radiation. The process of DNA repair during the foetal development remains relatively poorly studied (Pachkowski et al., 2011). Therefore, the aim of this study was to assess the efficiency of DNA double-strand break repair, and to analyze the sensitivity of human primary extraembryonic fibroblasts to ionizing radiation.

MATERIALS AND METHODS

In this study, 18 primary human extraembryonic fibroblast lines, which were isolated from gestational sacs of medical abortuses, were used. The biological material was collected upon the informed consent of female donors. The cells were cultured according to a standard method except for minor changes (Rooney and Czepulkowski, 1992). Extraembryonic human fibroblasts were irradiated at a dose of 1 Gy using a γ-ray therapeutic Theratron Equinox irradiator (Cancer Research Institute, Tomsk). The levels of DNA double-strand breaks were estimated using the number of γH2AX foci. For immunostaining, γH2AX primary mouse monoclonal and rhodamine-conjugated secondary rabbit antibodies diluted to 1 : 500 in 3% FBS (Novus, United States) were used. For nuclei staining, DAPI at a concentration of 0.3 μM was used (Sigma, United States). To prevent fading, the stained samples were mounted using Vectashield (Vector Labs, United States). The frequency of centromere-negative micronuclei, which arise from chromosomal DNA containing unrepaired DNA double-strand breaks, was assessed using a micronucleus test combined with the fluorescent in situ hybridization (FISH) using pancentromeric DNA probes as previously described (Fenech, 2007). The samples were analyzed using an Axio Imager Z2 microscope (Zeiss, Germany) coupled with a Metafer platform (Metasystems, Germany). Statistical analyses were carried out using the Mann–Whitney test and Statistica 6.0 software (Statsoft).

RESULTS

The number of γH2AX foci in the control varied from 0.56 to 1.93 foci per cell, and the mean number of foci per cell was 1.01 ± 1.42. The number of γH2AX foci 30 min after irradiation increased significantly to 10.21 ± 5.17 foci per cell ($p = 0.0006$) followed by its reduction to the level comparable to that of the control at 24 h after the treatment (0.77 ± 0.36 foci/cell). The mean repair efficiency of radiation-induced DNA double-strand breaks, which was estimated as the percentage of γH2AX foci that disappear between 30 min and 24 h after the treatment, was calculated at 97% in

a number of cell lines ($n = 9$). The frequency of centromere-negative micronuclei increased significantly after irradiation and was 45.33 ± 19.23 compared to $3.86 \pm 2.12\%$ in the control ($p = 0.000002$).

A search for markers of individual radioresistance in human cells, among which the efficiency of DNA double-strand break repair is considered, is an important task. In this work, however, no statistically significant correlation was observed between the number of residual γH2AX foci 24 h after irradiation and the frequency of radiation-induced micronuclei. We have previously demonstrated that the endogenous levels of γH2AX foci may serve as the marker of individual radioresistance in the lymphocytes of peripheral blood obtained from adult donors (Melnikov et al., 2013). In this work, however, no correlation between the endogenous and radiation-induced levels of γH2AX foci and the radiation-induced frequency of centromere-negative micronuclei was observed. It is possible that the responses of lymphocytes and extraembryonic fibroblasts to ionising radiation are cell-type specific. On the other hand, the frequency of radiation-induced centromere-negative micronucleus formation was correlated with that of the one observed spontaneously ($R = 0.52$, $p = 0.038$). It is likely that the ability of extraembryonic fibroblasts to repair radiation-induced DNA lesions reflects the repair capacity of endogenous DNA double-strand breaks. In somatic cells, DNA repair efficiency and radiosensitivity depend on a number of different factors, including the age of an individual. However, we observed no relationships between the term at which the pregnancy was terminated and the levels of γH2AX foci and micronuclei in extraembryonic fibroblasts, thus excluding the age-dependent effect on the efficiency of DNA repair.

CONCLUSIONS

This work provides the first example in which the relationships between the levels of the spontaneous γH2AX focus formation and the frequency of radiation-induced cytogenetic damage in human extraembryonic fibroblasts are studied. Comparative analyses between the negative effects of radiation and their DNA repair capacity demonstrate that the efficiency of repairing spontaneous DNA double-strand breaks reflects the repair capacity of radiation-induced DNA lesions in the fibroblasts.

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