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Biological properties of human bone marrow mesenchymal stem cells (MSCs) during ex vivo expansion for clinical use

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Abstract

Objectives: MSCs are multipotent cells with many potential clinical applications. However, the lack of a precise definition of cell preparation and the heterogeneity of the obtained product call for validation criteria of the final product of the expansion.

Aims: To estimate immunophenotype and genetic stability (karyotype and aneuploid cell frequency) of MSCs from healthy donors during ex vivo expansion.

Methods: The MSCs (n=70) were prepared by seeding bone marrow MNC in flasks in low glucose DMEM with 20% FCS. The immunophenotype of the cultured cells was analyzed: CD90, CD105, CD73, CD45, CD31, CD34, CD133. The karyotype was analyzed by G-banding technique. To analyze the level of aneuploidy, fluorescent in situ hybridization (FISH) with chromosome enumeration probe (CEP) studies were performed.

Results: In all cases the karyotype of the MSCs during expansion remained stable: 46,XY or 46,XX. The disomy rate for 6 and 8 chromosomes in the MSC cultures was 98%, and the tetrasomy rate was 0.9% and did not change during cultivation. The monosomy rate for sex chromosomes (X and Y) in the MSC cultures was higher than the rate of trisomy ($p \leq 0.05$). The rate of aneuploidy in the MSC cultures did not change during cultivation. FISH-analysis of MSC revealed 3 samples with abnormal clones (1 with trisomy 8, and 2 with monosomy X). These clones arose on early passages and remained in the cultures during expansion.

Conclusions: During the culturing procedures chromosome abnormalities can arise which can lead to long term consequences of cell therapy. Further research is required concerning different passaging techniques and culture conditions to better understand their effects on MSC chromosomal stability.

Keywords: mesenchymal stem cells, bone marrow, ex vivo expansion, immunophenotype, genetic stability

<-- Previous abstract Contents Next abstract -->

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Top