nature protocols
Recipes for Researchers

Roles for mesenchymal stem cells as medicinal signaling cells

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Understanding the *in vivo* identity and function of mesenchymal stem cells (MSCs) is vital to fully exploiting their therapeutic potential. New data are emerging that demonstrate previously undescribed roles of MSCs *in vivo*. Understanding the behavior of MSCs *in vivo* is crucial as recent results suggest these additional roles enable MSCs to function as medicinal signaling cells. This medicinal signaling activity is in addition to the contribution of MSCs to the maintenance of the stem cell niche and homeostasis. There is increasing evidence that not all cells described as MSCs share the same properties. Most MSCs reside in a perivascular location and have some functionalities in common with those of the pericytes and adventitial cells located around the microvasculature and larger vessels, respectively. Here we focus on the characteristics of MSCs that have been demonstrated to be similar to those of pericytes located around the microvasculature, defined as perivascular MSCs (pMSCs). Although we focus here on pMSCs, it is important to bear in mind that pericytes are found in many types of blood vessels, and that not all pericytes are thought to be MSCs.

MSCs *in vivo*

Sympathetic neuron
Osteoblast
Osteoclast
Endosteal niche
Bone-lining MSC
NES+ CD271+
Quiescent HSC
Periarteriolar pMSC
NES^{bright}NG2+α-SMA+
CD271+CD146+
Quiescent HSC
Perivascular niche (arteriole and sinusoid)
Perisinusoidal pMSC
NES^{dim}LepR+CD271+
CD146+
Active HSC
CXCL12
CXCR4
Microvessel
Endothelial cell
Erythrocyte

Bone marrow (BM)

In the BM, distinct nestin+ pMSCs and bone-lining MSCs are in close association with hematopoietic stem cells (HSCs), both at the perisinusoidal niche where pMSCs that express low levels of nestin (NES^{dim}) are associated with active HSCs, and at the endosteal and periarteriolar niches where pMSCs that express high levels of nestin (NES^{bright}) are associated with quiescent HSCs. The secretion of CXCL12 by nestin+ cells is, in part, regulated by the sympathetic nervous system (direct innervation) and modulated by circadian rhythms. CXCL12, among other factors, provides homing and retention signals for HSCs. In addition, it has been shown that nestin+ MSCs can contribute to skeletal remodeling by differentiating into osteochondral lineages. The *in situ* localization of MSCs within the BM has been defined based on the differential expression of CD146, CD271, NG2 and α-SMA.

Neural tissues

The existence of a perivascular niche for neural stem cells (NSCs) has also been described in the subventricular zone. It has been suggested that pMSCs may regulate the local niche by direct contact with NSCs and by secreting different types of neurotrophins, such as BDNF.

Lateral ventricle
Ependymal cell
NSC
Astrocyte
pMSC
Neurotrophins

The universal stem cell niche for adult tissues

A common feature of many adult stem cell (ASC) niches is proximity to the vasculature. In this regard, pMSCs may be involved in the regulation of the perivascular niche of ASCs across different tissues. This has been shown for BM, nervous system tissue, liver, muscle and other vascularized tissues.

Other niche cells
Adult resident/organ-specific stem cells
pMSC
Niche molecules

Immunomodulation
Affect DC maturation
Shift T_H1 to T_H2 response
Increase T_{reg} cells
T cell unresponsiveness
Immature Monocyte
Macrophage M2
IL-10
IDO, NO, PGE2, Il-6, TGF-β1...
Activated anti-inflammatory MSC 2
TLR3
TLR3 priming

Trophic
Angiogenic
Anti-apoptotic
Mitotic
Anti-scarring
VEGF, bFGF, HGF, IGF-1, CXCL12...

High
Inflammatory milieu
Low
Injury-inflammation

Anti-microbial
hCAP-18/LL-37 Cathelicidin
IFN-γ
TNF-α
Activated T cells
Macrophage M1
CXCL9/10, Rantes, MIP-1α/MIP-1β
Immature monocyte
LPS
TLR4
Activated pro-inflammatory MSC 1

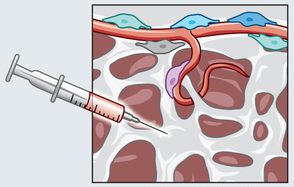
pMSC response to injury and inflammation

After microvascular damage, released pericytes differentiate into MSCs which become activated by sensing their local injury-dependent milieu. It has been suggested that toll-like receptor 3 (TLR3) priming induces the shift of MSCs to an anti-inflammatory phenotype (type 2 MSCs), in which immunomodulatory and trophic activities are elicited after the secretion of specific mediators and the polarization of monocytes into M2 macrophages. In contrast, when MSCs are subjected to TLR4 priming conditions, the MSCs shift to a pro-inflammatory phenotype (type 1 MSCs), resulting in the activation and polarization of members of the innate (e.g., monocytes into M1 macrophages) and adaptive (e.g., T lymphocytes) immune system. Thus, type 1 MSCs contribute to early reparative responses to tissue injury, whereas in later phases type 2 MSCs contribute to a more-regenerative resolution. In addition, the TLR4-type response, such as that mediated by bacterial infection, activates the secretion by MSCs of a potent antimicrobial peptide (hCAP-18/LL-37). The specific response of pMSCs is dependent on the type of injury and the tissue in which the cells reside; therefore, the response may involve fibrosis development if a quick repair of the injured tissue is needed.

Platelet
Cancer cell
pMSC

MSCs *in vitro* and therapeutic applications

MSCs can be isolated from BM and other vascularized tissues including fat, dental pulp and muscle. They are defined *in vitro* by a specific surface marker expression profile (blue box), their ability to adhere to plastic and form colonies (i.e., CFU-F cells), and their capacity for serial expansion. From an initial heterogeneous population, specific subpopulations can be obtained by either sorting with markers related to their roles *in vivo* (red box) or by priming them with stimulating solutions during expansion (e.g., FGF2). MSCs have the *in vitro* ability to differentiate into mesodermal lineages such as chondrocytes, osteoblasts, adipocytes and tenocytes, and this differentiation is achieved by supplementing cultures with lineage-specific soluble factors and specific microenvironmental cues. GMP-processed human MSCs (i.e., cells of clinical grade) are used in clinical trials for cell therapy, as the basis for novel therapeutic approaches for regenerative medicine. The availability and versatility of these cells make them an excellent option for a wide variety of clinical conditions associated with inflammation, ischemia, autoimmunity and trauma. When supplied exogenously, MSCs home to sites of injury, readopting their perivascular localization. At these sites, MSCs exert their local immunomodulatory and trophic activities.



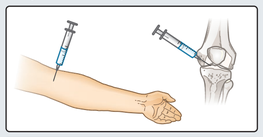
Bone marrow based on an original image from Servier Medical Art (www.servier.com/Powerpoint-image-bank)

MSCs
POS: CD90; CD73; CD105; CD44
NEG: CD34; CD45; CD14; CD19
Adherence to plastic
FGF2
Expansion
Tissue engineering
Multi-lineage *in vitro* differentiation
Chondrocyte
Osteoblast
Adipocyte
Other
Differentiation cocktail
CD146; CD271; NG2; Nestin; PDGFR-β
Cell priming
Subpopulations selection

Image reproduced from the cGMP Facility, Diabetes Research Institute and Cell Transplant Center, University of Miami

GMP-processed 'clinical-grade cells'

Recognition and engraftment to injury sites



MSCs for regenerative medicine
Cell therapy

Reestablished perivascular localization

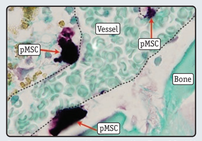
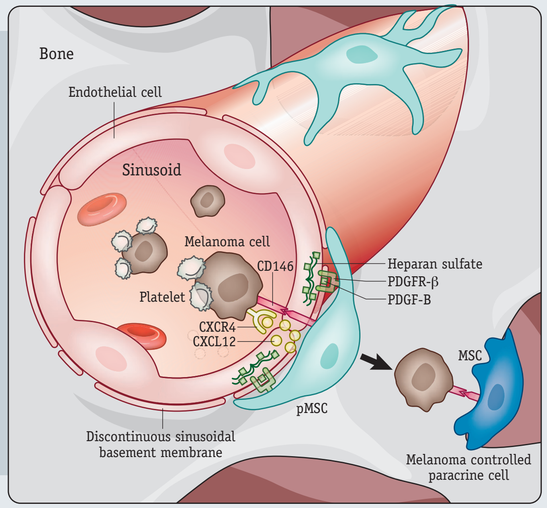


Image adapted from Lin P *et al. Mol. Ther.* **22**, 160–168 (2013), American Society of Gene & Cell Therapy

Immunomodulatory, trophic, antimicrobial

MSCs in cancer metastasis

PDGFR-β–expressing MSCs are attracted to an abluminal location by gradients of endothelial cell-secreted, heparan sulfate–bound PDGF-β. Once in their perivascular niche, they have a pivotal role in controlling the extravasation of circulating cancer cells (e.g., in melanoma and breast cancer) into bone and liver parenchyma. The molecular mechanisms underlying this regulatory process involve the action of (i) secreted chemokines (e.g., CXCL12) by pMSCs recruiting CXCR4-expressing cancer cells close to the endothelium; and (ii) intercellular adhesion molecules (such as CD146) expressed by both cells, generating a migratory cellular complex. These mechanisms may serve as a platform for the development of novel therapies aimed at controlling the establishment and progression of skeletal and liver metastasis by targeting pMSCs. Moreover, based on the known pericytic mimicry capability of angiotropic tumor cells, targeting of perivascular cells may aid in the control of metastatic dissemination of cancer cells that use this alternative mechanism of tumor spread.



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Abbreviations

α-SMA: Alpha smooth muscle actin; ASC: Adult stem cell; BDNF: Brain-derived neurotrophic factor; CCL5: C-C motif chemokine 5 (Rantes); CXCR4: Chemokine (C-X-C motif) receptor 4; CXCL9: Chemokine (C-X-C motif) ligand 9; CXCL10: Chemokine (C-X-C motif) ligand 10; CXCL12: Chemokine (C-X-C motif) ligand 12; DC: Dendritic cell; FGF2: Fibroblast growth factor 2; GMP: Good manufacturing practice; hCAP-18/LL-37: Human cationic antimicrobial protein; HGF: Hepatocyte growth factor; HSC: Hematopoietic stem cell; IDO: Indoleamine 2,3-dioxygenase; LPS: Lipopolysaccharide; IGF-1: Insulin-like growth factor-1; IL-6: Interleukin-6; IL-10: Interleukin-10; IFN-γ: Interferon gamma; LepR: Leptin receptor; MIP: Macrophage inflammatory protein; NES: Nestin; NG2: Neural/glial antigen 2; NO: Nitric oxide; NSC: Neural stem cell; PDGFR-β: Platelet-derived growth factor receptor beta; PGE2: Prostaglandin E2; pMSC: Perivascular mesenchymal stem cell; TH: T helper; TLR3: Toll-like receptor-3; TLR4: Toll-like receptor-4; TNF-α: Tumor necrosis factor alpha; VEGF: Vascular endothelial growth factor

References

Barry, F. & Murphy, M. Mesenchymal stem cells in joint disease and repair. *Nat. Rev. Rheumatol.* **9**, 584–94 (2013).
Bautch, V.L. Stem cells and the vasculature. *Nat. Med.* **17**, 1437–1443 (2011).
Bernardo, M.E. & Fibbe, W.E. Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell* **13**, 392–402 (2013).
Bunnell, B.A., Betancourt, A.M. & Sullivan, D.E. New concepts on the immune modulation mediated by mesenchymal stem cells. *Stem Cell Res. Ther.* **1**, 34 (2010).
Caplan, A.I. All MSCs are pericytes? *Cell Stem Cell* **3**, 229–30 (2008).
Caplan, A.I. & Correa, D. The MSC: an injury drugstore. *Cell Stem Cell* **9**, 11–15 (2011).
Caplan, A.I. & Sorrell, J.M. The MSC curtain that stops the immune system. *Immunol. Lett.*, **2**, 136-139 (2015).
Correa, D. *et al.* Sequential exposure to fibroblast growth factors (FGF) 2, 9 and 18 enhances hMSC chondrogenic differentiation. *Osteoarthritis Cartilage* **23**, 443–453 (2015).
Correa, D., Somoza, R.A., Lin, P., Schiemann, W.P. & Caplan, A.I. Mesenchymal stem cells regulate melanoma cancer cells extravasation to bone and liver at their perivascular niche. *Int. J. Cancer* **138**, 417–427 (2016).
Corselli, M. *et al.* The tunica adventitia of human arteries and veins as a source of mesenchymal stem cells. *Stem Cells Dev.* **21**, 1299–1308 (2012).
Crisan, M. *et al.* A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* **3**, 301–13 (2008).
Lin P., Correa, D., Kean, T.J., Awadallah, A., Dennis, J.E. & Caplan, A.I. Serial transplantation and long-term engraftment of intraarterially delivered clonally-derived mesenchymal stem cells to injured bone marrow. *Mol. Ther.* **22**, 160–168 (2013).
Lugassy, C. *et al.* Angiotropism, pericytic mimicry and extravascular migratory metastasis in melanoma: an alternative to intravascular cancer dissemination. *Cancer Microenviron.* **7**, 139–152 (2014).
Mendelson, A. & Frenette, P.S. Hematopoietic stem cell niche maintenance during homeostasis and regeneration. *Nat. Med.* **20**, 833–846 (2014).
Michurina, T.V. *et al.* Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* **466**, 829–834 (2010).
Putnam, A.J. The instructive role of the vasculature in stem cell niches. *Biomater. Science* **2**, 1562–1573 (2014).
Shen, Q. *et al.* Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell Stem Cell* **3**, 289–300 (2008).
Tormin, A. *et al.* CD146 expression on primary nonhematopoietic bone marrow stem cells is correlated with in situ localization. *Blood* **117**, 5067–5077 (2011).
Wong, S.-P. *et al.* Pericytes, mesenchymal stem cells and their contributions to tissue repair. *Pharmacol. Ther.* **151**, 107–120 (2015).

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