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COMMENTARY

Beyond genetic stability of mesenchymal stromal cells

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Mesenchymal stromal cells (MSCs) have been used in the clinic for more than a decade. However, this rapidly emerging area of research (352 clinical trials registered at ClinicalTrials.gov) is associated with much debate and controversy. A major issue is the safety and risk of transformation of MSCs during *ex vivo* expansion. Previous articles (1,2), now retracted (3,4), or analyses (5) reported the transformation or risk of transformation of MSCs from different sources during expansion. Although such transformation is likely a rare event (6,7), some genetic abnormalities, mainly aneuploidy, can appear during culture but are not related to the transformation per se (8). Consequently, from a regulatory point of view, we need to develop processes avoiding such transformation that use relevant controls to avoid the risk of transformation. A recent review, following a meeting of the European Medicines Agency in London to ensure the safety of MSC production, at least in pre-clinical settings, determined that cytogenetic abnormalities should be assessed (9). In this issue of *Cytotherapy*, three teams report on the genetic stability of *ex vivo*-expanded human MSCs.

MSCs used in these studies came from three different sources: two from adult tissue [bone marrow (BM MSCs) (10) and adipose tissue (ASCs) (11)] and one from a fetal source [chorionic villi (CV MSCs) (12)]. The culture processes differed. BM MSCs were expanded in a bioreactor (Quantum Cell Expansion System; Terumo BCT, Lakewood, CO, USA), and ASCs and CV MSCs were expanded in flasks. The media were similar, including fetal bovine serum, with the addition of conditioned medium for CV MSCs. Finally, a wide range of techniques were used to assess genetic stability, including conventional karyotyping, spectral karyotyping, fluorescent *in situ* hybridization, genome-wide array comparative genomic hybridization, microsatellite genotyping and micronucleus formation. Whatever the source of cells and process used, the studies found no (BM MSCs) or only rare (ASCs and CV MSCs) clonal cytogenetic abnormalities increasing with time during culture. Injection of BM MSCs and ASCs into immunodeficient mice did not produce tumors. These reports reinforce previous results (8,13) showing that human MSCs do not transform during *ex vivo* expansion, and aneuploidy is not related to transformation but rather to senescence. Microsatellite genotyping did not reveal any allele expansion or loss of heterozygosity. However, sensitivity of karyotyping with conventional techniques or spectral karyotyping was poor. Fluorescent *in situ* hybridization, requiring a knowledge of the target, has slightly increased sensitivity, and array comparative genomic hybridization detects small genomic abnormalities but only in a large percentage of cells (>20–30%). If sensitivity is the problem, existing techniques such as single nucleotide polymorphism and RNA sequencing could be used.

Despite the availability of numerous tools, are we looking in the right direction? Have we defined the real target? Transformation is an extremely rare event and has never been reported after >15 years of clinical use, so how can we predict something that has never happened? To study the potential transformation steps of expanded MSCs, we need transformed human MSC clones. Such studies could reveal mechanisms of transformation of human MSCs and help define relevant controls.

Transformation is only one side of the coin. The other side, senescence, should be avoided too.

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Senescence is related not only to risk of transformation but also to the risk of inefficiency and increase in side effects. As recommended by the European Medicines Agency and other experts, population doublings should be kept to a minimum (9). Although these three reports reinforce that human MSCs that are not overly expanded can be safely used, we look forward to studies giving deeper insight into transformation mechanisms of human MSCs to help establish better relevant controls.

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