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Original research paper

SUBLETHAL X-RAY IRRADIATION INDUCES GENETIC INSTABILITY IN HUMAN ENDOMETRIAL MESENCHYMAL STEM CELLS AT THE KARYOTYPE LEVEL*

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Abstract. *We aimed to study the karyotype structure of human adult stem cells after X-ray irradiation. Cultured endometrial mesenchymal stem cells (eMSC) isolated from desquamated endometrium of menstrual blood of the healthy woman were the object of this research. The eMSC at the 9th passage were irradiated with the sublethal X-ray dose (5Gy). Irradiated cells were cultivated under standard conditions and, at the 13th passage, they underwent to the karyotyping assay with the G-banding technique. The cytogenetic analysis revealed that the progeny of irradiated cells exhibited genetic instability. Most of analyzed cells had chromosomal abnormalities. Karyotypic changes were manifested mostly as aneuploidy and near-centromeric and other breaks. Within a particular karyotype, various chromosomes may be involved in breaks. Chromosome 1, 4 and X were not involved in chromosomal rearrangements randomly. About 80% of the control not irradiated eMSC metaphase plates had the standard karyotype at the same 13th passage. Deviations from the normal karyotype were random. Chromosomal breaks were not observed. Our findings show that sublethal X-ray irradiation of eMSC resulted in multiple disorders of the genetic apparatus at the karyotype level. The cells that survived irradiation entered replicative senescence and avoided immortalization or transformation.*

Key words: *Human stem cells, irradiation, X-rays*

1. INTRODUCTION

Ionizing radiation is an important type of exogenous stress that can induce changes in the cell genome [1]-[4]. Results of X-ray and gamma radiation can be gaps in one or two strands of DNA, the damage of its secondary structure, the formation of intra- and intermolecular crosslinks, the destruction of certain amino acids in proteins and nucleic acids bases. The modified DNA structure underlies the irradiation mutagenic and carcinogenic effect. The cells that retained mitotic activity had various types of karyotypical disorders: nuclear pyknosis, preudo-mitosis, cell division disorder and various chromosomal abnormalities. Morphological aberrations are presented as acentric fragments, dicentric and ring chromosomes, chromatid fragments and spaces [5]-[7]. Human lymphocytes irradiated by gamma-rays in the dose of 4.27 Gy exhibited changes in the karyotype structure (centric rings and fragments) in 90% of the cells [8]. At X-ray irradiation with the dose of 1 Gy, 28% of the cells had changes in the karyotype structure whereas, in the control, the adjustment met only 3% of the cells [9]. According to the studies on the Chernobyl accident, the number of human lymphocytes containing chromosomal aberrations was 4-6 times higher than the control level. The most common types of aberrations were single and paired fragments [10]. In liquidators irradiated with doses higher than 20 Gy, the frequency of dicentric chromosome and chromosomal rings significantly exceeded the control values. The cytogenetical analysis identified that chromosome 4 was repeatedly involved in rearrangements as the result of irradiation [11]-[14]. These data are of particular importance in connection with the use of X -rays in the clinic.

The purpose of this work was to study the influence of the X-ray sublethal dose on the karyotype of human mesenchymal stem cells in culture.

2. MATERIALS AND METHODS

2.1. Biological material

The study was performed on cultured endometrial mesenchymal stem cells (eMSC) derived from desquamated endometrium in the menstrual blood

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[15]. The cells were cultivated in the DMEM/F12 medium (Gibco, United States) with 10% bovine fetal serum (HyClone, United States), 1% antibiotic-antimycotic solution and 1% GlutaMAX (Gibco, United States) with 5% CO_2 at 37°C.

2.2. Irradiation

MSC at the 9th passage were irradiated with X-rays in the dose of 5Gy. The dose is sublethal and about 20 % cells remained viable. Irradiated cells were cultivated under standard conditions. At the 4th passage after irradiation (in total, cells have gone through 13 passages), they underwent to karyotyping assay with the G-banding technique.

2.3. G-banded karyotyping

Colcemid (stock solution 10 mg/mL) (Sigma, United States) was added to the cell culture that reached a confluence of 80% for 1-1.5 h. at 37°C. Then, the medium was removed, cells were detached with 0.05% trypsin (Biolot, Russia) and centrifuged (1300 rpm). The pellet was suspended and treated with 0.075M KCl hypotonic solution for about 1 h. The cell suspension was centrifuged, the sediment was resuspended and fixed by methanol mixed with acetic acid 3:1. The fixator was changed three times, the total fixation time was 1.5 h. The fixed cell suspension was dropped on cold and wet slides. The slides were air-dried for one week. Then, the chromosomes were G-banded with the Giemsa stain (Fluka, United States) after the preliminary trypsinization. Metaphase plates with well-spread chromosomes were assayed under the microscope Axio Scop (Carl Zeiss, Germany), objective 100×, ocular 20×. The chromosomes were identified according to the international nomenclature [16] and the atlas of human chromosomes [17].

2.4. SA-β-Gal activity

The enzyme activity is a marker of cellular senescence. 10^5 cells were plated in 3 cm Petri dishes and cultivated for 3 days. Then, the medium was removed, cells were washed with PBS (Sigma, United States), fixed with 4% formaldehyde and stained with senescence-galactosidase staining kit (Cell Signalling, United States) according to the manufacturer's instructions. SA-β-Gal activity was detected by cell blue staining and visualized under a microscope.

3. Results and discussion

The cytogenetic analysis revealed that the progeny of irradiated cells exhibited genetic instability. Most of analyzed cells had chromosomal abnormalities (Table). Most types of karyotypic changes were aneuploidy evident as one or three chromosome copies and chromosome breaks both in near-centromere regions and other regions (see Table 1, 2) . Chromosomes 1, 4 and X were engaged in chromosomal rearrangements not randomly (see Figure 1). It should be noted that the medical examination of Chernobyl liquidators revealed chromosome abnormalities in the peripheral blood lymphocytes with chromosome 4 being the most frequently involved [14].

Table 1. Changes in copies of chromosomes and chromosome arms in eMSC survived X-ray irradiation

CHROMOSOME NUMBER	QUANTITY OF METAPHASES WITH COPIES CHANGES IN 30 ANALYZED PLATES	
	1 copy	3 copies
1	-	1; 3p*; q**
2	-	1
3	-	1
4	2	-
5	5	5; qter***
6	-	2
7	-	2
8	3	-
9	6	-
10	2	2
11	3	3; q
12	6	-
13	6	3
14	2	5
15	-	4
16	4	1
17	2	2
18	4	2
19	-	-
20	3	3
21	9	-
22	9	-
X	7	-

* p—chromosome short arm; ** q—chromosome long arm; ***qter-terminal part of chromosome long arm

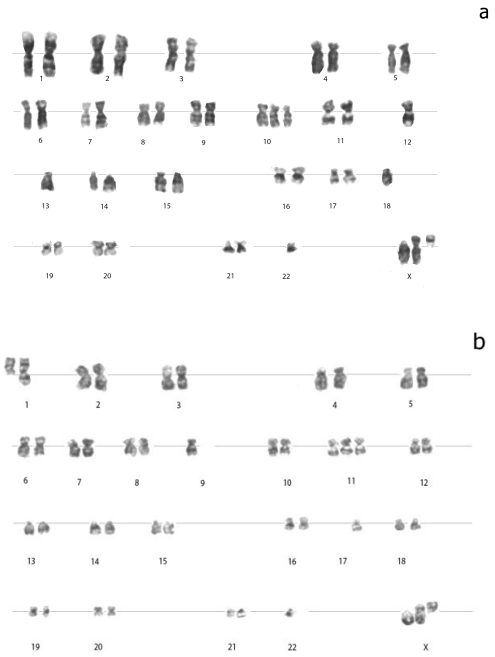

Figure 1. Karyotypes eMSC 4th passage after X-ray irradiation. In total, cells have gone through 13 passages. a -near-centromere breakage of chromosome X; trisomy of chromosome 10; monosomy of chromosomes 12,13,18,22. b - near-centromere breakage of chromosomes 1, X; trisomy of chromosome 11; monosomy of chromosomes 9, 17 and 22.

Table.2. Karyotype changes in eMSC survived X-ray irradiation

PLATE NUMBER OF	CHROMOSOMES HAVING BREAKS									TRANSLOCATION	COPY CHANGES IN CHROMOSOMAL MATERIAL	
	1	2	3	4	5	6	10	12	X		Monosomy	Trisomy
1	-	-	-	p+q	-	-	-	-	-	-	+	+
2	-	-	-	-	-	-	-	-	-	-	+	+
3	delq	-	-	-	-	-	-	-	-	-	+	+
4	-	-	-	-	-	-	-	-	delqter	-	-	+
5	delp; 2N+q	-	-	-	-	-	-	-	delq	-	+	+
6	-	-	-	-	-	-	-	-	-	-	+	+
7	p+q	2N+q	-	-	-	-	-	-	delqter	-	+	+
8	-	-	-	-	-	-	-	-	-	tr 14:17	+	+
9	-	-	-	-	-	-	-	-	-	-	+	+
10	-	-	-	-	-	-	-	-	-	-	+	-
11	delqter	-	-	delq	-	-	-	-		-	+	+
12	-	-	-	-	-	-	-	delqter	-	-	+	+
13	-	-	-	-	-	delqter	-	-	p+q	-	+	+
14	-	-	-	-	-	-	-	-	p+q	-	+	+
15	-	-	-	-	-	delqter	-	-	-	-	+	+
16	delpter	-	-	-	-			-	-	-	+	+
17	delqter	-	-	delqter	-	-		-	-	-	+	+
18	delq	-	-	-	-	-		-	-	-	+	+
19	-	-	-	-	-	-		-	p+q	-	+	+
20	-	-	-	-	-	-		-	-	-	+	+
22	-	-	delpter	-	-	-		-	-	-	+	+
23	-	-	-	delqter	-	-	delqter	-	-	-	+	+
24	-	-	-	p+q	del p+2/3 q	-	-	-	-	-	+	+
25	-	-	-	-	-	-	-	-	p+q	-	+	+
26	-	-	-	-	-	-	-	-	-	-	+	-
27	-		-	-	-	-	-	-	-	-	+	+
28	-		-	-	-	-	-	-	-	-	+	+
29	-	-	-	-	-	-	-	-	-	-	+	-
30	-	-	-	-	-	-	-	-	-	-	+	+

p—chromosome short arm; q—chromosome long arm; ter-terminal part of chromosome; del – deletion; tr – translocation; p+q – near centromeric break without chromosomal material loss; 2N – 2 normal copies; ter – terminal part of del p+2/3 q – deletion of p-arm and 2/3 of q-arm

The control, not irradiated eMSC at the 13th passage, had normal karyotype in 80% of the cases. Deviations from the normal karyotype were random. Chromosomal breaks were not registered (see Figure 2).

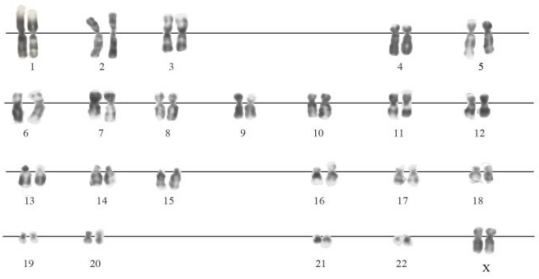

Figure 2. Normal G-banded eMSC karyotype, passage 13

Interestingly, that pattern of karyotyping changes evoked by X-rays in eMSC was later comparable with cytogenetic changes in these cells after the heat shock [18].

We provide evidence that the sublethal X-ray irradiation of eMSC results in the destabilization of the karyotype. Karyotypic changes in these cells did not cause their immortalization or transformation *in vitro*. Cells exposed to irradiation resumed proliferation and were able to divide during 6 passages after treatment. Totally they underwent 15 passages and then stopped to proliferate. Most cells (90%) in these cultures were positively stained with SA-β-Gal (see Figure 3A) which is an indication of the replicative senescence. At this moment untreated cells underwent approximately the same population doublings and also entered into the replicative senescence (Fig. 3B).

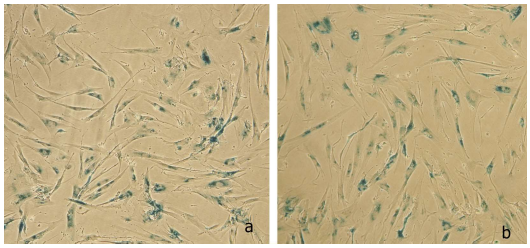

Figure 3. SA-β-Gal staining. A – long-term cultivated of eMSC survived X-ray irradiation; B—long-term cultivated untreated eMSC

Our findings show that X-rays induce karyotypic abnormalities involved preferentially chromosomes 1, 4, X. It should be kept in mind that the use of X-ray irradiation, which is widely used for therapeutic and diagnostic purposes, may be risky.

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