Ionizing Radiation-Induced Genomic Instability in CHO cells Is Followed by Selection of Radioresistant Cell Clones

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The DNA comet assay (neutral version) showed that irradiation of CHO cells in a dose of 1 Gy (γ-radiation, ^{60}Co) is followed by an increase in the degree of DNA fragmentation. These changes were observed immediately after irradiation and on days 7-21. On days 2-4 and 23-28 after irradiation, the degree of DNA fragmentation in the descendants of irradiated cell did not differ from that in control samples. The increase in the degree of DNA fragmentation on days 7-21 probably results from induction of apoptosis. This assumption is confirmed by the study of cell death. The sensitivity of cells to repeated irradiation in a dose of 10 Gy significantly increased on days 9, 11, 16, and 18 after irradiation. However, these cells were resistant to repeated irradiation on days 21-28. Our results confirm the hypothesis that genomic instability is a selective mechanism, which mediates the formation of radioresistant cell clones.

Key Words: *genomic instability; ionizing radiation; DNA damage; apoptosis; cell radiosensitivity*

The phenomenon of genomic instability manifested in increased frequency of newly formed genetic abnormalities is now intensively studied [3,6-8]. As any biological process, genomic instability may have a negative (neoplastic transformation, mutation, chromosomal instability, gene amplification, *etc*.) or positive role in the biological system (formation of hypervariable regions in the antibody paratope, appearance of alternative pathways for adaptation to environmental conductions due to genomic changes, *etc*.). Physicochemical changes in the molecule develop rapidly after exposure to ionizing radiation. However, the resultant changes can be observed in the delayed period after treatment (days, months, or years). Studying the mechanisms of these changes is an urgent problem of radiobiology.

Here we studied the delayed molecular and cellular effects of ionizing radiation in CHO cell progeny.

MATERIALS AND METHODS

Experiments were performed on culture of epithelial cells from Chinese hamster ovary (CHO line). The cell doubling time was 18-20 h.

The cells were cultured in complete DMEM medium containing 10% fetal bovine serum (FBS), 1% L-glutamine, and antibiotics (penicillin and streptomycin). The cells were grown in a CO_2 incubator at 37°C and 5% CO_2.

The cells were exposed to γ-irradiation in doses of 1 and 10 Gy depending on the experimental

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conditions. The exposure rate was 1.5 Gy/min. The study was conducted using an Altai device (^{60}Co as a γ-radiation source).

The degree of DNA fragmentation was evaluated by electrophoresis of DNA with agarose-immobilized single cells at neutral pH (DNA comet assay, neutral version) [1]. The number of DNA breaks (damage) in this method was directly proportional to the percentage of DNA migrating from the nuclear region and length of DNA migration in DNA electrophoresis with agarose-immobilized and lysed single cells. The slides were stained with acridine orange. The DNA comet assay was performed using a Lyumam I-2 luminescence microscope (LOMO) with a Mikmed-1600-3f system for microphotograph visualization (LOMO). The DNA comet assay was conducted with CometScore software (TriTek Corp.). The DNA comet tail moment (Olive tail moment) was calculated as a product of the distance from the nucleus center to the density center of the comet tail by DNA percentage in the tail.

Changes in radiosensitivity of irradiated cell progeny were estimated from the incidence of double-strand DNA breaks after repeated irradiation with 10 Gy at 4°C. Double-strand DNA breaks were evaluated by the DNA comet assay under neutral conditions.

The ratio of apoptotic cells was measured by the DNA halo technique [9]. Low-molecular-weight DNA fragments formed during apoptotic internucleosomal degradation of DNA easily diffused into agarose gel and formed a typical "halo" around the cell nucleus. This method allowed us not only to evaluate the degree of cell death, but also to differentiate the mechanism of cell death (apoptosis or necrosis). The slides were stained with a fluorescent dye SYBR-green. The degree of cell death was determined under a Lyumam R-8 microscope (LOMO).

The results were analyzed by Statistica 6.0 software. The data are expressed as mean values (3 independent experiments) and standard errors.

RESULTS

The radiation-induced genetic instability is observed in several generations of cells after exposure to ionizing radiation. These changes are manifested in increased frequency of newly formed genetic abnormalities, including mutations, chromosomal aberrations, chromosomal instability, neoplastic changes, and apoptosis [4,8].

Three independent experiments showed that the degree of DNA fragmentation increases by 1.5-2 times immediately after irradiation of cells in a dose of 1 Gy. The observed changes were mainly related to double-strand DNA breaks. On days 2-4 after irradiation, the degree of DNA fragmentation in irradiated cell progeny did not differ from the control (Fig. 1). The degree of *de novo* DNA fragmentation significantly increased on day 7 after irradiation, which served as the sign of radiation-induced genomic instability. The increase in the degree of DNA fragmentation was observed by the 21st day after irradiation. These changes were particularly pronounced on days 11 and 18. The degree of DNA fragmentation in irradiated cell progeny was shown to decrease and did not differ from the control on days 23-28 after irradiation (Fig. 1).

Cell apoptosis was studied by the DNA halo technique. In the progeny of irradiated cells, the ratio of dying cells significantly increased on days 7-21 (Fig. 2). Similarly to DNA fragmentation, the percentage of these cells did not differ from the

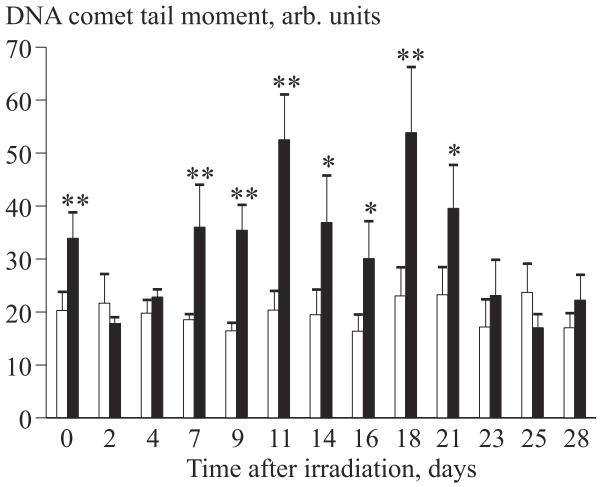

Fig. 1. Degree of DNA fragmentation in CHO cells at various stages after irradiation with 1 Gy. Here and in Figs. 2 and 3: light bars, control; dark bars, irradiation. *p<0.05 and **p<0.01 compared to the control.

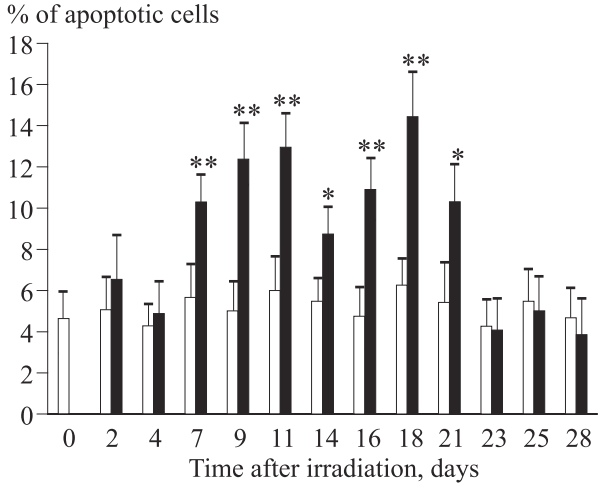

Fig. 2. Apoptosis in the progeny of irradiated CHO cells (1 Gy).

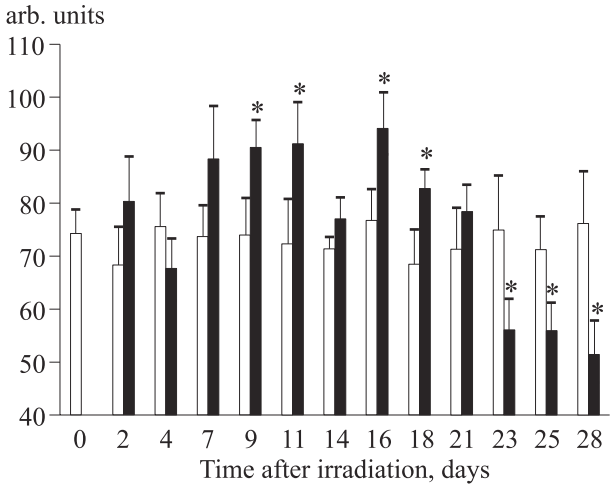

Fig. 3. Radiosensitivity of DNA in the progeny of CHO cells after irradiation with 1 Gy. Ordinate: increment of the tail moment (10 Gy: initial level).

control on days 23-28. The increase in the degree DNA fragmentation (DNA comet assay) is probably related to the presence of apoptotic cells with highly fragmented DNA.

Figure 3 shows changes in radiosensitivity of irradiated cell progeny. The data are presented as the difference (increment) between the DNA comet tail moment after repeated irradiation (10 Gy) and DNA comet tail moment without additional irradiation. The sensitivity of cells to repeated irradiation with 10 Gy significantly increased on days 9, 11, 16, and 18 after irradiation. The opposite effect was revealed on days 21-28 (resistance to repeated irradiation).

Our results suggest that genomic instability is a selective mechanism for the adaptation of cells to variations in environmental conditions. It results in a progressive normalization of cell function. Changes in the genome of irradiated cells contribute to destabilization of cell metabolism, which results in cell death or induction of cascade reactions to restore metabolic balance (stabilization of the cell) [2]. The concentration of reactive oxygen species increases in the progeny of irradiated cells [5]. They can induce DNA damage, including double-strand breaks. Selection in the population of irradiated cell progeny is directed to elimination of oxidative stress-sensitive cells and adaptation (and/or survival) of cells with high antioxidant activity and strong enzymatic system of DNA repair.

We conclude that the progeny of irradiated CHO cells (1 Gy) is characterized by an increase in the degree of genomic damage, high ratio of apoptotic cells, and elevated sensitivity of cells to repeated irradiation (days 7-21). The degree of genomic damage and ratio of apoptotic cells do not differ from the control. The progeny of irradiated cells is more resistant to repeated irradiation (days 23-28).

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