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COMMENTARY

Implications of long-term culture for mesenchymal stem cells: genetic defects or epigenetic regulation?

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See related research by Redaelli *et al.*, http://stemcellres.com/content/3/6/47

Abstract

Mesenchymal stem cells change dramatically during culture expansion. Long-term culture has been suspected to evoke oncogenic transformation: overall, the genome appears to be relatively stable throughout culture but transient clonal aneuploidies have been observed. Oncogenic transformation does not necessarily entail growth advantage *in vitro* and, therefore, the available methods - such as karyotypic analysis or genomic profiling - cannot exclude this risk. On the other hand, long-term culture is associated with specific senescence-associated DNA methylation (SA-DNAm) changes, particularly in developmental genes. SA-DNAm changes are highly reproducible and can be used to monitor the state of senescence for quality control. Notably, neither telomere attrition nor SA-DNAm changes occur in pluripotent stem cells, which can evade the 'Hayflick limit'. Long-term culture of mesenchymal stem cells seems to involve a tightly regulated epigenetic program. These epigenetic modifications may counteract dominant clones, which are more prone to transformation.

Genetic or epigenetic changes during culture expansion?

Mesenchymal stem cells (MSCs) require culture-expansion to achieve sufficient cell numbers, particularly for therapeutic applications. Long-term culture, however, is associated with extensive morphological and functional changes, such as loss of *in vitro* differentiation potential. These changes are continuously acquired and conflict with the high demands for standardization and safety in regenerative medicine. Cells in culture may accumulate stochastic mutations, thus bearing the risk of malignant transformation. Furthermore, MSCs acquire specific epigenetic changes during culture, which may account for the functional changes. For a better understanding of this process, Redaelli and coworkers [1] have recently compared genetic and epigenetic modifications in the course of culture expansion of MSCs.

The risk of malignant transformation: an unmet challenge in cellular therapy

So far, oncogenic transformation of MSCs has not been observed despite their extensive application in a wide variety of clinical trials. Two publications reported spontaneous transformation of MSCs with tumor formation in immunocompromised mice - but both were subsequently retracted as the results were based on contamination with immortalized cell lines [2,3]. The concerns, however, still remain: expansion of MSCs is associated with enormous proliferation rates in an artificial cell culture environment and it is well conceivable that this favors genetic defects. Particularly autologous transplant settings appear to be prone to transplant-associated tumor formation because potentially transformed cells would be less challenged by the immune system. In fact, several studies reported occasional existence of transient aneuploidy in MSCs but cells with abnormal karyotype did not persist [1,4]: these were lost upon prolonged culture and all MSC preparations ultimately entered senescence with proliferation arrest. Array-comparative genomic hybridization has been used in search of smaller deletions or duplications [1,5,6]. However, this method only detects dominant cell clones, which account for a significant percentage of the entire cell population. Yet oncogenic transformation is not necessarily associated with growth-advantage *in vitro.* Furthermore, point mutations or other subtle molecular events might also

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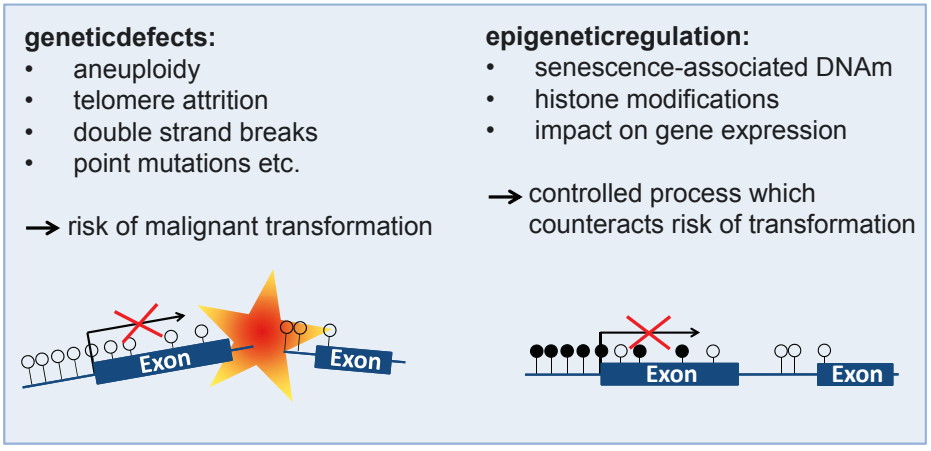

Figure 1. Molecular changes upon long-term culture of mesenchymal stem cells. DNAm, DNA methylation.

predispose to transformation. Therefore, the available methods for karyotypic analysis and genomic profiling cannot exclude the risk of transplant-associated tumor formation.

Epigenetic changes during culture expansion

Epigenetic modifications, such as DNA methylation (DNAm) and histone modification, play pivotal roles in the development, cellular differentiation and aging of the organism. They may also be relevant for functional changes during long-term culture of MSCs: specific CpG sites, particularly in developmental genes and homeobox genes, become differentially methylated at higher passages [1,5,7,8]. These senescence-associated DNAm (SA-DNAm) changes are extremely reproducible in different cell preparations and an 'Epigenetic-Senescence-Signature' can be used to monitor senescence for quality control [9]. SA-DNAm changes are enriched in intergenic regions and seem to be associated with repressive histone marks such as H3K9, H3K27 and polycomb-group protein targets [5,7]. These results indicate that long-term culture of MSCs is associated with a tightly regulated epigenetic program.

Senescence is not an inevitable fate - it may be advantageous

Cellular senescence is not an inevitable fate of all cells in culture: pluripotent cells, such as embryonic stem cells and induced pluripotent stem cells, can bypass the 'Hayflick limit' [10]. Their telomeres refrain from attrition. Notably, embryonic stem cells and induced pluripotent stem cells escape also from SA-DNAm changes [7]. Apparently, these primitive cells can be rejuvenated to grant their specific function for the organism. On the other hand, the barrier of senescence in somatic cells may be purposeful to reduce the risk of oncogenic transformation - it limits expansion of dominant and potentially malignant cells (Figure 1).

Conclusion

The genome of MSCs appears to be relatively stable throughout culture and so far malignant transformation upon MSC transplantation has not been observed in clinical trials. However, the fear of transplant-associated tumor formation still remains and it cannot be ruled out by the available molecular methods. On the other hand, long-term culture of MSCs induces tightly regulated epigenetic modifications. It is yet unclear how this process is regulated, but its purpose might be growth restriction of dominant clones - such as the transient clones with aneuploidy. Such dominant clones may acquire SA-DNAm changes faster than others and consequently disappear from culture due to enhanced senescence. Epigenetic changes might therefore antagonize some of the genetic defects during long-term culture of MSCs.

Abbreviations

DNAm, DNA methylation; MSC, mesenchymal stem cell; SA-DNAm, senescence-associated DNAm.

Competing interests

RWTH Aachen has submitted a patent application for the 'Epigenetic-Senescence-Signature'. The author declares that he has no other competing interests.

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