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Pericytes, or mesenchymal stem cells, is that the question?

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Abstract
For almost a decade, mesenchymal stem cells (MSCs) were believed to reside as perivascular cells *in vivo*. In this issue, Guimarães-Camboa et al. challenge this idea, and use lineage tracing to demonstrate that perivascular cells do not behave as tissue-specific progenitors in various organs, despite showing MSC potential *in vitro*.

Main Text
Mesenchymal stem cells (MSCs) have attracted considerable attention as promising tools for cell-based regenerative therapies. The prospect of being able to transplant, or even reactivate *in situ*, tissue-resident progenitors evokes great interest, particularly as alternative strategies, such as the use of induced pluripotent stem cells, prove increasingly challenging. However, little is currently known about the biology of such progenitors in their native microenvironment, and the nature and functions of MSCs *in vivo* remain unclear. In this issue of *Cell Stem Cell*, Guimarães-Camboa and colleagues provide new insights into the identity of these cells, and challenge the previous idea that they may correspond to perivascular cells *in vivo*.

Strictly, the term MSC refers to a sub-population of cells in the bone marrow (BM) that was found to be able to regenerate the BM stroma and its environment upon serial transplantation (Sacchetti et al., 2007). When grown *in vitro*, BM-MSCs behave as adherent, colony-forming cells with the ability to differentiate into all skeletal tissue lineages (chondrocytes, osteoblasts and adipocytes). Cells exhibiting the same characteristics as BM-MSCs *in vitro* have been extracted from many organs, leading to the hypothesis that MSCs exist in most tissues where they can participate in both tissue homeostasis and repair. When isolated and reintroduced *in vivo*, these cells contribute to multiple lineages. However, their ability to self-renew and differentiate into tissue-specific lineages within their endogenous environment, without any experimental manipulation, was not consistently proven, raising an ongoing and heated debate about their definition as MSCs, given that the term is restricted to native *in vivo* populations (Bianco, 2014). In 2008, Crisan and colleagues made a prominent advance towards the identification of MSCs *in vivo*, by finding that perivascular/mural cells, i.e. pericytes and vascular smooth muscle cells (vSMCs), extracted from several human tissues behave as MSCs *in vitro* and upon transplantation *in vivo*, while expressing MSC markers as shown by histological analysis (Crisan et al., 2008).

Since then, it was postulated that perivascular cells behave as MSCs *in vivo*. Further studies supported this model, showing that transplanted purified perivascular cells (Chen et al., 2015; Dellavalle et al., 2007) and, more importantly, genetically-traced pericytes and vSMCs (Feng et al., 2011; Goritz et al., 2011; Krautler et al., 2012; Tang et al., 2008) contribute to tissue-specific lineages *in vivo*.

In the current issue, Guimarães-Camboa and colleagues now challenge this concept and suggest that mural cells do not intrinsically behave as MSCs during aging and repair in multiple adult organs. Their work relies on the identification of Tbx18 as a gene specifically expressed in all mural cells of many adult organs in the mouse, including the brain, heart, skeletal muscle, and brown and white adipose tissues. Using a transgenic line they generated expressing an inducible Cre recombinase in Tbx18-expressing cells (Tbx18-CreERT2), they perform a tissue-wide lineage tracing study and follow the progeny of pericytes and vSMCs during both aging and post-injury tissue repair. Surprisingly, they find that over the course of two years, Tbx18 lineage-derived cells maintain their perivascular identity in brain, heart, muscle and fat, therefore suggesting that mural cells do not exhibit overt potential to give rise to other cell types during aging in these organs. In order to test whether such potential could arise in the context of tissue repair, the authors genetically-traced Tbx18 lineage-derived cells following injury (brain, heart, muscle) or under strong adipogenic stimuli. In all cases, marked pericytes and vSMCs did not contribute to other tissue-specific cell types, thus strongly suggesting that mural cells do not behave as MSCs in the studied organs.

This work raises important questions, both regarding the methods used to identify MSCs and to assess their potential *in vivo*, as well as about the biology of MSCs itself. Transplantation has been widely used as a technique to assess cell potency *in vivo*. However, discussions in the field have already suggested that the differentiation potential of transplanted cells could be affected by *ex vivo* manipulation, and cell-cell fusion events with host cells have also been reported. Genetic lineage tracing studies, on the other hand, offer the possibility to label cells and follow their progeny within their native microenvironment, and constitute the most reliable method to assess cell potency *in vivo*. However, the strategy of lineage tracing is highly dependent on the genetic tools at use. The discrepancies between the present work and previous studies may, therefore, mostly rely on the specificity of the transgenic lines used to mark mural cells *in vivo*. Notably, Guimarães-Camboa and colleagues show here that the PDGFRß-Cre line, previously used to follow the progeny of mural cells *in vivo* (Krautler et al., 2012; Tang et al., 2008) is not suitable for this purpose as PDGFRß is expressed throughout the embryo and in adult organs within non-mural cell types. Other studies using mouse lines, however, do show contribution of perivascular cells to tissue-specific cell types during postnatal development (Feng et al., 2011) and in post-injury responses (Feng et al., 2011; Goritz et al., 2011) with no reported lack of specificity. The divergence of their results with the present work may suggest that mural cells can behave as MSCs, but that this behavior is dependent on the organ and developmental stage. At present, it is clear that pericytes and vSMCs, best characterized by their morphology and

topology, are a highly heterogeneous population in terms of ontogeny, expression profiles and even cell type and functions. Therefore, mural cells may show different cellular potencies within different organs and with disparate developmental ages. With the advent of single cell technologies, the true heterogeneity and potential plasticity of perivascular cells will likely be revealed with much greater detail in the near future.

This work also raises important questions regarding MSC biology. First, it challenges the previous established idea that MSCs identify as perivascular cells. However, since Guimarães-Camboa and colleagues label a very high percentage, but not all mural cells, they may have overlooked the contribution of a small but nevertheless existing population of mural cells with progenitor properties. It also should be noted that the present conclusions rely on the use of antigen profiles defined by our current knowledge and understanding of mural cell identity, and that MSCs could constitute a subset of perivascular cells with distinct properties and markers. Finally, a major finding of this study is that cells possessing all the hallmarks of MSCs *in vitro* can lack any MSC potential *in vivo*. This finding puts into question much of the previous literature supporting the existence of multi-lineage progenitors, such as MSCs, in many adult organs based on the transplantation of isolated cells, and challenges the existence of MSCs itself in those organs. Further work will need to address this key issue, and assess the potential of putative MSCs *in situ*. As many clinical trials using MSCs as therapeutic agents are currently under way and show only limited success, the present study highlights a crucial need for a better definition and understanding of the potential of MSCs, both in their native environment and as therapeutic tools.

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