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The identity and properties of mesenchymal stem cells

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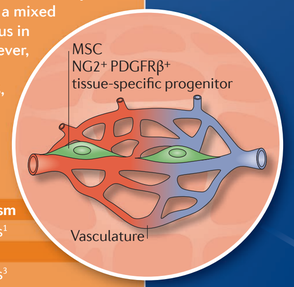
MSCs are self-renewing, multipotent precursors. They were originally found to reside in the stromal adherent fraction of the bone marrow, where they sustain the homeostatic turnover of non-haematopoietic stromal cells, regulate HSC maintenance and might contribute to vascular stability. The physiological roles of MSCs in anatomical locations other than the bone marrow remain largely undefined. MSCs can be expanded *in vitro* to generate mesenchymal stromal cell cultures, which, under appropriate conditions, can differentiate into adipocytes, chondrocytes and osteoblasts. In more recent studies multipotent mesenchymal stromal cell cultures have been derived from perivascular stem cells expressing pericyte markers in many postnatal tissues. The differentiation capabilities, extraordinary paracrine potential and ease of isolation of *in vitro*-expanded mesenchymal stromal cells have attracted great interest into, and efforts towards, the exploitation of MSCs and their expanded progeny as therapeutic agents for tissue regeneration and repair.

MSCs in postnatal tissues

MSCs were first identified in the adherent fraction of bone marrow stroma. They were termed CFU-Fs because of their ability to generate single cell-derived colonies, in analogy to their haematopoietic counterparts, CFU-Cs. CFU-Fs from almost all embryonic and postnatal tissues can be expanded *in vitro* to generate cell cultures that conserve trilineage potential. The role of MSCs in multiple anatomical locations, and whether they constitute a specific homogeneous cell type or a mixed population of tissue-specific cells heterogeneous in nature and origin, is not well understood. However, these progenitors express pericyte-specific cell-surface markers, such as NG2 and PDGFRß, and are located in perivascular regions of the different tissues in which they reside.



Markers defining cells enriched in MSC activity

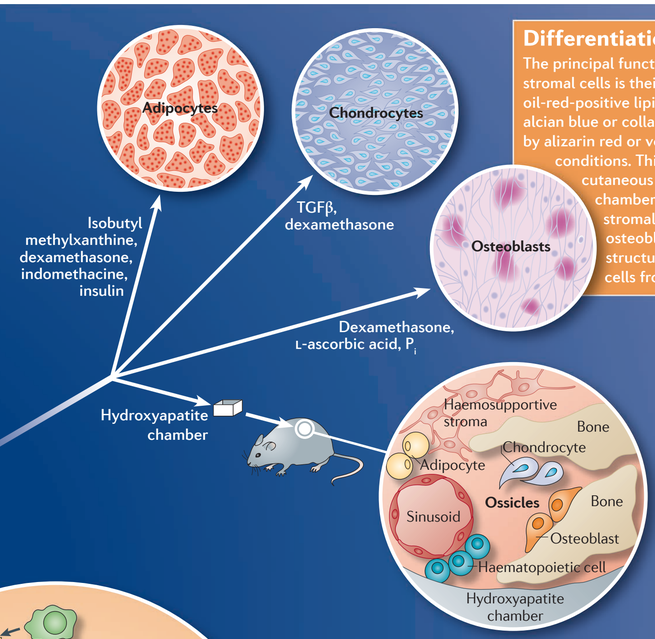
Marker	Anatomical location	Organism
CD146	Bone marrow	Humans[1]
PDGFRα–SCA1	Bone marrow	Mice[2]
CD146–NG2–PDGFRβ	Postnatal and embryonic tissues	Humans[3]
Nestin–GFP	Bone marrow	Nestin–GFP transgenic mice[4]

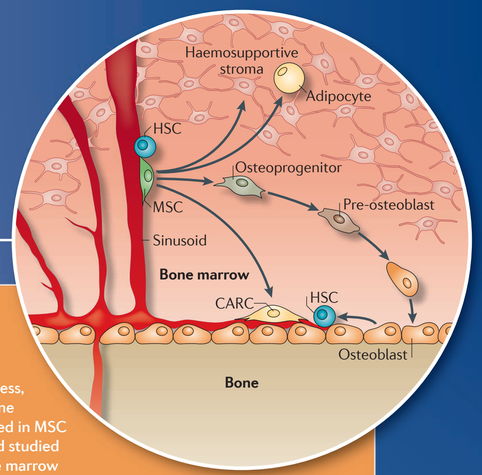
Mesenchymal stromal cell expansion *in vitro*

MSCs can be expanded *in vitro* when cultured in two-dimensional monolayers of adherent cells in specialized medium. The expanded cells, sometimes termed multipotent mesenchymal stromal cells, are defined by the expression of CD73, CD90 and CD105 and the lack of CD11b, CD19, CD34, CD45 and HLADR. Here we use the term mesenchymal stromal cells to refer to these *in vitro*-expanded cells.

Differentiation potential

The principal functional criterion defining multipotent mesenchymal stromal cells is their ability to give rise to mature adipocytes (in which oil-red-positive lipid vesicles accumulate), chondrocytes (identified by alcian blue or collagen-specific staining) and osteoblasts (identified by alizarin red or von Kossa staining) when placed in specific culture conditions. This trilineage capability can be probed *in vivo* by subcutaneous implantation inside ceramic cubes (hydroxyapatite chambers) in mice. Within these implants, mesenchymal stromal cells differentiate into adipocytes, chondrocytes, osteoblasts and haemosupportive stroma, giving rise to bony structures termed ossicles, which recruit haematopoietic cells from the recipient mice to the implant.

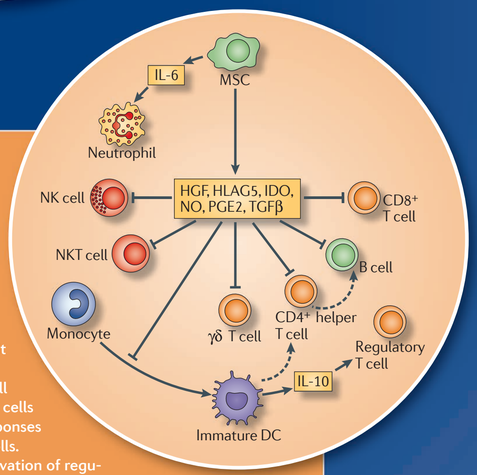




MSC roles *in vivo*

The study of MSCs in their native environment has been hindered by the inability to identify them *in situ*. Nonetheless, rare cell populations in the bone marrow that are highly enriched in MSC activity have been isolated and studied *in vitro* and *in vivo*. In the bone marrow parenchyma, MSCs lie in perivascular niches, where they associate with HSCs, exerting a key regulatory effect on early stages of haematopoiesis. MSCs enter differentiation pathways to replenish mature osteoblasts, adipocytes and haemosupportive stroma in the bone marrow. Recent studies have shown that bone marrow-residing nestin+ MSCs are innervated by sympathetic nervous system fibres and mediate neural control of haematopoiesis.

Immunoregulatory properties *in vitro*

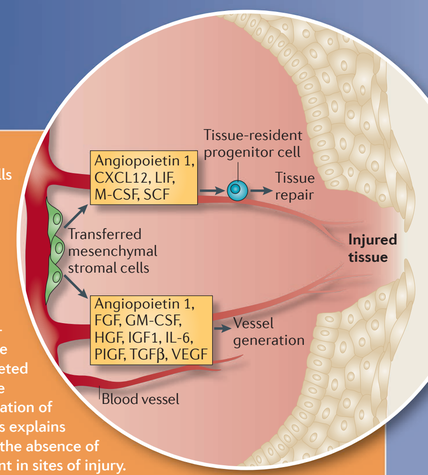


MSCs are endowed with remarkable immunoregulatory properties. When co-cultured *in vitro* they modulate the responses of neutrophils, NK cells and NKT cells, and suppress the maturation of DCs from monocytes, which may lead to defective antigen presentation to CD4+ helper T cells. MSCs have also been shown to inhibit the activation of CD4+ helper T cells (potentially leading to defective T cell help to B cells), the proliferation of B cells and the activation and cytotoxic responses mediated by γδ T cells and CD8+ T cells. Furthermore, MSCs promote the activation of regulatory T cells, which are a specialized subset of CD4+ T cells that can suppress the responses of other T cells.

The immunosuppresive effects of mesenchymal stromal cells mainly rely on their ability to secrete various soluble factors, such as IDO, NO and PGE2. Whether tissue-resident MSCs play a physiological part in directly modulating immune responses *in vivo* is still unknown.

Therapeutic potential

Expanded multipotent mesenchymal stromal cells are being extensively studied for their possible therapeutic properties in numerous pre-clinical and clinical settings. Studies initially focused on using their stem cell-like properties for tissue regeneration and repair. However, it is now well established that their beneficial effects are mostly derived from the secretion of immunomodulatory and cytoprotective factors that contribute to the regeneration of injured tissues. The current hypothesis is that paracrine factors secreted by mesenchymal stromal cells provide protective microenvironmental cues and promote the activation of local tissue-resident progenitor populations. This explains why favourable effects can be observed even in the absence of prolonged mesenchymal stromal cell engraftment in sites of injury.



Systemic infusion of mesenchymal stromal cells has proved beneficial in different pre-clinical models of acute lung injury, myocardial infarction, diabetes as well as renal and hepatic failure. Some of the human conditions for which the safety and efficacy of mesenchymal stromal cell-based therapies are being, or will soon be, studied in clinical trials include acute graft-versus-host disease, multiple sclerosis, osteogenesis imperfecta, stroke, spinal injury, systemic lupus erythematosus and cardiovascular disease.

Abbreviations

CARC, CXCL12-abundant reticular cell; CFU-Cs, colony-forming unit-cells; CFU-Fs, colony-forming unit-fibroblasts; CXCL12, CXC-chemokine ligand 12; DC, dendritic cell; FGF, fibroblast growth factor; GFP, green fluorescent protein; GM-CSF, granulocyte macrophage colony-stimulating factor; HGF, hepatocyte growth factor; HLA, human leukocyte antigen; HSC, haematopoietic stem cell; IDO, indoleamine 2,3-dioxygenase; IGF1, insulin growth factor 1; IL, interleukin; LIF, leukaemia inhibitory factor; NG2, nerve/glial antigen 2; NK, natural killer; NKT, natural killer T; NO, nitric oxide; PGE2, prostaglandin E2; MSC, mesenchymal stem cell; PDGFR; platelet-derived growth factor receptor; P_i, inorganic phosphate; PlGF, placental insulin growth factor; SCA1, surface cell antigen 1; SCF, stem cell factor; TGFβ, transforming growth factor-β; VEGF, vascular endothelial growth factor.

References

1. Sacchetti, B. *et al. Cell* **131**, 324–336 (2007).
2. Morikawa, S. *et al. J. Exp. Med.* **206**, 2483–2496 (2009).
3. Crisan, M. *et al. Cell Stem Cell* **3**, 301–313 (2008).
4. Mendez-Ferrer, S. *et al. Nature* **466**, 829–834 (2010).

Further reading

Salem, H. K. & Thiemermann, C. *Stem Cells* **28**, 585–596 (2010). | Meirelles Lda, S. *et al. Cytokine Growth Factor Rev.* **20**, 419–427 (2009). | Nombela-Arrieta, C., Ritz, J. & Silberstein, L. E. *Nature Rev. Mol. Cell Biol.* **12**, 126–131 (2011). | Uccelli, A., Moretta, L. & Pistoia, V. *Nature Rev. Immunol.* **8**, 726–736 (2008).

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