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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

identified in our study only in MSCs, and was previously reported to recur in immortalized MSCs as well (Takeuchi et al., 2009); interestingly, this monosomy is the most common in bone and soft tissue tumors (Figure 2E in Ben-David et al., 2011). Moreover, some mesenchymal tumors have been suggested to originate from mutated MSCs in vivo (reviewed in Mohseny and Hogendoorn, 2011). Thus, the recurrent aberrations in MSCs may simply imply an ability to confer growth advantage, both to stem cells in vitro and to tumors in vivo, or they may indicate the beginning of a spontaneous transformation in culture. More studies of the functional consequences of these aberrations are needed to distinguish between these possibilities.

Finally, the question remains of whether acquisition of large chromosomal aberrations by approximately 1 out of 25 MSC cultures is alarming or reassuring. Until the functional consequences of these aberrations are elucidated, this question will remain outstanding. Notably, however, our analysis did not look at small genomic changes or point mutations (both of which are reported to occur in PSCs), so it is likely to be an underestimate of the real prevalence of genomic aberrations in MSCs. Our analysis also reflects only aberrations that became established in most of the cells in culture, and mosaic MSC cultures with chromosomal abnormalities are probably common, as has been recently demonstrated by Estrada et al. (2011). In this FISH-based analysis of the chromosomal integrity of human MSCs, the authors also observed a clear tendency toward increased aneuploidy with passage. We would therefore argue that the genomic stability of cultured MSCs should not be defined as "robust."

In conclusion, we agree with Sensebé et al. that a balanced view of the fundamental issue of genomic stability of adult stem cells should be provided. This desirable balance should bind all parties not to overinterpret our findings, and not to rush into hasty conclusions; it should also bind all parties not to disregard these findings, and to directly confront the concerns that they raise. Recently, the International Stem Cell Initiative (ISCI) surveyed 125 independent human ESC lines, provided by 38 laboratories in 19 countries, using karyology and high-resolution SNP arrays to identify common genetic changes that occur during prolonged culture (Amps et al., 2011). We believe that the MSC field would benefit from a similar large-scale prospective analysis, and we reiterate to our recommendation to monitor the genomic stability of MSCs propagated in culture on a regular basis.

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