Location of Chromosomes in the Nucleus of Human Mesenchymal Stem Cells

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For evaluation of the spatial structure of chromatin in nuclei of mesenchymal SC we determined the position of centromeres and individual chromosomes in interphase nucleus of mesenchymal SC. More than 300 nuclei in 7 cultures of mesenchymal SC were analyzed. Centromeres of chromosomes 6, 8, and 11 lie at a longer (0.68, 0.67, 0.7), while centromere of chromosome 18 at a shorter radial distance (0.49). Homologues of each chromosome had different radial distances. No differences in radial distances of centromeres were detected between mesenchymal SC from the adipose tissue and BM. After passage 8, distal displacement of chromosome 6 centromere (from 0.66 to 0.72) was observed, which probably indicates aging or spontaneous differentiation of cells.

Key Words: *chromosome territory; structure of interphase nucleus*

Mesenchymal SC (MSC) are used in regenerative medicine due to their availability, simple culturing procedure, and high proliferative and differentiation potentials. MSC can be isolated from all tissues of the body. For applied purposes, BM or adipose tissue (AT) are most widely used. Understanding of the mechanisms of the maintenance of their stem capacities based on the cell type-specific gene expression pattern is essential for more effective use of MSC.

An important role in the regulation of gene expression is played by epigenetic mechanisms, in particular, by spatial structure of the chromatin in the nucleus. Interphase nuclei of all higher eukaryotes represent highly organized cell organelles. Chromosome territories (CT), structural elements of the nucleus represent domains of the nucleus containing chromosome material visualized by labeled DNA hybridization and 3D microscopy. The existence of CT as independent structural units was proven in 1980 [6]; however, the mechanisms of the formation and maintenance of mutual arrangement of CT unique for different cell types of higher eukaryotes and cause-effect relationships between CT structure and gene activity in the corresponding chromosomes remain unclear. Until now little is known about nucleus structure in the majority of cell types, including MSC, the cells of crucial practical importance. Apart from many common features, MSC cultures have individual and tissue-specific peculiarities depending on their source.

Here we studied the location of centromeres of individual chromosomes in interphase nucleus with consideration for MSC heterogeneity.

MATERIALS AND METHODS

Bone marrow and adipose tissue MSC were isolated and cultured as described earlier [1,5]. MSC from AT were kindly provided by Bank of Stem Cells (Department of Health Care, Moscow) or isolated by us. MSC from BM were kindly provided by ReMeTeks Company. All patients signed informed consent for participation in the study.

Before FISH analysis, the preparations were fixed as described previously [1].

For FISH analysis of interphase nuclei, centromere probes (Vysis, Inc.) for chromosomes 6, 8, 11, 18

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were used. Denaturation, hybridization, and washout were performed according to manufacturer's instruction. The nuclei were stained with DAPI. The preparations were examined under an AxioImager microscope equipped with a set of interference filters (Zeiss) using FISH analysis software (Fish View System, Applied Spectral Imaging).

The cells were washed with PBS from culture medium, harvested with EDTA-trypsin solution, and centrifuged; the supernatant was discarded and the pellet was resuspended in PBS, the cells were counted, and 10^5 cells were taken for flow cytofluorometry on a CyFlow ML cytofluorometer (Partec).

RESULTS

Seven different MSC cultures at different passages were analyzed (Table 1). Passages before and including the 4th passage and after the 8th passage were considered as early and late passages, respectively. Chromosome 18 (carrying low number of genes and located at the periphery of the nucleus) was selected for the analysis as one of the most frequently used in these studies; chromosome 6 was selected as a chromosome carrying a number of genes related to undifferentiated status of SC and genes participating in some (osteo- and adipogenic) differentiation pathways typical of MSC. The data on localization of chromosome 8 and 11 centromeres were also available for some cultures.

Analysis of MSC culture on a flow cytofluorometer revealed two subtypes of cells, small and large, which is typical of MSC cultures [2-4]. Cells in MSC cultures differed not only by the size, but also by their shape and cytoplasm vacuolation [4]. There is a positive correlation between cell size and nucleus size, therefore not only chromosome location, but also nucleus size were taken into account during the analysis in order to compare chromosome location in nuclei of different size.

Previous studies showed heterogeneity of cells in MSC culture from AT by nucleus size [2]. During FISH analysis, the area of nuclei stained with DAPI was measured. Then, the size of analyzed nuclei was taken into account during the analysis of radial distances. The nucleus size distribution did not fit the normal law, while the range of variations for this parameter was great: the minimum and maximum values differed by several times. Each culture contained numerous cells with medium-size nuclei and ~25% cells with small and large nuclei.

For evaluation and comparison of chromosome location in the interphase nuclei, radial distance, a nondimensional relative parameter equal to the ratio of the distance from nucleus center to FISH signal to the distance from nucleus center to its edge along the radius passing through the analyzed signal, was determined. The distributions of radial distances differ from the normal distribution. No differences were revealed in chromosome locations between the analyzed cultures. The medians of radial distances of chromosome 6, 8, 11, and 18 centromeres were 0.68, 0.67, 0.7, and 0.49, respectively. Centromere 18 in the same culture was located at a lesser distance than centromere 6 ($p<0.001$, Kolmogorov–Smirnov test; Table 2). No differences in centromere location in nuclei of different size were revealed except one culture, where one chromosome 6 homologue was located closer to the center in smaller nucleus (0.5 *vs.* 0.65; $p<0.05$). The absence of differences in this case can be a false-negative result related to low number of observations and low number of small and large nuclei. In further

TABLE 1. Characteristics of Analyzed Cultures

Culture No.	Passage	Source	Measured nuclei	Analyzed chromosomes			
				6	8	11	18
MSC-1	Early	BM	–	+			+
MSC-2	Early	BM	+	+			
MSC-3	Early	BM	+	+	+		
MSC-3	Late	BM	–	+	+		
MSC-4	Late	BM	+	+		+	
MSC-5	Early	AT	+	+			
MSC-6	Early	AT	+	+			+
MSC-7	6	AT	+	+			+
MSC-7	Late	AT	+	+			+

experiment, preliminary separation of cells by the size before sorting on a flow cytofluorometer is advisable. Comparison of early and late cultures revealed distal displacement of chromosome 6 (from 0.66 to 0.72, p=0.042; Mann–Whitney test; Fig. 1). This displacement can reflect processes of aging and/or spontaneous differentiation in culture, because chromosome 6 carries genes responsible for non-differentiated state of MSC, *e.g. OCT4* typically expressed by MSC [8], and genes responsible for typical pathways of MSC differentiation (including spontaneous differentiation) towards adipo- and osteogenic lineage cells, *BMP6, HDAC2,* and *RUNX2*.

Analysis of homologous chromosomes showed that one of the homologues is always located closer to the nucleus center than the other (p~0.001). Only in 2 of 16 analyzed pairs, the distances from the center for chromosome 6 homologues differed insignificantly: 0.58 and 0.64 (p>0.1); 0.57 and 0.67 (p>0.1). For other chromosome homologues and analyzed cultures, differences in the position of homologues differed significantly (Table 2). The differences in the location of homologues are often explained by their different activity.

Thus, we showed that centromeres of chromosomes 6, 8, and 11 lie at a longer (0.68, 0.67, 0.7), while centromere of chromosome 18 at a shorter (0.49) radial distance. The difference in centromere location for each chromosome homologues and the absence of differences in radial distances for the analyzed chromosomes in MSC cultures originating from different sources were demonstrated. Long-term culturing (>8 passages) is accompanied by changes in nucleus structure: the centromere of chromosome 6 is displaced distally, which can reflect cell aging or spontaneous differentiation.

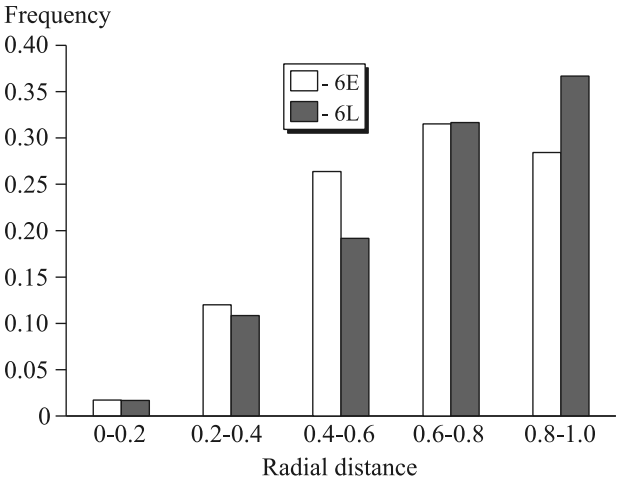

Fig. 1. Frequency distribution for radial distances of chromosome 6 centromeres during early (E) and late (L) passages.

Further study of the structure of interphase nuclei in MSC culture at different terms of culturing and during differentiation is required for better understanding of peculiarities and displacement of chromosome territories in stem and differentiated cells and mechanisms of epigenetic regulation of genome activity and maintenance of stem cell properties via remodeling of the volume structure of chromatin.

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TABLE 2. Medians of Radial Distances for Chromosome 6, 8, 11, and 18 Centromeres

Culture	Chromosome and homologues								Chromosomes			
	6pr	6d	8pr	8d	11pr	11d	18pr	18d	6	8	11	18
MSC-1 E	0.57	0.67					0.43*	0.73*	0.64			0.52
MSC-2 E	0.44*	0.79*							0.61			
MSC-3 E	0.64*	0.88*	0.54*	0.86*					0.72	0.68		
MSC-3 L	0.58*	0.84*	0.57*	0.80*					0.71	0.66		
MSC-4 L	0.62*	0.82*			0.58*	0.81*			0.72		0.70	
MSC-5 E	0.59*	0.79*							0.74			
MSC-6 E	0.58	0.64					0.40*	0.60*	0.59*			0.48*
MSC-7 6	0.53*	0.78*					0.46*	0.67*	0.67			0.53
MSC-7 L	0.61*	0.87*					0.34*	0.60*	0.73*			0.43*

Note. E: early passage; L: late passage; pr: proximal homologue; d: distal homologue for a certain pair. *Pairs with significance of differences ≤0.025.

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