Cell Stem Cell
Letters

Limited Acquisition of Chromosomal Aberrations in Human Adult Mesenchymal Stromal Cells

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In their recent article entitled "Large-Scale Analysis Reveals Acquisition of Lineage-Specific Chromosomal Aberrations in Human Adult Stem Cells," Ben-David and colleagues conclude that human adult mesenchymal stromal cells (MSCs) have a 4% probability of acquiring chromosomal abnormalities (Ben-David et al., 2011). Moreover, the authors propose, based on their analysis, that transplantation of adult stem cells may result in tumor formation, an assertion that calls into question the safety of MSC-based cell therapy. Their study is based on a comprehensive evaluation of acquired chromosomal aberrations performed using gene expression profiles for a large series of human stem cells from different origins (neural stem cells, MSCs, and pluripotent stem cells). However, in our opinion some of the points within the reported results and in the presented conclusions should have been discussed in a more balanced way, particularly those that relate to adult MSCs, which are already being used for clinical trials looking at immunoregulation and tissue repair.

Overall, Ben-David et al. identified MSC genetic instability in 4 studies out of the 22 that they analyzed. In one of them (GSE18934_GSM469130; Ben-David et al.'s Table S1), the MSCs presenting chromosomal aberrations were derived from fetal liver, and thus should not be considered postnatal stem cells of adult origin. In another (GSE9520; Ben-David et al.'s Table S1), Ben-David et al. report three different MSC preparations displaying the same monosomy 6q appearing at the beginning of the second passage at day 2, but later disappearing at day 7. This culture development is not discussed by Ben-David et al., but would seem to be at odds with their general interpretation that "in MSCs chromosomal aberrations can take over the culture in as few as seven passages" and that "multipotent stem cells are prone to acquire advantageous chromosomal aberrations that enable them to rapidly outgrow the normal cell population." On the contrary, these results suggest a selective pressure against monosomy 6q. The findings of Ben-David et al. that only 4% of examined MSC lines carry a detectable genomic abnormality is in agreement with those of Tarte et al. (2010), who reported a longitudinal study of 20 clinical-grade MSC preparations obtained using two distinct and well-defined culture conditions and showed that the cells do display some chromosomal abnormalities, i.e. trisomies of chromosomes 5, 8, and/or 20, as detected using conventional karyotype and FISH analysis. However, as for the monosomy 6q, these trisomies did not appear to confer any selective mitogenic advantage in vitro and disappeared rapidly after the second or the third passage. Finally, all MSC batches reached senescence without recurrence of these chromosomal abnormalities and no cell transformation could be documented either in vitro or in immunocompromised mice. All these observations argue against the idea that acquisition of lineage-specific chromosomal aberrations confers a growth advantage in human adult stem cells.

Finally, Ben-David et al. cite certain potentially misleading articles to support their arguments. The potential transformation of MSCs in culture reported by Røsland et al. (2009) was related to a cross-contamination by various cancer cell lines (Torsvik et al., 2010). The same problem occurred in a study performed on adipose tissue-derived MSCs that was subsequently retracted (de la Fuente et al., 2010, addressed by Garcia et al., 2010), underlining the absolute requirement to validate, prior to scientific publication, that the cells analyzed at the end of a culture period actually derive from those used to initiate the culture. It also seems misleading to state that "transplantation of human adult stem cells may result in tumor formation" by referencing an article in which cells used were fetal neural cells and not adult cells (Amariglio et al., 2009), and another article showing tumor formation only in immunocompromised mice injected with NSCs derived from an olfactory bulb adjacent to meningioma (Casalbore et al., 2009).

In conclusion, we would argue that genomic stability of cultured adult stem cells and MSCs in particular is robust and not as significant a source of concern as was suggested by Ben-David et al. (2011). Thus, we would suggest that a more balanced view of the fundamental issue of genetic stability of adult stem cells should be provided to all parties with an interest in this field, including researchers, physicians, and regulatory authorities.

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Significant Acquisition of Chromosomal Aberrations in Human Adult Mesenchymal Stem Cells: Response to Sensebé et al.

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Our recent article (Ben-David et al., 2011) presented a comprehensive evaluation of chromosomal aberrations in pluripotent and multipotent cell types. We reported that ~9% of the pluripotent and neural stem cells (PSCs and NSCs, respectively), and ~4% of the mesenchymal stem cells (MSCs), that we analyzed harbored large chromosomal aberrations. We found that each stem cell type was prone to acquire distinct recurrent chromosomal abnormalities, aberrant cells could outgrow the normal cells in culture within several passages, and the common aberrations in stem cell cultures resembled characteristic aberrations of tumors from the same cell lineages. Importantly, we detected some aberrations that had been overlooked—and, consequently, not controlled for—in the original studies reporting the cell lines. We therefore concluded that the genomic integrity of stem cells should be monitored carefully before using the cells in a clinical setting. We anticipated that this article would provoke discussion, and we welcome the questions raised by Sensebé et al. (2012).

First, we would like to clarify terminological ambiguity. In order to distinguish them from embryonic stem cells (ESCs), we used the generic terms "adult stem cells" and "multipotent stem cells" alternately, as commonly used to refer to stem cells derived from adult, newborn, or fetal tissues, all of which are cell sources under evaluation for clinical application. In order to prevent potential confusion, the exact origin of all MSC samples analyzed was mentioned throughout the article. Importantly, seven out of the eight MSC samples that harbored chromosomal aberrations had been derived from adult tissues (Table S1 in Ben-David et al., 2011). Moreover, the same aberration found in the fetal-liver-derived MSC line (GSE18934_GSM469130) was independently identified in an adult-bone-marrow-derived MSC line (GSE6460_GSM148485). Therefore, the chromosomal aberrations we identified are shared by MSCs of various origins.

Sensebé et al. discuss the observations by us and others that some chromosomal aberrations do not confer any growth advantage to the cells in vitro. However, such findings do not preclude other aberrations from being advantageous, as we found in the case of aberrations in chromosomes 7q and 17q, which appeared in a bone-marrow-derived MSC line by passage 21 and took over the culture by passage 28 (GSE7637_GSM184649-53). Moreover, in the case of monosomy 6q, the aberration did not simply disappear from culture; rather, our analysis shows that it most likely still existed at the later passage, but didn't meet the stringent criteria for statistical significance, probably due to mosaicism in culture. Because this monosomy was independently identified twice in our analysis (Table S1 in Ben-David et al., 2011), and was also found to recur in late passages of adipose-tissue-derived MSCs (Buyanovskaya et al., 2009), we do not think it should be dismissed. Together, these findings are in line with recent studies of PSCs demonstrating two types of genomic aberrations: transient aberrations that occasionally appear in culture, but are disadvantageous, and thus disappear throughout culture propagation (Hussein et al., 2011); and advantageous recurrent aberrations, which rapidly accumulate in culture in a clonal manner (Amps et al., 2011; Mayshar et al., 2010). In MSCs, Sensebé et al. discuss the former type; however, the latter type was also reported by us and others (Buyanovskaya et al., 2009; Estrada et al., 2011; Ueyama et al., 2011). Thus, these two manifestations of genomic instability exist simultaneously in stem cell cultures.

In this context, it is important to point out our finding that independent MSC lines of different origins lost one copy of chromosome 13. This monosomy was