CHARACTERIZATION OF NON-IMMORTALIZED MESENCHYMAL STEM CELL LINE ISOLATED FROM HUMAN EPICARDIAL ADIPOSE TUSSUE

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A new non-immortalized mesenchymal stem cell (MSC) line from human epicardial adipose tissue has been obtained from fibroblast-like cells previously isolated from such tissue of a 50-year-old donor during coronary artery bypass grafting and was named ADH-MSC. The analysis of the main characteristics was carried out in the process of long-term cultivation from the 8th to the 16th passages. In the course of such cultivation, the proportion of senescent cells increased. According to the activity of β-galactosidase, the proportion of senescence cells reaches 66% to the 16th passage. In addition to increasing activity of β-galactosidase, to the 16th passage, there were other signs indicating the onset of the active phase of replicative senescence: an increase in cell size and their spreading, a significant decrease in the plating efficiency and index of proliferation, an extension of the time of doubling of cell population. Overall, the results obtained confirm the limited lifespan of ADH-MSC cells, which is typical of non-immortalized cell populations. Karyotypic analysis was carried out at the 8th, 12th and 16th passages. It has been shown that there is a normal human karyotype at the 8th passage, but karyotypic heterogeneity significantly increases by the 12th passage and enhances to the 16th passage exceeding the permissible level of chromosomal abnormalities in normal MSCs. Formation of new structural variants of karyotype (SVK) has been shown. The predominant participation of the short arm of one of the chromosome 21 homologues in both clonal and non-clonal rearrangements and as well as in dicentrics by the type of telomeric associations was found. A decrease in the frequency of polyploids during long-term cultivation was shown. On the 8th and 16th passages, the presence of surface antigens CD44, CD73, CD90, CD105, HLA-ABC typical for human MSCs and the absence of CD34, CD45, HLA-DR were revealed. The marker of undifferentiated human embryonic stem cells (ESC) – SSEA-4 presents at the 8th passage. But the presence this marker is significantly reduced on the 16th passage. The presence of markers of early differentiation of ESC in the derivatives of the 3 germ layers has been shown for cells of ADH-MSC line. It is shown that the cells of the ADH-MSC line have the ability to differentiate at the 8th and 16th passages in osteogenic and chondrogenic directions with the same intensity. However, the intensity of adipogenic differentiation is reduced at the 16th passage compared at the 8th passage. In general, the presented results confirm the status of MSCs for the derived line and indicate significant changes occurring in the process of early replicative senescence that is possibly associated with impaired microenvironment in which there were cells from a donor having heart disease. Early replicative senescence and karyotypic instability may be associated with more significant changes of the DNA repair system in the ADH-MSC line compared to other lines.

Keywords: human mesenchymal stem cells, proliferation, replicative senescence, surface cell markers, karyotype, differentiation