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Are nestin-positive mesenchymal stromal cells a better source of cells for CNS repair?

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ABSTRACT

In recent years there has been a great deal of research within the stem cell field which has led to the definition and classification of a range of stem cells from a plethora of tissues and organs. Stem cells, by classification, are considered to be pluri- or multipotent and have both self-renewal and multi-differentiation capabilities. Presently there is a great deal of interest in stem cells isolated from both embryonic and adult tissues in the hope they hold the therapeutic key to restoring or treating damaged cells in a number of central nervous system (CNS) disorders. In this review we will discuss the role of mesenchymal stromal cells (MSCs) isolated from human olfactory mucosa, with particular emphasis on their potential role as a candidate for transplant mediated repair in the CNS. Since nestin expression defines the entire population of olfactory mucosal derived MSCs, we will compare these cells to a population of neural crest derived nestin positive population of bone marrow-MSCs.

1. Introduction

Friedenstein was the first to identify that single cell suspensions of bone marrow (BM) stroma could generate colonies of adherent fibroblast-like cells *in vitro* (Friedenstein et al., 1968). These colony-forming unit fibroblasts (CFU-Fs) were found to be capable of osteogenic differentiation and provided the first evidence that clonogenic stem cell precursors existed of the bone lineage (Friedenstein et al., 1968, 1970). Later these stromal cells were classified as stem cells, since single cells could transdifferentiate into multi-lineage cells of bone and osteogenic tissue (Friedenstein, 1980). Their eventual capability of generating the osteogenic, chondrogenic and adipogenic mesenchymal lineages meant they were then given the title of mesenchymal stem cells (Caplan, 1991, Fig. 1). It was also shown that whilst they cannot make hematopoietic stem cells (HSCs), they do physically support them and promote their differentiation (Dexter, 1982; Owen, 1988). Interestingly, Caplan discussed the concept of cell transplantation therapy using MSCs therapeutically, but as a source of bone and connective tissue (Caplan, 1991). This became more pertinent when it was shown that MSCs only express the class I major histocompatibility complex (MHC-1) but not class II or co-stimulatory molecules such as CD40, CD80 and CD86 making them less likely to raise an immune response (Le Blanc, 2003). It has also been suggested that due to their limited pluripotent potential, MSCs should be re-named and termed "mesenchymal stromal cells" to avoid the excessive promotion of their stem cell potential (Horwitz et al., 2005; Pacini and Petrini, 2014). Therefore, in this review the abbreviation MSC is referred to as mesenchymal stromal cells (MSCs).

1.1. MSCs and their origins

MSCs are known to be present in virtually all postnatal organs and tissues including heart, lung umbilical cord, peripheral blood, adipose tissue, muscle, cartilage, synovium, dental pulp, BM, tonsil, placenta, thymus and olfactory mucosa (OM) (da Silva Meirelles et al., 2006; Kuhn and Tuan, 2010; Lindsay et al., 2013, 2016; Xie et al., 2015). However, whether they permanently reside in such tissues, or can circulate in the blood or even exist in perivascular spaces to reach different tissues is still not known (Pacini and Petrini, 2014). By definition MSCs must i) adhere to plastic, ii) express specific cell surface markers and iii) differentiate in a multipotential manner along the osteogenic, chondrogenic, and adipogenic lineages (Dominici et al., 2006). A panel of markers are used to define MSCs including CD73 (ecto-5′nucleotidase) CD90

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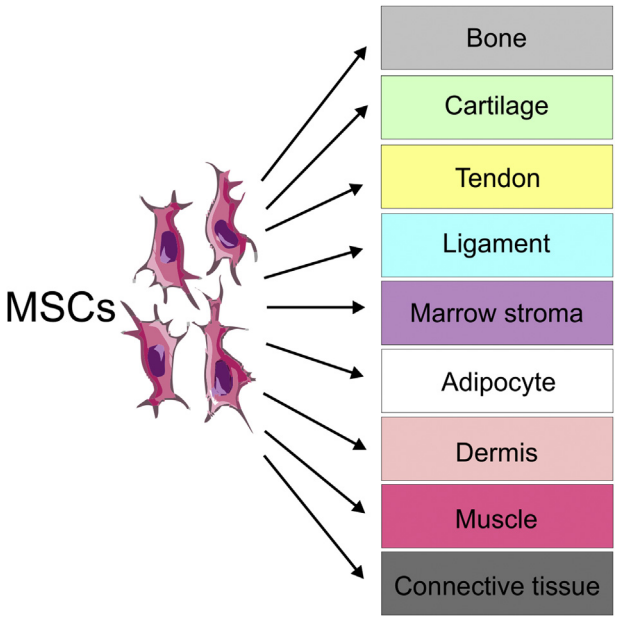

Fig. 1. Differentiation of MSCs based on Caplan, 1991. MSCs have the capacity to differentiate into osteogenic, chondrogenic and adipogenic mesenchymal lineages.

(Thy-1), CD105 (endoglin), CD166 (ALCAM), CD271 (p75NFGR/NTR), CD44 and STRO-1. However, none of these are specific and will also label a range of other cell types including endothelial cells, epithelial cells, fibroblasts, T cells and certain neural cell types (Kuhn and Tuan, 2010; Xie et al., 2015). MSCs also lack expression of CD34 (hematopoietic progenitor and endothelial cell marker), CD45 (pan-leukocyte marker), CD11b or CD14 (monocyte and macrophage markers), CD19 or CD79a (B cell markers), and HLA-DR (marker of stimulated MSCs) (Mo et al., 2016). Initially their purification from BM was carried out by differential adherence to plastic since only the MSCs from stroma will adhere. However, there are now specific isolation kits available based on cell surface antibodies and magnetic selection which can be used to highly enrich for MSCs from a variety of different tissue sources, including BM. To add to the complexity, MSCs share cell-surface markers and localisation with pericytes, making their true classification and distinction even more complex (Crisan et al., 2008). Importantly, in the context of their therapeutic potential, these cells are widely available, have a high capacity to self-renew and are easily propagated in culture in substantial enough numbers. However the lack of standardised protocols for their expansion and isolation makes results difficult to interpret (Pacini and Petrini, 2014).

1.2. MSCs from the human olfactory mucosa

The uniquely regenerative properties of the olfactory system (Graziadei and Monti Graziadei, 1983) has meant that this tissue has gained much interest for the transplant mediated repair of the CNS (Barnett and Riddell, 2007; Lindsay et al., 2010; Roet and Verhaagen, 2014; Tabakow et al., 2013). Some of the transplantation studies have incorporated the use of the entire OM, while others have used the purified glial cell population, known as olfactory ensheathing cells (Li et al., 1997; Ramón-Cueto et al., 2000). We undertook a study to identify the stem cell population(s) from this tissue, since many researchers were already transplanting cells from OM into patients (Lima et al., 2006; Mackay-Sim et al., 2008; Geraghty, 2008). We identified MSC-like cells from the lamina propria of the human OM using CD271 purification and selection, which we termed OM-MSCs (Lindsay et al., 2013; Johnstone et al., 2015). Detailed comparison was made with classical BM-derived MSCs which were isolated and maintained using identical methods and culture conditions (Lindsay et al., 2013; Johnstone et al., 2015). We demonstrated that the OM-MSCs adhered to plastic, expressed classical markers and differentiated into bone and fat lineages in a similar manner to BM-MSCs. Furthermore, using a micro (mi)RNA array we showed that they were 64% homologous with a similar core subset of miRNAs (Lindsay et al., 2016). We and others have also shown that while they were identical in their expression of a panel of CD markers, a greater proportion of OM-MSCs expressed nestin immunoreactivity; 100% of OM-MSCs express nestin compared to around 50% of BM-MSCs (Lindsay et al., 2010; Johnstone et al., 2015; Delorme et al., 2010, Fig. 2, Table 1). The relevance of nestin-positive MSCs within the BM is now being evaluated by researchers. Nestin is a class VI intermediate filament protein which was originally identified as a stem cell marker for neuroepithelial cells (Lendah et al., 1990), although, it has been reported to label a range of cells from neural stem cells, fibroblasts and reactive glia (Kishaba et al., 2010; Toft et al., 2013; Xie et al., 2015). Table 1 summarises the comparative differences reported to date on OM-MSCs and BM-MSCs. Since OM-MSCs have only recently been identified, very few direct comparisons of their biological properties to BM-MSCs have been reported. Although, we have directly compared the two types of MSCs abilty to promote CNS myelination *in vitro* in the table, comparative data on myelination potential *in vivo* is limited. BM-MSCs have been shown to increase the number of oligodendrocytes, and enhance remyelination in EAE but similar data for OM-MSCs has not been published (see review of Cohen, 2013).

1.3. Nestin-positive OM- and BM-MSCs

Interestingly, a subpopulation of BM-MSCs have also been reported to express nestin (Tondreau et al., 2004; Wiese et al., 2004) and more detailed studies demonstrated that the nestin-positive MSCs are similar to early progenitor cells that are able to self-renew and differentiate into bone, fat and adipose (Mendez-Ferrer et al., 2010). These early progenitors have been hypothesised to be “mesodermal progenitor cells” or MPCs by other researchers (Petrini et al., 2009; Pacini and Petrini, 2014). The nestin-positive MSCs have been shown to co-localize with HSCs supporting their maintenance and homing (Isern and Mendez-Ferrer, 2011). Using transgenic mice that express the regulatory elements of the nestin-promotor (Nes-GFP) it was demonstrated that the nestin-positive MSC subpopulation originate from the neural crest and have special HSC niche functions, while the nestin-negative MSCs originate from the mesoderm and give rise to bone and cartilage (Isern et al., 2014). Other epithelial tissues have also been suggested to contain neural crest derived mesenchymal progenitors including the human oral mucosa (Davies et al., 2010), oral gingivae (Xu et al., 2013), dental pulp tissue (Volponi et al., 2010) and airway epithelium (Ortega-Martínez et al., 2015). Furthermore, nestin-positive MSCs were identified in airway epithelium within the perivascular areas and in connective tissue that is in close proximity to the airway epithelium (Ortega-Martínez et al., 2015). These authors suggest that these MSCs circulate in the bloodstream, transmigrate through blood vessels and localize in the epithelium to participate in its turnover by being able to generate several different types of lung tissues. This could be a general feature of many different types of mucosa that exist throughout the body, where rapid turnover of cells is required after damage or during normal cell turnover. The importance of isolating nestin-positive neural crest derived MSCs for therapy over nestin-negative MSCs is not yet fully known. Moreover, the various