The landscape of cellular aging: long-term culture of mesenchymal stem cells is associated with specific changes in their DNA-methylation profile

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Abstract

Mesenchymal stem cells (MSC) can only be culture expanded for a limited time until they reach a senescent state, the so-called "Hayflick-limit", which is accompanied by growth arrest, cell enlargement and reduced differentiation capacity. Therefore, culture-associated changes in MSC may hamper their therapeutic potential. In this study, we have analyzed genetic and epigenetic changes upon long-term culture of MSC from human adipose tissue. The fibroblastoid colony-forming unit (CFU-f) frequency and the differentiation potential were already significantly impaired within the initial passages. Relevant chromosomal aberrations were not detected by karyotyping and SNP-microarrays and this supports the notion that human MSC possess relatively little genomic instability. Subsequently, we have compared DNA-methylation profiles with the Infinium HumanMethylation27 Bead Array and the profiles differed markedly in MSC derived from adipose tissue and bone marrow, indicating that the epigenetic makeup of MSC is highly dependent on the tissue of origin. Highly consistent senescence-associated modifications at specific CpG sites, especially in developmental genes, occurred already within the early expansion phase (between the passages 5 and 10). Remarkably, these DNA-methylation changes are highly enriched in regions with repressive histone modifications such as trimethylation of H3K9, H3K27 and EZH2 targets. These results indicate that cellular aging is not just a random accumulation of cellular defects, but that it is precisely regulated by epigenetic means in the course of culture expansion.