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COMMENTARY

Transition of mesenchymal stem/stromal cells to endothelial cells

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Abstract

Mesenchymal stem/stromal cells (MSCs) are heterogeneous. A fraction of these cells constitute multipotent cells that can self-renew and mainly give rise to mesodermal lineage cells such as adipocytes, osteocytes and chondrocytes. The ability of MSCs to differentiate into endothelial cells remains controversial. Isolation and *in vitro* manipulation of MSCs before clinical application are important steps. High numbers of MSCs are needed, requiring the *in vitro* expansion of these clinically important cells. To this end, a well-controlled procedure for MSC isolation and maintenance in culture is necessary.

A study published in the current issue of *Stem Cell Research and Therapy* shows that the phenotypic characteristics and multi-lineage differentiation efficiency of mesenchymal stem/stromal cells (MSCs) derived from human Wharton's jelly (WJ) are dependent on the isolation methods and culture conditions used [1]. MSCs are multi-lineage mesodermal progenitor cells selected by their capacity to adhere to plastic and proliferate in long-term cultures of unfractionated cell suspensions. Initially described in the adult bone marrow, MSCs now can be derived from many other developing and adult tissues including fat, skeletal muscle, dental pulp, placenta and umbilical cord. MSCs have a defined phenotype, expressing the cell surface markers CD44, CD73, CD90 and CD105. However, such a retrospective isolation *in vitro* results in high cell heterogeneity in the culture dish, even more so since isolation procedures and culture conditions differ significantly from one laboratory to another. Such differences in cell processing may generate variable results, as evidenced by the present study.

The endothelial differentiation potential of MSCs is controversial and may depend on their tissue origin [2,3]. Besides their capacity to differentiate into adipocytes, bone and cartilage, which will not be discussed here, Corotchi and colleagues compare in their study the endothelial differentiation capacity of WJ-MSCs isolated and cultured along distinct protocols [1]. Unfractionated cells derived from WJ are either seeded directly into endothelial cell medium or first cultured in xeno-free MSC medium before endothelial cell medium, prior to phenotypic and functional detection of endothelial cells. Unfractionated WJ cells directly cultured in endothelial cell medium upon enzymatic dissociation express, five passages later, endothelium-related genes and proteins and form vascular networks *in vitro*. The emergence of such endothelial cells could be due to contamination by endothelial cells or progenitors upon isolation. Indeed, endothelial cell marker mRNAs (very similar to that seen in human endothelial cells derived from the umbilical cord vein) are found in the cell suspension upon isolation but before culture, although these are not detected as proteins.

As extensively discussed by Corotchi and colleagues, there is no clear evidence showing that WJ-MSCs are able to differentiate into endothelial cells as previously shown from other tissues [2-4]. Simultaneous isolation of rare endothelial cells or endothelial progenitor cells and MSCs from WJ could explain these results. Endothelial cells would preferentially grow in these culture conditions. Interestingly, after five passages in endothelial cell medium, most WJ-derived cells not only express the endothelial cell markers CD31, CD34, von Willebrand factor, CD144, Tie-2, and vascular endothelial growth factor receptors 1 and 2 (Flk1), but also typical MSC markers such as CD44, CD73 and CD105. Intriguingly, CD90 is absent. Multicolor flow cytometry analysis would provide further information about the

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frequency of cells coexpressing endothelial and MSC markers. This information leads to raising questions of whether some endothelial (progenitor) cells were able to transdifferentiate toward MSCs and whether some MSCs were truly able to differentiate into endothelial cells. Transdifferentiation in both ways was indeed previously reported in other systems [5,6].

It would be very interesting to know whether the mesenchymal–endothelial like cells described in this study maintain their capacity to differentiate into adipocytes, osteocytes and chondrocytes. Myoendothelial cells were described in the adult human skeletal muscle, which coexpress endothelial and myogenic cell markers and can regenerate muscle [7]. Importantly, these myogenic endothelial cells are also able to differentiate toward bone and cartilage [8]. Hemogenic endothelial cells coexpress hematopoietic and endothelial cell markers and generate definitive self-renewing hematopoietic stem cells *in vivo* (reviewed in [9]). However, the cells derived by Corotchi and colleagues from human WJ and coexpressing both endothelial and mesenchymal cell markers are only described in culture. It would be interesting to test whether a common mesenchymal–endothelial progenitor is present *in situ* in human WJ, or whether the presence of such cells is an artifact of cell culture. A common progenitor for endothelial cells and adipocytes has been reported in human white adipose tissue [10].

A second culture condition was tested in this study to determine whether WJ cells can differentiate into endothelial cells. Corotchi and colleagues show that WJ-MSCs do not differentiate into endothelial cells if they are first cultured in xeno-free MSC medium followed by endothelial cell medium. These observations were independent of the protocol used for cell isolation. Endothelial cell markers were completely absent and no capillary-like structures developed in Matrigel [1]. In contrast, all typical MSC markers were expressed. However, osteogenic, chondrogenic and adipogenic assays were not performed after endothelial cell medium was added. It would thus be informative to test whether the multipotentiality of MSCs is maintained in these endothelial cell culture conditions, as is the case in xeno-free MSC medium.

The lack of endothelial differentiation of WJ-MSCs was also recently reported [11]. Importantly, Choi and colleagues showed that WJ-MSCs cultured in these conditions act as a perivascular niche and stabilize blood vessels *in vivo*. Besides displaying an MSC phenotype, these WJ-MSCs express perivascular cell markers and are found localized within the perivascular wall of newly formed functional blood vessels when transplanted into an ischemic mouse. In contrast, transplantation in the same mouse model of human endothelial cells derived from the umbilical cord vein also gave rise to blood vessels but these were not functional, suggesting a key paracrine function of perivascular WJ-MSC-derived cells in vessel stabilization [11]. These data are no surprise. The affiliation between MSCs and perivascular cells in human tissues was recently described [12]. Pericytes purified to homogeneity by flow cytometry from multiple human organs and cultured over the long term become indistinguishable from conventional MSCs in terms of morphology, growth properties, phenotype, and developmental potential [12]. Pericytes/MSCs were also derived from human pluripotent stem cells that, upon transplantation into ischemic mouse limbs, promoted vascular recovery and skeletal muscle regeneration [13]. Pericytes/MSCs did not differentiate into endothelial cells, but were incorporated within the perivascular wall of *de novo* formed functional blood vessels of host origin. Moreover, human pluripotent stem cell-derived pericytes can develop in culture into chondrocytes, osteoblasts and adipocytes similar to conventional MSCs and purified pericytes. Similarly, perivascular cells purified from human skeletal muscle improved cardiac anatomy and function when transplanted into the ischemic mouse heart [14].

In conclusion, Corotchi and colleagues show that isolation protocols and culture conditions can give rise to different results, in terms of cell lineage development, starting with the same unfractionated human WJ cells. The cells used by the authors are highly heterogeneous upon initial isolation and different cell types can be enriched upon culture, namely MSCs or endothelium. Whether MSCs differentiate into endothelial cells remains controversial. Interestingly, this group describes for the first time a population of cells that coexpress endothelial and mesenchymal cell markers. These are functional endothelial cells but whether they can still differentiate into adipocytes, chondrocytes and osteoblasts was not tested. Phenotypically, these cells isolated from human umbilical cord are reminiscent of mesoangioblasts, the mesenchymal-like cells mainly associated with the walls of large vessels. Although it is thought that mesoangioblasts are similar to pericytes of small vessels, they express endothelial cell markers such as Flk-1 and are able to differentiate into endothelial or mesodermal cell lineages [15]. Whether the mesenchymal–endothelial-like cells described herein by Corotchi and colleagues emerge as a consequence of culturing conditions or whether they exist natively *in vivo* in human WJ prior to culture deserves further investigation.

Abbreviations

MSC: Mesenchymal stem/stromal cell; WJ: Wharton's jelly.

Competing interests

The author declares that she has no competing interests.

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