



Correction of genetic instability of the genome by fractional irradiation of MDBK cells

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ABSTRACT

With respect to given contradictory data in radiobiology, that according to some authors, irradiation of cells of plant and animal origin initiates DNA changes and according to others, repeated irradiation of cells in small doses has a stimulating effect on metabolism without increasing the purity of mutations, we conducted the present study, with the aim to study the stability of the genome of MDBK cell line against a background of single and double (fractional) irradiation. The object of the study was a continuous MDBK cell line (Madin-Darby Bovine Kidney Cell, cattle, kidney), which was grown in medium 199, manufactured by Federal State Budget Institution FCTRB-ARRVI (Kazan) with 10% bovine serum and the addition of antibiotics (benzylpenicillin sodium salt, kanamycin at 100 units/ml) (streptomycin sulfate at 100 µg/ml) according to standard procedure. A monolayer culture of MDBK cells in the logarithmic growth phase (3-4 days after culture) served as contacting cells. As a result, a radio-modified cell line, called MDBK-2, was obtained. Moreover, in an unirradiated MDBK cell culture, the level of chromosomal breakdowns was in the range of 1.03-3.1% with respect to the total number of studied anaphases. Based on the obtained data, it can be concluded, that during prolonged cultivation of MDBK cells, irradiated in a small (adaptive) dose and re-irradiated in 3-minute higher dose (5.95 Gy), there is a significant decrease in the yield of aberrant cells, induced by a test dose of γ -rays.



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INTRODUCTION

The results of numerous studies in the field of molecular, cellular radiobiology and biotechnology showed, that with simultaneous and fractionated irradiation of cell cultures (lymphocytes, splenocytes, fibroblasts, etc.), a similar pattern is observed, well known, when γ radiation acts on mammalian cells, as a hypersensitivity and induced radioresistance phenomenon with an increase in the yield of chromosome aberrations (Oka *et al.*, 1989; Brabander *et al.*, 2009). In experiments with fractionated irradiation of corneal brain cells and mouse embryonic stem cells was shown, that the expression of the genes of the p53 signaling pathway, induced

by DNA damage, is detected in the irradiated cells, or it leads to a cell cycle block, or apoptosis, or DNA repair (Viñas *et al.*, 2004). Chronic irradiation of splenocytes, blood lymphocytes, on the one hand, inducing DNA change and genome instability, increase in survival due to damage repair and the development of adaptation processes in irradiated cells, and on the other hand, to exclude multiple irradiation of phytogetic and zoogenic cells in small and large doses has a stimulating effect on their growth and development, without increasing the frequency of somatic mutations (Monica *et al.*, 2012; Zai *et al.*, 2013; Singh *et al.*, 2015).

Based on the above, it seemed relevant to study the change in the state of damage to cell chromosomes, induced by irradiation in small (adaptive) and increased (testing) doses of ^{137}Cs γ -rays.

MATERIALS AND METHODS

The object of the study was a continuous MDBK cell line (Madin-Darby Bovine Kidney Cell, cattle, kidney), which was grown in medium 199, manufactured by Federal State Budget Institution FCTRB-ARRVI (Kazan) with 10% bovine serum and the addition of antibiotics (benzylpenicillin sodium salt, kanamycin at 100 units/ml) (streptomycin sulfate at 100 $\mu\text{g}/\text{ml}$) according to standard procedure. A monolayer culture of MDBK cells in the logarithmic growth phase (3-4 days after culture) served as contacting cells. Cells were irradiated on a Puma installation ^{137}Cs VO Isotope, (Russia) at an exposure dose power of $2.58 \times 10^{-5} \text{ A}/\text{kg}$ in two different modes: single with a dose of 6 Gy and double with the same dose at the beginning: 0.05 Gy then re-irradiation in an interval of 3 min, irradiation with a dose of 5.95 Gy. As a result, a radio-modified cell line, called MDBK-2, was obtained.

The discovery of such effects of exposure of ionizing radiations as a decrease in sensitivity to them (adaptive response) or an increase in radiosensitivity dictates the need to study the mechanisms of formation of long-term effects of radiation exposure. A number of researchers consider, that the mechanisms of changing cellular radiosensitivity are closely related to the mechanisms of induction and realization of another irradiation effect - radio-induced instability of the genome, accompanied by an increase in the frequency of occurrence of chromosomal aberrations and gene mutations in subsequent generations, leading to increased cell death (Udalova *et al.*, 2015).

Given these considerations, we carried out cytogenetic studies of the initial (MDBK) and radio-modified (MDBK-02) cell cultures.

Control preparations were held during the irradiation time in borate buffer at 4°C . Cell survival was assessed by their growth curves after irradiation and by determining the density of cells in a fixed field of view under a microscope (magnification $\times 200$) (Singh *et al.*, 2015).

DNA fragmentation (chromosome breaks - PX) in the cells directly (after 30-60 s, 4°C) and 1 hour after irradiation (1-hour cell incubation at 37°C in 199 medium with CRS serum) was determined by the modified method of single cell DNA electrophoresis at neutral pH (basic conditions for electrophoresis: $1\text{V}/\text{cm}$, 4°C 20 min).

Statistical processing of the results was carried out using the Statistica 6.0 program. The results of studies, carried out in 3-series MDBK are presented as arithmetic mean \pm standard error. Student's t-test was used to assess the differences (Carrasco-Pancorbo *et al.*, 2008; Mahmoudi *et al.*, 2014; Rao and Kumar, 2015)

RESULTS AND DISCUSSION

It was found, that when irradiating a cell culture in a monolayer in the dose range from 0.05 to 1.0 Gy, the survival of irradiated cells decreases. At doses, starting from 6.0 Gy and higher, a gradual decrease in survival followed, and at doses of 9.0-10.0 Gy, a significant (1.83-2.42 times) increase in the death of MDBK culture cells was observed.

The results of the second series of experiments, carried out by double irradiation of MDBK cells showed, that fractionated cell irradiation had a radiomodifying effect, increasing both the density and concentration of cells, and their proliferative activity.

The results of cytogenetic studies of single and double γ -ray irradiated cells in small and large doses showed, that a single irradiation of cells, depending on the irradiation dose, starting with a dose of 3.0 Gy and higher, leads to a significant increase in the number of chromosomal aberrations in the form of bridges, fragments and cells with double minichromosomes (DMC). Moreover, in an unirradiated MDBK cell culture, the level of chromosomal breakdowns was in the range of 1.03-3.1% with respect to the total number of studied anaphases.

It is important, that the increase in the number of chromosomal aberrations at low doses of irradiation (0.05-1.0 Gy) occurred, mainly due to bridges (1.13%) and double minichromosomes (1.07) and less due to fragments, which testifies an increased ability of chromosomes to repair damage. With an increase in the irradiation dose, a change in the nature and correlation of chromosomal abnormali-

Table 1: Cytogenetic characteristics of the initial (MDBK) and radio modified cells (MDBK-2) under long-term cultivation

| Passage | The number of cells with chromosomal aberrations (%) | | | | | |
|---------|--|-----------|-----|-----------|------------|------------|
| | MDBK | | | MDBK-2 | | |
| | Bridges | Fragments | DMC | Bridges | Fragments | DMC |
| 4 | 1.03±0.10 | - | - | 1.31±0.49 | 1.73±0.27* | 0.9±0.13 |
| 14 | 1.01±0.05 | - | - | 1.29±0.29 | 1.21±0.31* | 0.51±0.15 |
| 20 | 1.00±0.07 | - | - | 1.17±0.19 | 0.53±0.07* | 0.23±0.06 |
| 25 | 1.01±0.01 | - | - | 1.11±0.23 | 0.09±0.01* | 0.03±0.003 |
| 30 | 1.02±0.03 | - | - | 1.03±0.31 | - | - |

*-P<0.05; -lack of effect

ties options occurred. So, with a single irradiation of the culture with doses of 6.0 Gy and higher, an increase in chromosomal aberrations in the form of breaks (fragmentations) of chromosomes up to 9.19% (P<0.001) was observed, against 1.03-3.1% in the control. A significant excess of the content of aberrant cells with fragments compared to bridges indicates a reduced ability of chromosomes to associate (damage repair) with the resulting fragments or with each other.

Double (0.05 Gy, interval + 3 min 3.0; 5.0; 7.0 and 10.0 Gy) irradiation had a significantly smaller violation of the chromosomes of MDBK cells, with a tendency to decrease chromosomal aberrations in the form of bridges, fragments and double minichromosomes and the most important is the decrease in the number of fragments, caused by chromosome break, leading to apoptotic cell death. Probably, the increase in the radioresistance of cells of the studied culture upon repeated irradiation occurs as a result of the inclusion of repair processes when a certain level of cell damage is reached.

In the 3rd series of experiments, the question was studied - how much radioinduced chromosome abnormalities are inherited in the next generations of cells during long-term culture passages, i.e. whether these abnormalities are stable or temporary, not associated with a violation of the genome of cells.

The results of cytogenetic studies are presented in the Table 1. The experiments were carried out in 3 replicates and the given figures are average over 3 replicates. The Table 1 shows, that at the 4th passage of cultivation, the cell population turned out to be heterogeneous with respect to the quantitative and qualitative composition of chromosomes, which was expressed in the excess of the number of altered chromosomes in the form of bridges, fragments, and double minichromosomes compared to the control. However, starting from 14th passage, there was a

tendency to decrease the number of aberrant cells, and by the 23rd passage their number slightly differed from the control (P>0.05). By the 30th passage, the heterogeneity by the number of cells with altered chromosomes (fragments, bridges and double minichromosomes) was similar to that of the control values.

Based on the obtained data, it can be concluded, that during prolonged cultivation of MDBK cells, irradiated in a small (adaptive) dose and re-irradiated in 3-minute higher dose (5.95 Gy), there is a significant decrease in the yield of aberrant cells, induced by a test dose of γ -rays.

The obtained results may testify, that the development of an adaptive response (AR) in cycling MDBK cells after double irradiation in an adaptive and tested dose is accompanied by an increase in the DNA repair efficiency due to fragmentations and chromosome breaks, caused by radiation, by reuniting the ends of its strands at the break sites. Probably, the initiation of AR leads mainly to the activation of a slower (during long-term cultivation) by stable repair of DNA chromosome breaks (fragmentations) by homologous recombination.

The study of the karyological stability of doubly γ - rays irradiated cells (MDBK-2) during long-term cultivation under suspension conditions showed, that at the level of 15th passage there was a slight decrease in the value of the modal class (27%) while maintaining its number (40 chromosomes) with an interval of variation of the number of chromosomes in cells from 38 to 77.

CONCLUSION

Thus, the obtained data testify a high level of karyological stability of the MDBK-2 cell population, obtained on a γ -irradiation decontaminated nutrient medium and subjected to double irradiation with γ -rays in adaptive (0.05 Gy) and tested (5.95

Gy) doses. Based on the above data, the MDBK-2 cell culture can be classified as a karyotype-stable cell line.

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