PERSPECTIVES

Functional markers and the 'homogeneity' of human mesenchymal stem cells

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Stem cells are characterized by the abilities to self-renew, generate large numbers of progeny and differentiate into at least one mature cell type. Bone marrow serves as a reservoir for several classes of adult stem cells. In addition to haematopoietic stem cells (HSCs), which can reconstitute the haematopoietic system of a myeloablated host, bone marrow contains a diverse population of marrow stromal cells (Herzog *et al.* 2003). Included among these are mesenchymal stem cells (MSCs), which *ex vivo* can be isolated as a relatively *homogeneous* and undifferentiated cell population that produces multiple mature cell types including fat, bone and cartilage (Pittenger *et al.* 1999). Under appropriate, but ill-defined conditions, human MSCs (hMSCs) can also differentiate into other cell types, including excitable cells with neuronal-, myogenic and cardiomyogenic-like phenotypes. Because MSCs are multipotent and readily expandable *in vitro*, these cells have already been employed in early clinical studies, including the treatment of human myocardial infarction. Transplantation of autologous or allogeneic MSCs therefore represents a novel form of cellular therapy, which shows substantial promise for the treatment of a number of human diseases.

A number of fundamental questions relating to the biology of undifferentiated MSCs remain unanswered. These involve the homogeneity of the cells used for therapy, the best way of introducing (local or systemic) the cells for therapeutics, the survival and homing capacity of the cells to host tissues following transplantation, and the differentiation potential of these cells *in vitro versus in vivo*. Ultimately, scientists and clinicians must demonstrate that MSCs and their derivatives *function* in a normal physiological way *in vivo*.

In this issue of *The Journal of Physiology*, Heubach *et al.* (2004) have begun the early and critical electrophysiological characterizations of undifferentiated hMSCs. This study lays the groundwork for the identification of *functional* markers prior to and following differentiation of hMSCs both *in vitro* and *in vivo*. Heubach *et al.* have identified and characterized several inward and outward cell currents typically present in a 'homogeneous' population of MSCs obtained either from primary cell isolates or from a commercial source. The authors demonstrate the presence of two outward currents (I_r and I_s) in the majority of hMSCs, and the I_r current was further characterized as a large-conductance voltage- and Ca^{2+}-activated K^+ current. Inward currents were, however, only present in a *subpopulation* of hMSCs. Even though cells employed in this study were considered relatively homogeneous ($CD29^+$, $CD105^+$, $CD166^+$, $CD34^{--}$ and $CD45^{-/lo}$), the finding that hMSCs displayed a mixed distribution of channels argues for a heterogeneous cell population. Specifically, the L-type Ca^{2+} channel, which is critical for excitation–contraction coupling mechanisms in adult heart, was only found at a low frequency in hMSCs. Similarly, Kawano *et al.* previously demonstrated that store-operated Ca^{2+} currents could be recorded in a majority of cells, but that only 15% of hMSCs demonstrated a dihydropyridine-sensitive calcium current typical of L-type calcium channels (Kawano *et al.* 2002).

Since we know that some MSCs can form heart cells, do subpopulations of hMSCs or epigenetic modifications of hMSCs in culture permit differentiation to excitable cell (cardiac) precursors? A cardiomyogenic cell line has previously been established by culturing marrow stromal cells from mice in the presence of the DNA demethylation agent 5-azacytidine, showing that epigenetic modifications can be crucial to the formation of cardiomyocytes (Makino *et al.* 1999). Alternatively, the identification of small numbers of cells that express distinct patterns of ion channels argues for the presence of poorly defined precursor/stem cell populations or differentiated cells in hMSC cultures. In this regard, it would be useful to extend the present study to test for the presence of connexins (Cx40, 43 and 45) and functional gap junctions. Oyamada *et al.* previously showed that undifferentiated embryonic stem cells express gap junctions (Oyamada *et al.* 1996), and several groups have shown that gap junctions are important for the differentiation process to cardiomyocytes. The presence of gap junctions in a subpopulation of hMSCs might argue for the presence of an excitable cell precursor.

Finally, the development of clonal assays and cell sorter-based separation techniques proved critical to the isolation of candidate stem cell populations from the haematopoietic system of mice. These advances ultimately led to the identification of two classes of multipotent cells – long-term and short-term reconstitutive HSCs and to the delineation of haematopoietic stem and progenitor cell lineage hierarchies (Weissman *et al.* 2001). This in turn led to important medical advances in cancer therapy, transplantation and autoimmunity. Multipotent hMSCs have been isolated in clonal assays, but the isolated clones did not differentiate with an equal degree of plasticity (Pittenger *et al.* 1999). Together with the current functional findings of Heubach *et al.* (2004) these results suggest that unique subpopulations may be present in hMSC cultures that preferentially differentiate to selected mature cell types. Clearly, further basic research is required, and the use of cell surface or functional markers may prove critical to this evaluation. Only after we have a better understanding of the basic mechanisms and the specific cells required for directed differentiation will we be able to fully exploit hMSC multipotentiality for therapeutic purposes.

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