EXPERIMENTAL GENETICS

Aneuploidy of Stem Cells Isolated from Human Adipose Tissue

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 9, pp. 320-323, September, 2008
Original article submitted April 28, 2008

The incidence of autosomes 6 and 8 aneuploidy in stem cell cultures derived from adipose tissue was evaluated at different stages of culturing. Monosomy was more incident than trisomy during the early passages. Distribution of cultures by the incidence of aneuploidy in different chromosomes was virtually the same. Clones with chromosome 6 monosomy were detected in two cultures during late passages.

Key Words: *aneuploidy; FISH; interphase cells; stem cells*

The majority of methods of cell therapy include culturing of stem cells (SC) for increasing their number or committing their development in a certain direction. It is a very important stage and in order to predict the safety of cell therapy in the delayed period of treatment, it is essential to evaluate the SC chromosome stability, as clones of cells with chromosome or genome changes can become the potential cause of malignant tumor formation [4,5,7]. The data on the regularities of SC chromosome and genome variability *in vitro* are scanty and contradictory [2]. Accumulation of experimental data on the types and quantitative evaluation of genome changes in different SC cultures at different terms and under different culturing conditions are needed.

We studied aneuploidy by the FISH method in interphase cultured mesenchymal SC from human adipose tissue during early (2-5) and late passages (9-15).

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MATERIALS AND METHODS

Specimens of adipose tissue from the anterior abdominal wall were obtained during cosmetic liposuction or other surgical interventions. The age of donors was 26-55 years. The tissue was washed 3-4 times in phosphate buffered saline (pH 7.4) and suspended in an equal volume of the same buffer with 1% bovine serum and 0.1% collagenase-1 (Worthington Biochemical Corp.). Tissue disaggregation was carried out on a magnetic stirring device at 37°C for 1 h and then centrifuged for 5 min at 300-500*g*. The supernatant with mature adipocytes was discarded. Cell precipitate containing the fraction of stromal and vascular cells (fibroblasts, pericytes, endothelium, *etc.*) was cultured. The cells were seeded in 90-mm plastic Petri dishes (Cornig) in DMEM/F12 growth medium supplemented with 10% FCS (HyClone) and 1% insulin-transferrin-selenite (Paneco). The cultures were in-

0007-4888/08/1463-0344

cubated in a CO_2 incubator (5% CO_2, 37°C) for 48 h until cell adhesion, after which the medium was replaced and the cells were cultured to 75-90% confluence, then harvested with trypsin, and seeded into new dishes. Inoculation density was 1.5×10^3 cell/cm^2 for all passages.

The cells were harvested from the flask wall with versen (1 min) and then with 0.25% trypsin (3-7 min at 37°C), after which DMEM (5 ml) was added and the cell suspension was put into centrifuge tubes. After centrifugation, the supernatant was discarded. The cells were treated with 0.55% KCl (10 min at 37°C), 3-5 drops of fixative was added to arrest hypotonization before centrifugation. A mixture of methyl alcohol and glacial acetic acid (3:1) was used as fixative, fixation was carried out by the standard method in 3 portions of the fixative.

Interphase FISH analysis was carried out using α-satellite DNA probes CEP6 (D6Z1) and CEP8 (D8Z1) (Vysis Inc.) for chromosomes 6 and 8. The preparations were denaturated with 70% formamide at 73°C for 3 min and hybridized overnight at 37°C. On the next day, the preparations were washed with 0.4×SSC solution (70°C) on a water bath. The nuclei were contrasted with DAPI. FISH preparations were analyzed under an AxioImager microscope with a set of interference filters (Zeiss) using Fish View System software for FISH analysis (Applied Spectral Imaging, GmbH).

The incidence of aneuploidy in some cultures was evaluated twice: during early (2-5) and late passages (9-15).

RESULTS

A total of 17,560 cells in 18 cultures were analyzed. Nuclei with one, two, and three signals were counted. The incidence of nuclei with nullisomy (without signals) varied from 0 to 0.2%. Nullisomy

TABLE 1. Incidence of Aneuploidy in Cultures of Early Passages

Culture No. by protocol	Cell number	Disomy, %	Monosomy, %	Trisomy, %
Chromosome 6				
3	998	97.19	2.60	0.20
4	987	99.39	0.20	0.40
5	1009	99.21	0.69	0.10
7	1006	97.12	2.68	0.20
8	992	96.07	3.63	0.30
10	1066	96.72	2.91	0.37
12	1083	97.04	1.94	1.01
15	1028	98.54	0.68	0.78
17	993	98.89	0.20	0.91
18	1004	97.31	1.49	1.19
19	1055	99.71	0.19	0.09
Mean	11221	97.93±0.38	1.56±0.38	0.50±0.12
Chromosome 8				
3	996	96.99	2.91	0.10
4	988	98.68	1.31	0
5	1008	98.71	0.99	0.30
7	1003	96.51	3.39	0.10
8	992	95.56	3.93	0.50
10	1067	96.63	3.00	0.37
12	1083	94.92	3.60	1.48
15	1029	98.44	0.48	1.07
17	994	97.48	1.91	0.60
18	1004	95.72	3.58	0.70
19	1057	98.01	0.57	0.09
Mean	11221	97.06±0.40	2.33±0.40	0.48±0.14

TABLE 2. Distribution of cultures by Incidence of Aneuploidy during Early Passages (Number of Cultures)

Incidence of aneuploidy, %	Monosomy		Trisomy	
	chromosome 6	chromosome 8	chromosome 6	chromosome 8
0-0.5	3	1	7	7
0.5-1.0	2	2	2	2
1.0-1.5	1	1	2	2
1.5-2.0	1	1	0	0
2.0-3.0	3	2	0	0
More than 3.0	1	4	0	0

estimation was excluded from the analysis because of possible ineffective hybridization. Tetrasomic nuclei were also excluded from the analysis because of impossibility of differentiating between tetrasomy in a diploid cell and tetraploidy or because of cases when the cell was in the late S or early G_2 phase of cell cycle.

The incidence of aneuploidy during the early passages is presented in Table 1. The mean number of normal diploid cells with two pairs of chromosomes 6 and 8 was 97-98%. The incidence of monosomy in 11 cultures varied within a wide range: from 0.19 to 3.63% for chromosome 6 (mean 1.56±0.38%) and from 0.48 to 3.93% (mean 2.33±0.40%) for chromosome 8. The difference between the means for monosomy in chromosomes 6 and 8 was insignificant ($p>0.05$).

The incidence of trisomic cells for chromosome 6 varied in different cultures from 0.09 to 1.19% (mean 0.50±0.12%), for chromosome 8 from 0 to 1.48% (mean 0.48±0.14%). The difference between the incidence of cells with chromosomes 6 and 8 trisomy was insignificant ($p>0.05$).

Comparison of the incidence of monosomic and trisomic cells showed significant differences between these values for both chromosomes ($p<0.01$). The incidence of monosomy was 3-5-fold higher than that of trisomy. Paired comparison of the incidence of mono- and trisomies in individual cultures showed that the level of chromosome 6 trisomy was higher than that of monosomy in cultures Nos. 4, 15, and 17, while for chromosome 8 it was higher only in culture No. 15. Predominance of monosomy over trisomy indirectly indi-

TABLE 3. Incidence of Aneuploidy in Cultures at Late Passages

Culture No. by protocol	Cell number	Disomy, %	Monosomy, %	Trisomy, %
Chromosome 6				
1	1021	98.63	0.49	0.88
2	710	80.14	19.86	0
3	999	97.20	2.30	0.50
4	482	77.38	21.58	1.04
5	1480	91.35	5.81	2.84
7	999	99.40	0.50	0.10
18	491	98.37	1.42	0.20
Chromosome 8				
1	1022	99.12	0.29	0.59
2	706	98.44	0.85	0.71
3	998	94.89	4.41	0.70
4	476	97.90	0.63	1.47
5	1489	94.29	3.09	2.62
7	999	98.50	1.40	0.10
18	491	99.80	0	0.20

cates that along with failure of chromosomes to part, there is another possible source of aneuploidy in SC cultures during the metaphase stage: chromosome lagging behind at the anaphase stage.

Comparison of the distribution of the resultant series of individual values (Table 2) can provide an important characteristic of fluctuations in aneuploidy incidence for different chromosomes (in our study chromosomes 6 and 8).

Statistical analysis showed that distribution of cultures by the incidence of monosomy and trisomy was virtually the same for chromosomes 6 and 8 ($p>0.05$).

The data on the incidence of aneuploidy during the late passages are summed up in Table 3. Coinciding numbers of cultures in the protocols (Nos. 3, 4, 5, 7, 18) at the early and late passages indicate the same cultures which were analyzed twice.

The range of monosomy and trisomy incidence for both chromosomes during the late passages was greater in comparison with early passages. For example, the incidence of chromosome 6 monosomy varied from 0.49 to 21.58%. The mean cannot be correctly estimated for this wide range, because low and high incidence can reflect different phenomena occurring during SC culturing. For example, the question about evaluation of clone formation in these mosaic cultures has to be solved in the analysis of the late passage cultures with high incidence of aneuploidy. The presence of clones in cultures Nos. 2 and 4 (Table 3) can be seen from the fact that the greater part of monosomic cells ($^1/_5$) were detected for chromosome 6, but not for both chromosomes (as would be the case if it were just high chromosome changeability). The incidence of trisomic cells in these cultures (Nos. 2 and 4) was low, and low incidence of monosomy and trisomy was noted for chromosome 8. If the mosaic (clonal) type of cultures Nos. 2 and 4 causes no doubts, it remains unclear at what incidence of aneuploid cells the cultures with clones can be referred to mosaic ones. The difficulty of this problem is explained by the fact that the regularities of the dynamics of cell populations, in which abnormal cells appear, remain little studied; another problem is the technology of culture reinoculation. It seems that appropriate statistical validation is needed for solving these problems.

Published data [2] and our findings indicate that the hypotheses [3,6] on genetic stability of SC are not always confirmed. All types of chromosome and genome mutations are detected in adult human SC cultures [1]. As the conditions of culturing and reinoculation of cell cultures are usually similar, the appearance of abnormal clones can be explained by their selective advantage in multiplication. Clinical use of cultures with chromosome and genome changes can lead to undesirable delayed effects, while genetic screening of cell transplants using DNA probes for detection of aneuploidy or other markers of genetic instability of cultures will presumably prevent them.

Hence, our findings indicate that genome changeability in cultured SC manifests during the early (2-5) passages derived from adipose tissue. Monosomic cells by one chromosome were detected in about 2% cases, trisomic cells in 0.5% cases. Culture distribution by the incidence of aneuploidy in different chromosomes was virtually the same. High level of mosaicism, which could be mistaken for clone formation, was observed in some cultures during late passages (after 9). Clones with chromosome 6 monosomy, constituting about 20% cells in the entire culture, were detected in 2 of 7 cultures.

The study was supported by the Russian Foundation for Basic Research (grants Nos. 06-04-49135-a, 07-04-12157-ofi), grant from President of the Russian Federation (NSh-1006.2006.7), and State Contract No. 02.512.11.2135).

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