

Characterization and expression of senescence marker in prolonged passages of rat bone marrow-derived mesenchymal stem cells

ABSTRACT

The present study is aimed at optimizing the in vitro culture protocol for generation of rat bone marrow- (BM-) derived mesenchymal stem cells (MSCs) and characterizing the culture-mediated cellular senescence. The initial phase of generation and characterization was conducted using the adherent cells from Sprague Dawley (SD) rat's BM via morphological analysis, growth kinetics, colony forming unit capacity, immunophenotyping, and mesodermal lineage differentiation. Mesenchymal stem cells were successfully generated and characterized as delineated by the expressions of CD90.1, CD44H, CD29, and CD71 and lack of CD11b/c and CD45 markers. Upon induction, rBM-MSCs differentiated into osteocytes and adipocytes and expressed osteocytes and adipocytes genes. However, a decline in cell growth was observed at passage 4 onwards and it was further deciphered through apoptosis, cell cycle, and senescence assays. Despite the enhanced cell viability at later passages (P4-5), the expression of senescence marker, β -galactosidase, was significantly increased at passage 5. Furthermore, the cell cycle analysis has confirmed the in vitro culture-mediated cellular senescence where cells were arrested at the G0/G1 phase of cell cycle. Although the currently optimized protocols had successfully yielded rBM-MSCs, the culture-mediated cellular senescence limits the growth of rBM-MSCs and its potential use in rat-based MSC research.