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Prospective isolation of resident adult human mesenchymal stem cell population from multiple organs

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Abstract Mesenchymal stem/stromal cells (MSCs) have the potential to form colonies in culture and reside in adult tissues. Because MSCs have been defined using cells cultured in vitro, discrepancies have arisen between studies concerning their properties. There are also differences between populations obtained using different isolation methods. This review article focuses on recent developments in the identification of novel MSC markers for the in vivo localization and prospective isolation of human MSCs. The prospective isolation method described in this study represents an important strategy for the isolation of MSCs in a short period of time, and may find applications for regenerative medicine. Purified MSCs can be tailored according to their intended clinical therapeutic applications. Lineage tracing methods define the MSC phenotype and can be used to investigate the physiological roles of MSCs in vivo. These findings may facilitate the development of effective stem cell treatments.

Keywords Mesenchymal stem/stromal cells · Skeletal stem cells · Isolation · Multipotency · Regenerative medicine

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Introduction

Stem cells derived from human somatic tissue are used in the treatment of a variety of diseases and injuries. Somatic stem cells exhibit tissue organization and contribute to homeostasis in multiple organs. The most popular cell source is bone marrow (BM) stem cells, which consist of hematopoietic stem cells (HSCs) and mesenchymal stem/stromal cells (MSCs). HSCs are present in the BM niche in a state of quiescence [1–3]. MSCs reside in the non-hematopoietic fraction and can be cultured to form fibroblast-like colonies (colony-forming unit fibroblasts: CFU-Fs) in vitro [4–7]. MSCs are found in the BM [8, 9], umbilical cord blood [10, 11], placenta [12, 13], dental pulp [14, 15], adipose tissue [16–19], and synovium [20–22]. BM-MSCs are thought to function in the maintenance of BM homeostasis, restoration of injured bone, and regulation of differentiation in HSCs [23]. The definition of MSCs depends on in vitro culture conditions, and thus varies among researchers in the stem cell field. In general, MSC refers to adherent cultured cells that can differentiate into bone, fat, and cartilage [24]. However, adherent culture conditions on plastic dishes inevitably change the expression of surface markers and the biological properties of stem cells [25, 26]. Indeed, most MSC marker information is obtained from cultured MSCs [27], which makes it difficult to identify MSC-specific markers and analyze physiological functions in vivo.

Recently, a number of studies have addressed such problems using flow cytometry and transgenic mouse (Fig. 1). Tissues are dissected or crushed to make them physically small, and the pieces of tissue are incubated for a few hours in the presence of enzymes that degrade the matrix component of tissues. It is common to use a collagenase reagent to acquire cells from tissue samples. However, obtaining different

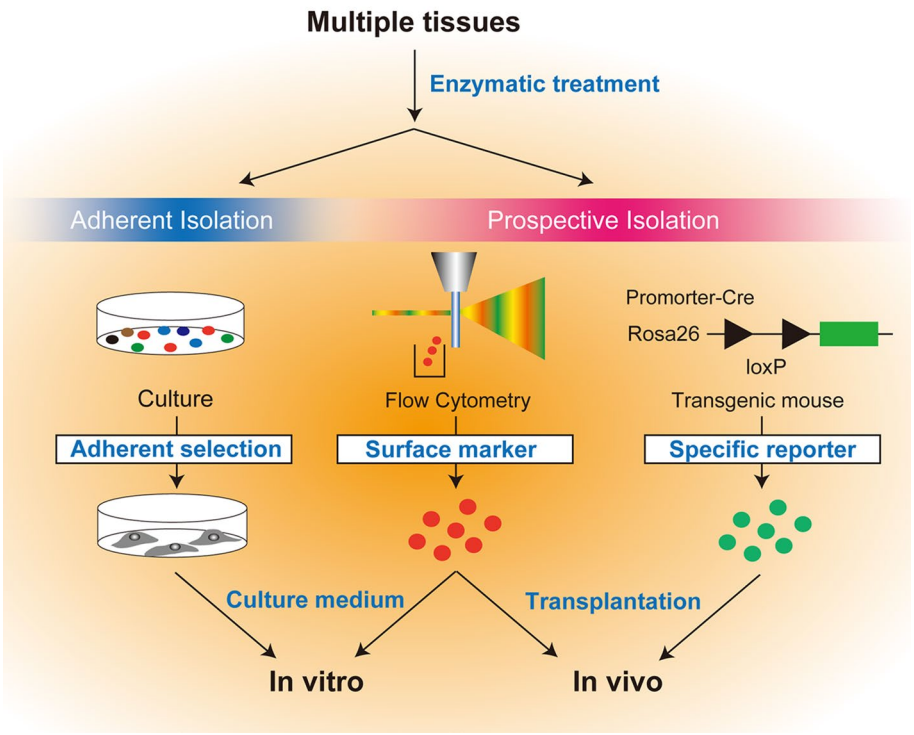

Fig. 1 Prospective isolation methods for MSCs from multiple tissues. Traditional MSC isolation uses adherent cultures on the dish (*left*). Prospective identification of MSCs using flow cytometric identification of cell surface markers (*center*) and transgenic mouse expressing tissue-specific reporters allows the isolation of a pure population of multipotent MSCs (*right*)

populations depends on the collagenase concentration and treatment time. In tissues containing red blood cells, the enzyme-treated cells are resuspended in water or lysis buffer to remove the red blood cells. At this stage, the cell population is heterogeneous (see enzymatic treatment in Fig. 1). In the case of the BM, whole BM cell populations may contain various mature cells, such as fat cells, macrophages, endothelial cells, blood cells, and fibroblasts. Stem cell-specific markers are needed to isolate tissue stem cells from these heterogeneous cell populations. The cells are stained with a stem cell-specific monoclonal antibody on ice (see surface marker in Fig. 1). In mouse tissue, stem cells are prospectively isolated and identified according to fluorescence generated by tissue-specific-promoter-Cre/Floxed reporter mice (see specific reporter in Fig. 1). Stem cell-specific markers can be used to obtain uniform MSCs and trace cell fate in vivo.

MSCs are defined as self-renewing populations in multiple organs

MSCs are selected based on adherent potency in culture conditions containing fetal bovine serum. Friedenstein and others reported the existence of MSCs by CFU-F in the BM [4, 5, 28, 29]. Pittenger confirmed that MSCs have multipotency for adipocytes, chondrocytes, and osteoblasts, clonally [24]. Thereafter, a large number of studies correlated MSC phenotypic features and investigated the therapeutic potential of MSCs. Elahi et al. reported that the term

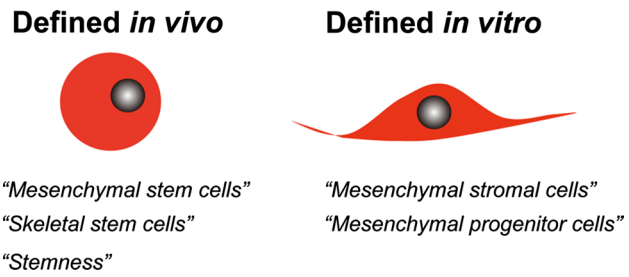

Fig. 2 The term of "mesenchymal stem cells" vs "mesenchymal stromal cells". Numerous articles use the word "stem"; however, "stemness" has been used as a stricter term to define MSCs with tissue repair ability in vivo

"mesenchymal stem cell" yielded more than 357,000 hits in Google Scholar (Google Scholar; July 2015) [30]. Colony-forming cells have since been called "mesenchymal stem cells" or "mesenchymal stromal cells." It appears that the term "stem cell" has become more popular than the term "stromal cell" in the last 20 years in MSCs studies. Interestingly, "stemness" has been shown to be a stricter term for MSCs involved in tissue repair (Fig. 2) [30]. Recently, "skeletal stem cells (SSCs)" has supplanted the term "tissue stem cells" as the new term for these cells [31, 32]. A web search showed that the term "skeletal stem cell(s)" yielded more than 46 hits in article titles in November 2015. SSCs self-renew and generate osteoblasts, chondrocytes, and reticular marrow stromal cells, but not adipocytes [33, 34].

Recently, many reports have demonstrated the presence of neural crest stem cells in various adult tissues, and suggested that they are present not only in embryos but also in multiple organs during adulthood [35, 36]. The neural crest is a transient embryonic tissue that originates at neural folds during vertebrate development. Neural crest cells migrate from the dorsal neural tube and migrate to various locations, where they differentiate into a vast range of cells, including neurons and glial cells of the peripheral nervous system, smooth muscle cells, bone, and cartilage cells. The characteristics of neural crest stem cells are similar to those of MSCs [37]. Cre/loxP-mediated lineage analysis also suggests that a subpopulation of adult BM-MSCs may have a developmental origin in the murine neural crest [38, 39]. Furthermore, neural crest-derived cells have been confirmed to exist in skin [40], cornea [41], and heart [42] in adult mouse. These organs may, thus, represent additional sources for the supply of MSCs. In order to avoid problems related to culture procedures, it is important to identify specific markers for MSCs to enable their rapid isolation and to define the common features of MSCs [43].

Prospective identification and isolation of human MSCs

Various surface makers have been used to isolate human MSCs (Table 1). The first isolation method used a monoclonal antibody against Stro-1 to efficiently isolate fibroblast-like cells from fresh BM [44]. The Stro-1-positive compartment includes non-hematopoietic cells, but the antibody is also reactive with red blood cells. A number of studies have examined MSC isolation using CD73-positive [45], CD49a-positive [45], CD105-positive [46], and MSCA-1-positive [47], SSEA4-positive [48, 49] populations. In 2002, a direct isolation method for MSCs was performed using an antibody against CD271 (low-affinity nerve growth factor receptor: LNGFR) [50]. LNGFR is not reactive to red blood cells and hematopoietic progenitor cells, which has made LNGFR one of the more popular markers for the isolation of human MSCs. Some studies have used the LNGFR marker in combination with MSCA-1-positive [51], CD56-positive [52, 53], CD140b-positive [54], CD146-positive/negative [55], and SSEA4-positive [56] populations. In addition, high CFU-F frequency is associated with a population double-positive for CD106 (VCAM-1) and Stro-1 antibodies [57]. These cells differentiate into bone and fat on ectopic transplantation in vivo. In 2013, our group reported that the LNGFR and THY-1 double-positive population (i.e., the LT population) have a high CFU-F frequency in the BM [8]. CFU-F can be classified into three different cell groups based on proliferation ability. Rapidly expanding MSC clones (RECs) are undifferentiated cells with proliferation and differentiation ability that are maintained during long-term subculture in vitro. RECs are highly associated with the VCAM-1^{hi}-positive population. Aomatsu et al. recently demonstrated that the novel SCRG1/BST1 axis determines the fate of hMSCs by regulating their kinetic and differentiation potentials [58]. SCRG1/BST1 preserves self-renewal potential and the expression of stem cell markers such as LNGFR, THY-1 and VCAM-1 [58]. Cell–cell adhesion in human MSCs enhances the expression of VCAM-1 via PDGFRb [59].

Table 1 Surface markers, existence, and CFU-F potential of human BM-MSCs

Marker	Percentage in BM	CFU-F	References
STRO-1+	5.0 % (Glycophorin A^- gated)	1/90,909	Simmons et al. [44]
$CD73^+$	5.2 % ($CD45^-CD14^-$ gated)	1/39,062	Boiret et al. [45]
$CD49a^+$	4.4 % ($CD45^-CD14^-$ gated)	1/28,011	Boiret et al. [45]
$CD105^+$	2.3 %	1/15,873	Aslan et al. [46]
$MSCA\text{-}1^+$	2.8 %	1/833	Gronthos et al. [47]
$LNGFR^+$	2.3 %	1/631	Quirici et al. [50]
$LNGFR^+$, $CD146^+$	0.02 % ($CD45^-$ gated)	1/64	Tormin et al. [55]
$LNGFR^{Bright+}$, $PDGFRb^+$	– %	1/52	Buhring et al. [54]
$LNGFR^+$, $CD146^{-/low}$	0.01 % ($CD45^-$ gated)	1/32	Tormin et al. [55]
$LNGFR^{Bright+}$, $MSCA\text{-}1^+$, $CD56^+$	8.5 % ($MSCA\text{-}1^+$ gated)	1/14	Battula et al. [52]
$STRO\text{-}1^{Bright+}$, $VCAM\text{-}1^+$	1.4 %	1/11	Gronthos et al. [57]
$LNGFR^+$, $SSEA4^+$	0.1 % (Lin^-CD45^- gated)	1/6	Matsuoka et al. [56]
$LNGFR^+$, $THY\text{-}1^+$	0.04 %	1/6	Mabuchi et al. [8]
$LNGFR^+$, $THY\text{-}1^+$, $VCAM\text{-}1^{hi+}$	0.004 %	1/3	Mabuchi et al. [8]

The ratios of marker population to CFU-F formation in the BM based on the reported data are shown. LNGFR: CD271, MSCA-1: STRO-3 Tissue non-specific alkaline phosphatase, VCAM-1: CD106, PDGFRb: CD140b, and THY-1: CD90

Based on a study of BM-MSCs, we found that LNGFR and THY-1 markers can be used to effectively isolate these cells from other tissues. In one study, we isolated LNGFR^{+} THY-1^{+} MSCs from synovium using flow cytometry [20]. The synovium contains a higher percentage of LNGFR^{+} THY-1^{+} MSCs. We examined the colony formation and differentiation abilities of BM- and synovium-derived MSCs isolated from the same patients. Both MSC types exhibit a marked propensity to differentiate into specific lineages. BM-MSCs preferentially differentiated into bone, while in the synovium-MSC culture, we observed enhanced adipogenic and chondrogenic differentiation. In another study, we examined human dental pulp stem/progenitor cells (hDPSCs) [14]. hDPSCs are attractive candidates for regenerative therapy because they can be easily expanded to generate CFU-Fs [15, 60, 61]. We identified a dental pulp tissue-specific cell population based on the expression profiles of two cell-surface markers, LNGFR and THY-1. Prospectively isolated dental pulp-derived LNGFR^{Low+} THY-1^{High+} cells represent a highly enriched population of clonogenic cells; notably, isolated cells exhibit long-term proliferation and multilineage differentiation potential in vitro. These cells also express known mesenchymal cell markers and promote new bone formation reparative of critically sized calvarial defects in vivo. These findings suggest that LNGFR^{Low+} THY-1^{High+} dental pulp-derived cells provide an excellent source of material for bone regeneration strategies. These data suggest that the tissue from which MSCs are isolated should be tailored according to their intended clinical therapeutic application. In human MSCs, the LNGFR marker has been validated by many researchers (Table 1) [62, 63], the next step should be improved selection and characterization of human MSC populations using comprehensive analysis [64].

MSC lineage tracking in vivo

In past reports describing the use of mouse MSCs, MSCs accumulated at injury sites and released trophic factors, such as prostaglandin E2 [65], TNF-α stimulated gene/protein 6 [66–68], and soluble TNF receptor 1 [69]. It is thought that MSCs respond to inflammation and have specific roles in immune regulation, lymphopoiesis, and bone homeostasis [70, 71]. These reports used cultured MSCs; accordingly, it is unknown whether the response is similar under physiological conditions. To investigate the physiological role of MSCs in vivo, two in vivo tracing strategies can be employed (Fig. 1); one method is to transplant the purified MSCs isolated using specific surface markers [43]. We used phenotypic, morphological, and functional criteria to identify and prospectively isolate a subset of MSCs (PDGFRα^{+} Sca-1^{+} CD45^{-} TER119^{-}: PaS cells) from adult mouse BM [72, 73]. PaS cells mainly differentiate into fat cells in adipose tissue, hematopoiesis support cells (CAR cells) [74], and osteoblasts in the BM [73]. The other method uses lineage tracing in transgenic mice, using transcription markers such as Nestin-GFP/cre [75], Ebf2-cre [76], and LepR-cre [77]. These techniques and histologic analyses have been used to identify MSCs locally, examine cell fate, and evaluate their physiological role in vivo. The cultured MSCs are trapped in pulmonary capillaries. However, freshly isolated MSCs survive in the BM and adipose tissue [73]. In an analysis of LepR-cre and Ebf2-cre mice, the group found that MSCs participate in turnover in bone and adipose tissue in adult BM [77, 78]. Worthley and colleagues demonstrated that the expression of the bone morphogenetic protein antagonist gremlin 1 defines a population of SSCs in the BM [79]. Chan and colleagues searched for unique gene expression patterns in the transcriptome of stem/progenitor cells to identify potential regulators of mouse SSC lineage commitment [80]. Inducing SSC formation with soluble factors and subsequently regulating the SSC niche to specify its differentiation toward bone, cartilage, or stromal cells could represent a paradigm shift in the therapeutic regeneration of skeletal tissues. Additional studies are needed to clarify the physiological role of the mesenchymal (or skeletal) lineage in vivo [81].

Conclusions

Many clinical studies have been performed using MSCs. However, cultured MSCs include heterogeneous cell groups, which have an effect on therapeutic outcomes. Isolation methods should be adapted such that only true MSCs are obtained. It is extremely important to determine the physiological functions of homogeneous MSC populations directly isolated and analyzed from multiple organs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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