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REVIEW

Mesenchymal stromal cells: misconceptions and evolving concepts

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Abstract

Nearly half a century has passed since the publication of the first articles describing plastic-adherent cells from bone marrow, referred to initially as colony-forming unit fibroblasts, then marrow stromal cells, mesenchymal stem cells and most recently multipotent mesenchymal stromal cells (MSCs). As expected, our understanding of the nature and biologic functions of MSCs has undergone major paradigm shifts over this time. Despite significant advances made in deciphering their complex biology and therapeutic potential in both experimental animal models and human clinical trials, numerous misconceptions regarding the nature and function of MSCs have persisted in the field. Continued propagation of these misconceptions in some cases may significantly impede the advancement of MSC-based therapies in clinical medicine. We have identified six prevalent misconceptions about MSCs that we believe affect the field, and we attempt to rectify them based on current available data.

Key Words: *adult stem cells, marrow stromal cells, mesenchymal stem cells, multipotent mesenchymal stromal cells*

Introduction

Over the past several decades, concepts regarding the nature and function of mesenchymal stromal cells (MSCs) have undergone numerous major paradigm shifts. Pioneering studies by Friedenstein and colleagues first revealed that MSCs were capable of sustaining hematopoiesis and functioned as progenitors of adipogenic, chondrogenic and osteogenic lineages, properties exploited in early clinical trials (1–3). As interest in MSCs expanded, studies conducted in experimental animal models revealed the cells also possessed potent tissue reparative properties. Initial studies attributed this activity to direct cell replacement via the transdifferentiation of transplanted MSCs. However, subsequent work by many laboratories revealed that MSCs promote tissue repair via paracrine action. In recent years, the therapeutic potency of MSCs has been attributed to the secretion by cells of a large number of factors that possess angiogenic, trophic, neuro-regulatory, immunomodulatory, and anti-inflammatory activity. However, as concepts became outmoded and replaced with new paradigms, many misconceptions related to the nature and biology of MSCs arose. In this article, we identify at least six misconceptions (Figure 1) that have persisted over the years and serve as potential impediments to the successful therapeutic application of MSCs. Where possible, we attempt to clarify these misconceptions based on available published literature.

Misconceptions about MSCs

MSCs isolated from different tissues are equivalent

Although initially isolated from bone marrow (4) and then adipose tissue (5), MSCs or MSC-like cells have been identified in many tissues and organs. The apparent ubiquitous presence of MSCs in most tissues is attributed to their similarity to peri-vascular cells *in vivo*. This concept originated from studies demonstrating that bone marrow-derived MSCs express antigens common to endothelial cells and pericytes, such as STRO1 (6), CD146 and 3G5 (7), and conversely that post-capillary venule pericytes from bone marrow (6) and peri-vascular cells in most blood vessels exhibit MSC-like characteristics (7–9).

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Box 1

Misconceptions about the nature and biology of MSCs

1. MSCs isolated from different tissues are equivalent.
2. MSCs can be defined by their surface epitopes.
3. Cloning MSCs provides homogeneous preparations of cells.
4. Mouse MSCs can be isolated and expanded under the same conditions as human MSCs.
5. The properties of MSCs in culture reflect their properties *in vivo*.
6. MSCs should not be tested in clinical trials until their mechanism of action to produce therapeutically beneficial effects are fully defined.

Figure 1. General misconceptions relating to MSCs.

Subsequent studies have shown that peri-vascular cells, pericytes and fibroblasts from different tissues closely resemble the surface phenotype of MSCs, exhibit similar genome-wide expression profiles based on cluster analysis of microarray data and share similar functional properties based on qualitative *in vitro* assays (9,10). Nevertheless, closer scrutiny of these data reveal marked differences in expression levels of lineage-restricted messenger RNAs between pericytes and MSCs (10), and more rigorous *in vivo* assays demonstrate clear differences in function between cells from different tissues (11,12). For example, MSCs in general lack the contractility of pericytes, and in one study ectopic transplantation of bone marrow-derived MSCs yielded heterotopic bone tissue, whereas dental pulp-derived MSCs produced dentin and pulp tissue (12). Similarly, the capacity to generate bone and cartilage is weaker for placental and adipose-derived MSCs compared with bone marrow-derived MSCs, and the contribution to muscle fiber formation *in vivo* is greater with post-natal skeletal muscle pericytes than bone marrow-derived MSCs (13).

Several laboratories have demonstrated a neuro-ectodermal origin for MSCs. For example, Takashima *et al.* (14) demonstrated that $Sox1^{+}$ neuro-epithelial cells via a neural crest intermediate give rise to mesenchymal derivatives with properties of MSCs. Similarly, Mendez-Ferrer *et al.* (15) demonstrated that cells isolated based on expression of the neuro-epithelial marker Nestin are precursors of MSCs and can serially regenerate heterotopic osseous tissue *in vivo*. Generation of MSCs from the neural crest likely occurs via an epithelial-to-mesenchymal transition. Forced expression of the potent epithelial-to-mesenchymal transition inducer TWIST in mammary epithelial cells generated mesenchymal derivatives with MSC-like properties (16). Because TWIST plays an important role as a self-maintenance factor in MSCs (17), it may represent a useful "marker" of MSC origin and function. A neuro-ectodermal origin may also explain early results indicating that MSCs share specific traits with neural cell lineages (18). Pericytes within brain (19), thymus (20) and heart tissue (21) also reportedly derive from neural crest derivatives. These findings may explain why in some tissues MSCs and pericytes exhibit similar phenotypic and gene expression profiles.

The prevailing evidence suggests that MSCs (and pericytes) originate from several distinct developmental programs and progenitor cells. Although MSCs from different tissues share similarities in phenotypes and gene expression profiles, differences in function may be distinguished experimentally, provided that the assays are sufficiently rigorous. Consequently, not all MSCs are equivalent, and the functional attributes of populations isolated from different tissues should be carefully evaluated before implementation in clinical therapy.

MSCs are defined by their surface epitopes

Many laboratories have devoted much effort over the years to identify antigens that associate the developmental potential of MSCs with a specific phenotypic trait. MSCs express a large complement of integrin receptors (CD29, CD49a through CD49f, CD51), adhesion molecules (CD44, CD105, CD106, CD146, CD166), enzymes (CD39, CD73), growth factor receptors (CD140b, CD271, CD340, CD349), intermediate filaments (vimentin, nestin, desmin, neurofilament) and embryonic antigens (SSEA1, SSEA4), but no single molecule uniquely defines the population. Prospective isolation of MSCs with antibodies against STRO1 (6), CD271 (22) or CD146 (23), or selection for nestin-expressing cells (15) all yield the entire complement of colony-forming unit fibroblasts from marrow. Most of these antigens identify MSCs but not uniquely. Consistent with this result, analysis by a European consortium identified a complement of 113 transcripts and 17 proteins that distinguished MSCs from hematopoietic, endothelial and periosteal cells and synovial fibroblasts (24). Although the MSC committee of the International Society for Cell Therapy (25) stated in 2006 that human "MSCs must express CD105, CD73 and CD90, and lack expression of CD45, CD34, CD14 or CD11b, CD79α or CD19 and HLA-DR surface molecules," at the time this definition was recognized as being limited because the epitopes CD105, CD73 and CD90 are expressed on many different cells. The problem of ascribing a surface phenotype to MSCs is confounded further by the fact that populations exhibit significant donor-to-donor and intra-population heterogeneity (see later) and radically alter their features as they are expanded in culture (23,26,27) and after