Spontaneous Aneuploidy and Clone Formation in Adipose Tissue Stem Cells during Different Periods of Culturing

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Cytogenetic analysis of 13 mesenchymal stem cell cultures isolated from normal human adipose tissue was carried out at different stages of culturing. The incidence of chromosomes 6, 8, 11, and X aneuploidy and polyploidy was studied by fluorescent *in situ* hybridization. During the early passages, monosomal cells were more often detected than trisomal ones. A clone with chromosome 6 monosomy was detected in three cultures during late passages.

Key Words: *stem cells; aneuploidy; clone formation; interphase nuclei; fluorescent in situ hybridization (FISH)*

Mesenchymal stem cells are in the focus of attention of many scientists. This interest can be explained by the possibility of using stem cells (SC) in medical practice. It seems that by their characteristics, MSC are the most promising autologous material for cell and gene engineering therapy. These cells are characterized by high proliferative potential during long-term culturing, due to which SC can be obtained in the needed volumes; moreover, MSC differentiation in osteo-, chondro-, myo-, adipogenic, and other directions can be easily induced. Stem cells were detected in virtually all organs and tissues of adult organism, but they are more abundant in the bone marrow and adipose tissue [3,9].

It was noted that, long-term culturing of SC is sometimes fraught with serious changes in the genome at the molecular and chromosomal levels of its organization. Structural and quantitative abnormalities of the chromosome set, amplification of some DNA sites, methylation of promotors, and telomer erosion with disorders in telomerase activity were detected [1,5,7].

These genetic changes can affect cell differentiation potential and lead to malignant degeneration of cells after their transplantation. Quantitative disorders in the chromosome set (from hypoploidy to hyperploidy) are a common sign in many malignant tumors. It seems therefore that chromosome analysis should be the leading test system in SC quality control and culture screening for the risk of malignant transformation.

We studied the quantitative disorders in the chromosome set of MSC from the adipose tissue at different stages of culturing using a molecular cytogenetic method — dual-color interphase FISH (fluorescent *in situ* hybridization) analysis.

MATERIALS AND METHODS

Adipose tissue MSC cultures at different periods of culturing (early passages 2-5 and late passages 9-15; ReMeTex) were used in the study. Methods for obtaining MSC cultures, conditions of culturing, and methods for making cytological preparations were described previously [1].

The incidence of aneuploid (monosomal and trisomal) and polyploid cells was studied by 3 autosomes (chromosomes 6, 8, 11) and X chromosome. Interphase FISH analysis, a highly accurate method for detection and identification of chromosomes and their quantitative disorders, was carried out using chromosome-specific DNA probes: CEP6 (D6Z1), CEP8

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(D8Z1), CEP11 (D11Z1), and CEPX (DXZ1; Vysis). The resultant cytological preparations were subjected to denaturation with 70% formamide (72°C, 3 min) and hybridized overnight at 37°C. On the next day, the preparations were washed with 0.4×SSC (saline sodium citrate buffer) at 70°C on a water bath. Cell nuclei were contrasted with DAPI (4,6-diamidino-2-phenylindole dihydrochloride). In most cases, hybridization reaction on the preparations was carried out with chromosome pairs 6 and 8 or 11 and X. The preparations were analyzed under an AxioImager microscope (Zeiss Inc. filters) using FISH View System, Applied Spectral Imaging software.

At least 1000 interphase nuclei were analyzed for each cell culture from different individuals, except several cultures of late passages, in which for technological reasons more than 100 cells were analyzed. Hybridization signals in the nuclei were counted in accordance with accepted criteria [6,7].

The cells were divided into 5 classes by the number of signals: class 1) nullisomy (0 signals for individual or pair of the studied chromosomes; reflects mainly the efficiency of hybridization); class 2) monosomy (1 signal from the studied chromosome in the nucleus); class 3) disomy (2 signals); class 4) trisomy (3 signals by the studied chromosome in the nucleus); class 5) tetrasomy (4 signals; presumably, in the majority of studied cells this indicated not double aneuploidy, but polyploidy of the chromosome set with the number of signals coinciding for two chromosomes in the same preparation).

The results were processed using Statistica 8, Excel XP, and Excel Binomial software.

RESULTS

Incidence of aneuploidy in early passage cultures. Thirteen cultures were analyzed for chromosomes 6 and 8, 11 and X (Tables 1, 2).

The percentage of nuclei with nullisomy by chromosomes 6 and 8 was 0 and 0.1%, respectively, and 0% for chromosomes 11 and X. Nullisomic cells were

TABLE 1. Incidence of Aneuploid Cells in MSC Cultures

Passage, culture No.	*n*	Monosomy, %		Trisomy, %		*n*	Monosomy, %		Trisomy, %	
		chromosome 6	chromosome 8	chromosome 6	chromosome 8		chromosome 11	chromosome X	chromosome 11	chromosome X
Early passages										
1	939	1.9	0.9	0.0	0.0	929	1.8	1.6	0.0	0.1
2	782	0.6	0.8	0.0	0.0	1034	1.3	1.8	0.0	0.0
3	1003	2.6	2.9	0.2	0.1	1024	0.3	0.9	0.3	0.0
4	1001	0.2	1.3	0.4	0.0	1007	0.0	0.3	0.2	0.0
5	1011	0.7	1.0	0.1	0.3	1014	0.6	0.0	0.0	0.0
7	1010	2.7	3.4	0.2	0.1	1026	1.3	0.9	0.1	0.1
8	1010	3.6	3.9	0.3	0.5	1016	0.3	0.8	0.0	0.1
10	1067	2.9	3.0	0.4	0.4	922	0.3	0.1	0.3	0.8
12	1083	1.9	3.6	1.0	1.5	1024	1.6	0.7	0.0	0.1
15	1045	0.7	0.5	0.8	1.1	1036	1.4	0.6	0.4	0.4
17	1005	0.2	1.9	0.9	0.6	1019	0.7	0.5	0.1	0.5
18	1018	1.5	3.5	1.2	0.7	1049	3.0	1.0	0.3	0.2
19	1057	0.2	0.6	0.1	0.1	1008	0.5	—	0.0	—
Late passages										
1	1026	0.5	0.3	0.9	0.6	908	0.4	1.4	0.1	0.1
2	710	19.9*	0.8	0.0	0.7	834	0.0	0.6	0.0	0.0
3	1000	2.3	4.4*	0.5	0.7	1021	0.1	1.6	0.1	0.1
4	489	21.2*	0.6	1.0	1.4	141	0.0	0.0	0.0	0.0
5	1531	5.6*	3.0	2.7	2.5	821	0.4	0.1	0.0	0.4
7	1002	0.5	1.4	0.1	0.1	1038	0.7	0.5	1.0	0.6
18	492	1.4	0	0.2	0.2	360	1.4	0	0.0	0

Note. *n*: cell number. *Cultures with the number of aneuploid cells surpassing 95% CI. «—»: culture originating from a man.

TABLE 2. Range of Fluctuations in the Percent of Aneuploid and Polyploid Cells in MSC Cultures of Early (EP) and Late Passages (LP)

Chromosome, type of anomaly		Mean incidence, %		95% CI	
		EP	LP	EP	LP
Chromosome 6					
	monosomy	1.5±1.2	1.1±0.8	0.8-1.9	0.5-2.3
	trisomy	0.4±0.4	0.3±0.3	0.3-0.7	0.2-1.0
	polyploidy	0.6±0.6	0.3±0.2	0.5-1.1	0.2-0.7
Chromosome 8					
	monosomy	2.1±1.3	1.5±1.5	0.9-2.2	0.1-3.0
	trisomy	0.4±0.5	0.8±0.9	0.3-0.8	0.6-1.7
	polyploidy	0.5±0.6	0.9±1.1	0.5-1.1	0.7-2.3
Chromosome 11					
	monosomy	1.0±0.8	0.4±0.5	0.6-1.4	0.3-1.1
	trisomy	0.1±0.2	0.2±0.4	0.1-0.3	0.2-0.8
	polyploidy	0.6±1.2	0.2±0.5	0.8-1.9	0.3-1.0
Chromosome X					
	monosomy	0.8±0.5	0.6±0.7	0.4-0.9	0.4-1.5
	trisomy	1.2±0.2	0.3±0.2	0.2-0.4	0.1-0.5
	polyploidy	0.5±1.1	0.4±0.5	0.8-1.9	0.3-1.0

not included in further analysis because of their negligible quantity. The incidence of tetrasomal (polyploid) cells was the same for the studied chromosomes: 0.5-0.6% (0-2.7% in different cultures).

The mean incidence of chromosome 6 monosomy was 1.5% (Table 1), its estimated upper threshold value (at 95% confidence interval, CI) was 1.9% (Table 2); a value higher than this threshold would indicate genetic instability; for chromosome 8 this value was 2.1% (95% CI, 0.9-2.2), for chromosome 11 it was 1.0% (95% CI, 0.6-1.4), and for chromosome X this value was minimum: 0.8% (0.4-0.9). Fluctuations in the incidence of monosomy in different cultures were significant, but the difference in the means was negligible for the studied chromosomes.

Trisomic cells were less incident than cells with monosomy: 0.4% for chromosome 6 (95% CI, 0.3-0.7), 0.4% for chromosome 8 (95% CI, 0.3-0.7), 0.1% for chromosome 11 (95% CI, 0.1-0.3), and 0.2% for chromosome X (95% CI, 0.2-0.4). No significant differences between the cultures were detected.

Higher incidence of monosomy compared to trisomy is theoretically expected, because, in addition to the mechanism of chromosome nondisjunction common for both aneuploidy types and leading to their equal proportion, monosomy can be caused by the mechanism of chromosome lag in the mitosis anaphase. The mean incidence of monosomy/trisomy ratio for chromosome 6 was 1.6:0.5% (3-fold more incident monosomy than trisomy), for chromosome 8 it was 2.2:0.4% (5-fold higher), for chromosome 11 1.0:0.1% (almost 10-fold higher), and for chromosome X 0.8:0.18% (4-fold higher). Analysis of the relationships between the aneuploid cell percent proportion based on Spearman ranked correlation coefficient showed that this characteristic is significant for the ratio of cells with monosomy by chromosomes 6 and 8 (R=0.64; p=0.03) and with trisomy by the same chromosomes (R=0.75; p=0.007). The percent of cells with disomy correlated for chromosomes 6 and 8 (R=0.80; p=0.001) and chromosomes 11 and X (R=0.89; p=0.001). Coefficient of correlation between the percentage of cells with tetrasomy (polyploidy) for chromosome pairs 6 and 8, 11 and X approached 1 (p=0.00001). On the other hand, the correlation between the percentage of cells with monosomy and trisomy for chromosomes 11 and X was positive, but negligible (R=0.48; p=0.12). On the whole, the data on correlations between different types of quantitative disorders indirectly attest to similar mechanisms of their appearance.

Incidence of aneuploidy in cultures of late passages. Of 13 cultures studied during early passages, 7 cultures were studied during late passages for chromosomes 6, 8, 11, and X by dual-color FISH analysis (Tables 1, 2).

The incidence of aneuploid cells varies significantly. For more profound study, we compared the incidence of aneuploidy surpassing the upper threshold CI in MSC cultures of early and late passages (Table 3).

In two cultures (Nos. 2 and 4), chromosome 6 monosomy reached 19.9 and 21.2%, respectively, in

TABLE 3. Comparative Analysis of Cultures of Early and Late Passages with Aneuploidy Percentage Surpassing the Upper CI Threshold

Culture	Passage	Cell number	Cells with signals (number of signals)					*p*
			0	1	2	3	4	
2	3	782	0	5	777	0	0	0.001
	14	710	0	141	569	0	0	
3	4	1003	4	29	966	1	3	0.07
	11	1000	2	44	947	7	0	
4	4	1001	0	7	1001	1	1	0.001
	9	1531	0	86	1352	42	51	
5	5	1011	0	7	1001	1	1	0.001
	10	1531	0	86	1352	42	51	

Note. Cultures 2, 4, and 5: chromosome 6 monosomy. Culture 3: chromosome 8 monosomy.

culture No. 5 it was 5.6%, which surpassed the upper CI threshold (Table 2). The incidence of chromosome 6 monosomy in all three cell cultures differed significantly from that in the same cultures at early passages (Table 3; p=0.001).

The incidence of chromosome 8 monosomy varied within a wide range, being 4.4% in culture No. 3. Though this was higher than the upper threshold CI (3%), no statistically significant differences between the early and late passages were detected (p=0.07). Longer culturing or studies by other markers of MSC genetic instability are needed to clear out the potential of cells with this type of aneuploidy to selective proliferation.

The incidence of cells with chromosome 11 monosomy and trisomy was lower than that for chromosomes 6 and 8: a trend to a reduction in comparison with the early passages was observed in the majority of cultures.

The incidence of chromosome X aneuploidy at late passages virtually did not differ from that in the early passage cultures.

Hence, two cell populations were detected in 3 cultures of adult MSC during late (9-15) passages: one with diploid (normal) chromosome set, the other with aneuploid set (monosomy by chromosome 6 with 80:20% ratio for cultures Nos. 2 and 4 and with 94:6% ratio for culture No. 5). It is obvious that serious genetic disorders in these cultures led to appearance of monosomal cells leading to their selective advantage in multiplication and clone formation. The lowest incidence of monosomal cells in the clones (Tables 1, 3) was observed in culture No. 5 (5.6%), which can be regarded as the beginning of selective multiplication of the clone with chromosome 6 monosomy. In cultures No. 2 (passage 14) and No. 4 (passage 9), the clone with chromosome 6 monosomy could reach 20%, if proliferation rate of aneuploid cells more than 2-fold surpassed that of diploid cells.

These findings, our previous results [1,2], and published data [8,10] disagree with the opinion of some scientists on genetic stability of SC and safety of their use without cytogenetic testing. The data on different types of chromosome changeability in cultured MSC necessitate more profound studies of SC genetics and creation of a system of their quality control for prevention of untoward consequences of therapeutic use of these cells.

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