Assessment of DNA Damage in Human Bone Marrow Cells and Multipotent Mesenchymal Stromal Cells

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Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 2, pp. 118-120, April, 2011
Original article submitted February 17, 2011.

We carried out a comparative analysis of DNA damage (percentage of DNA in comet tail) and frequencies of comets in apoptotic cells in BM samples and cultures of BM multipotent mesenchymal stromal cells at different terms of culturing (passages 3-11). The levels of DNA damage in mesenchymal stromal cells remained unchanged during culturing (3.5±0.9 and 4.4±1.2%) and did not differ from those in BM cells (3.6±0.8%). In BM samples, 10-28% atypical cells with high level of DNA damage were detected. In mesenchymal stromal cells, 2.8±0.9 and 3.6±1.8% apoptotic cells were detected at early and late passages, respectively.

Key Words: *stem cells; multipotent mesenchymal stromal cells; DNA-comet assay; apoptotic cells; genetic stability*

Therapy with SC is considered to be a perspective method for the treatment of various diseases. During recent decades, SC are intensively studied in genetic, cell, and animal experiments and at different stages of clinical trials. However, the safety of this therapy and the absence of delayed adverse effect have not been proven yet.

The problem of genetic stability of SC is still poorly studied. According to some experimental studies, SC are more genetically stable compared to differentiated cells [4,10,11], which can be explained by more accurate functioning of the reparation system and timely induction of apoptosis in cells with irreparable DNA damages. At the same time, genomic, chromosome, and gene mutations were detected in SC during culturing [1]. Rapid growth and division of genetically abnormal SC can lead to clone formation and development of malignant neoplasms [9]. This necessitates evaluation of genetic stability of SC before their transplantation as a measure ensuring safety of cell therapy. Assessment of DNA damage in SC seems to be promising (apart from evaluation of chromosome variability) for solving this problem.

Here we compared the levels of DNA damage in BM cells and BM multipotent mesenchymal stromal cells (MSC) at different terms of culturing.

MATERIALS AND METHODS

We analyzed BM samples from 5 healthy donors and 6 MSC cultures (passages 3-4 and 10-11). All donors signed informed consent for using BM samples for scientific purposes.

BM samples were frozen and stored in liquid nitrogen until use. MSC were cultured under standard conditions [3]. BM were rapidly defrosted on a water bath at 37°C. MSC cultures were twice washed from culture medium with cold PBS and harvested with a silicon policeman. BM and MSC cells were transferred into tubes and centrifuged at 5000 rpm for 5 min. The supernatant was discarded and the pellet was diluted with a buffer to a concentration of $1\text{-}5\times10^5$/ml and stored in a refrigerator at 4°C. DNA damage was studied by DNA-comet assay (alkaline version) as described previously [2].

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Microscopy was performed using an AxioImager A1 epifluorescent microscope (Zeiss; ×200). The digital images of DNA-comets were analyzed using C2ASP 1.2.2 software [6]. The analysis included at least 100 DNA-comets in each micropreparation. DNA content in DNA-comet tail (in %) was used as the measure of DNA damage. DNA-comets of typical shape with DNA content in the tail >70% (Fig. 1) were considered as apoptotic and counted separately. The data were processed statistically using Student *t* test.

RESULTS

Analysis showed that DNA damage in BM cells (Table 1) was 3.6±0.8% (2.1-6.5%), which agrees with a previous report [7], where this parameter in BM cells from healthy volunteers was 2-9%. Analysis of microscopic images (Table 1) showed that atypical DNA-comets without head and with diffuse tail looking as a halo constituted 10-28% (Fig. 2). These DNA-comets cannot be analyzed and are called ghost cells. They are often detected among cells subjected to cytotoxic influence of reactive oxygen species inductors, *e.g.* hydrogen peroxide. The presence of these DNA-comets in BM samples is probably related to cell damage with reactive oxygen species during cryopreservation [12].

DNA damages in MSC cultures were evaluated at early (3-4) and late (10-11) passages (Table 2). In one MSC culture (No. 2), increased level of DNA damage was detected at passage 10 (16.5% DNA in comet tail). The incidence of apoptotic cells also increased (to 18.3%). The causes of these high levels of DNA-damage in one of 6 MSC cultures remain unknown. This can be related to individual characteristics of the donor and isolation and culturing conditions. This culture was excluded from the analysis.

TABLE 1. Levels of DNA Damage and Atypical DNA-Comets in BM Cells

Sample No.	DNA in comet tail, %	Atypical DNA-comets, %
1	3.4	26.6
2	6.5	21.1
3	2.5	19.3
4	2.1	10.1
5	3.4	28.1
Mean value	3.6±0.8	21.1±3.2

The level of DNA damage for other cultures varied in a wide range (Table 2); the mean values for early and late passages were 3.5±0.9 and 4.4±1.2%, respectively. Comparative analysis of DNA damages in BM cells and MSC cultures at different passages revealed no significant differences (Tables 1 and 2).

The incidence of apoptotic DNA-comets in different MSC cultures (Table 2) was 2.8±0.9% at the early passages and did not change during culturing. At the late passages this parameter was 3.6±1.8. These values considerably surpassed the corresponding parameters in human peripheral blood cells, where apoptotic DNA-comets were rare (<1-2%). High proliferative activity of SC determined by shortening of G1 phase of the cell cycle leads to impairment of the control over DNA damages; under these conditions the cells with non-repaired DNA damage are eliminated via apoptosis. According to a previous report [10], SC damaged by chemical and physical agents did not stop their division upon transition from G1 to S stage of the cell cycle, but died later via apoptosis.

TABLE 2. Levels of DNA Damage and Atypical DNA-Comets in MSC Cultures

Culture No.	DNA in comet tail, %		DNA-comets of apoptotic cells, %	
	passages 3-4	passages 10-11	passages 3-4	passages 10-11
1	3.7	3.5	4.4	3.2
2	7.0*	16.5*	2.4*	18.3*
3	5.9	3.5	5.6	0.4
4	4.9	9.1	1.9	10.2
5	0.6	2.7	0.8	4.1
6	2.6	3.1	1.0	0
Mean value	3.5±0.9	4.4±1.2	2.8±0.9	3.6±1.8

Note. *Excluded from the analysis of the mean value.

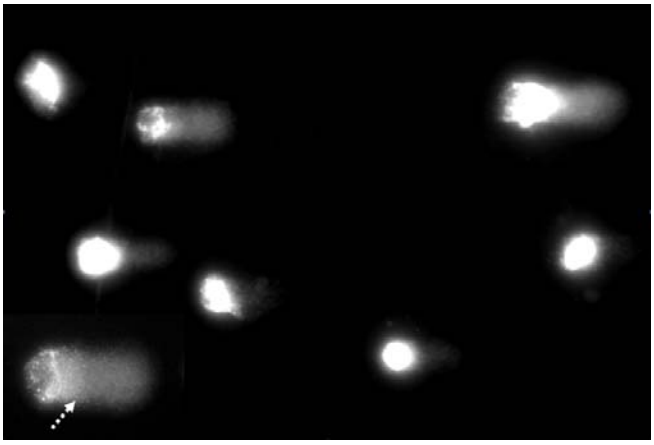

Fig. 1. DNA-comets of cultured MSC (micropreparation, SYBR Green I staining, ×200). Arrow shows DNA-comet of apoptotic cell (DNA content in comet tail >70%).

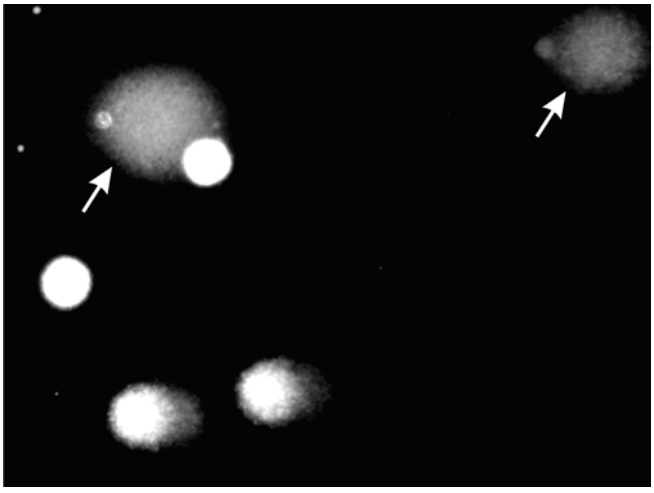

Fig. 2. DNA-comets of BM cells (micropreparation, SYBR Green I staining, ×200). Arrows show atypical DNA-comets.

Inadequate detection of DNA damage and its delayed reparation can lead to accumulation of cells with genetic errors affecting gene expression and cell phenotype, which increases the probability of differentiation and malignization of SC. However, initiation of apoptosis in response to damage can lead to reduction of SC number in the body and impairment of tissue regeneration. In humans, interrelationship of these processes manifests in some hereditary diseases (xeroderma pigmentosum, trichothiodystrophy, Cockayne syndrome, Weber syndrome, *etc.*) characterized by disturbances in DNA reparation system and increased incidence of tumors [5,8]. Understanding of the mechanisms underlying DNA-damage aftereffects in SC will help to develop methods for SC protection and maintenance of their count.

Thus, the level of DNA damage in BM cells does not differ from that in cultured MSC at different stages of culturing. The presence of highly damaged DNA in BM cell samples substantiates the need of improvement of cryopreservation methods for maximal protection of the cell material. One of the analyzed MSC cultures can be characterized as genetically instable due to high level of DNA damage and incidence of DNA-comets of apoptotic cells. Increased level of apoptotic cells in MSC cultures attests to intensive elimination of damaged cells, which can be related to the effect of isolation and culturing conditions. Analysis of DNA damages can be considered to be an important parameter in complex evaluation of genetic stability of SC cultures intended to cell therapy.

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