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Novel aspects of parenchymal–mesenchymal interactions: from cell types to molecules and beyond

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Mesenchymal stem or stromal cells (MSCs) were initially isolated from the bone marrow and received their name on the basis of their ability to differentiate into multiple lineages such as bone, cartilage, fat and muscle. However, more recent studies suggest that MSCs residing in perivascular compartments of the small and large blood vessels play a regulatory function supporting physiologic and pathologic responses of parenchymal cells, which define the functional representation of an organ or tissue. MSCs secrete or express factors that reach neighbouring parenchymal cells via either a paracrine effect or a direct cell-to-cell interaction promoting functional activity, survival and proliferation of the parenchymal cells. Previous concept of 'epithelial–stromal' interactions can now be widened. Given that MSC can also support hematopoietic, neuronal and other non-epithelial parenchymal lineages, terms 'parenchymal–stromal' or 'parenchymal–mesenchymal' interactions may better describe the supportive or 'trophic' functions of MSC. Importantly, in many cases, MSCs specifically provide supportive microenvironment for the most primitive stem or progenitor populations and therefore can play a role as 'stem/progenitor niche' forming cells. So far, regulatory roles of MSCs have been reported in many tissues. In this review article, we summarize the latest studies that focused on the supportive function of MSC. This thread of research leads to a new perspective on the interactions between parenchymal and mesenchymal cells and justifies a principally novel approach for regenerative medicine based on co-application of MSC and parenchymal cell for the most efficient tissue repair. Copyright © 2013 John Wiley & Sons, Ltd.

KEY WORDS—mesenchymal stromal cells; MSC; niche; parenchymal–mesenchymal interaction; trophic effects

INTRODUCTION

In the 1970s, clonogenic mesenchymal stem or stromal cells (MSCs) or fibroblast colony-forming units were first described as fibroblast precursors from bone marrow (BM) by Friedenstein *et al*.[1] In the 1990s, Caplan named these cells 'mesenchymal stem cells' on the basis of their clonogenicity and ability to undergo multilineage differentiation.[2] Initially, MSCs only referred to a group of cells derived from BM, which are non-haematopoietic stem cells.[3] Nowadays, MSCs are usually defined as a group of progenitor cells capable of differentiating into a number of mesenchymal lineages.[2] MSCs may be expanded for several passages in medium with or without serum while maintaining their capacity to differentiate into multilineage such as osteogenesis, chondrogenesis, myogenesis and adipogenesis. Such features make MSCs an attractive cell source for clinical applications, including cell-based therapies and tissue engineering.[4]

Adipose tissue is derived from the embryonic mesenchyme and contains stromal cells that can be easily isolated. In 2001, Zuk *et al*. reported that a group of multipotent cells can be isolated from the stromal vascular fraction of collagenase digested human adipose tissue.[5] These cells are called adipose tissue-derived stromal cells or adipose stem cells (ASCs). After expansion, ASCs can differentiate into adipocytes, osteoblasts, chondrocytes and myocytes in a proper differentiation environment.[6] From that point on, many studies have emerged to describe the multilineage potential of ASCs isolated from diverse animal models including mouse,[7] rat,[8] rabbit,[9] dog[10] and pig.[11] Characterizations of ASCs showed that they are very similar to MSCs derived from BM in terms of *in vitro* expansion and multilineage differentiation. Thus, ASCs are also considered as a group of MSCs, derived from another source, different from the BM. Compared with BM derived MSCs, ASCs are easier to isolate; large numbers of ASC can be harvested from a small volume of adipose tissue with minimal discomfort for a patient. These features are suitable

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for autologous cell transplantation.[6] Another advantage of ASCs is that they can be expanded to relatively larger numbers because of their proliferative capacity while maintaining their functionality after *in vitro* culture and cryopreservation. Thus, it is currently accepted that ASCs could be a promising alternative for BM-derived MSCs working as an abundant cell source for the clinical applications of musculoskeletal tissue engineering. More recently, it has became clear that besides the BM and adipose tissue, new evidences indicated that MSCs exist in virtually all organs,[12] including the brain, spleen, liver, kidney, lung, BM, muscle, thymus, pancreas, amniotic fluid and so on (Figure 1).

Our group and others have recently described MSC-like cells derived from human embryonic stem cells. Embryonic stem cell-derived MSC have very similar surface antigen profile to BM-derived MSC and can differentiate into osteogenic, adipogenic and chondrogenic lineages.[13]

Although the exact biological functions of MSC are not completely understood, their distribution across the entire organism indicates that the biological role of MSC is much broader than just making bone, cartilage or fat. Moreover, differentiation of MSC to these lineages never occurs in organs such as the brain, pancreas or kidney, indicating the different functional roles MSC may play in these organs *in vivo*. In the current review, we discuss multiple mesenchymal populations with respect to their ability to support parenchymal cells isolated from various organs and tissues. Indeed, MSC populations derived from the stromal compartment of different organs may not be functionally identical to those isolated from the BM. Moreover, it is highly unlikely that various stromal cells isolated from fat, skin and other anatomical regions represent 'true' stem cells, and therefore, a more accurate and broad term, 'mesenchymal stromal cells', will be used in this review.

THE *IN VIVO* ORIGINS OF MSCS

Despite the advances in the research of MSCs in the past few decades, their natural origins *in vivo* are still not completely understood. As one of the most promising types of adult stem cells for cell-based therapies, MSCs are mainly defined by functional tests performed with *in vitro* expanded cells. This definition of MSCs may be elusive by experimental artefacts introduced by the artificial culture conditions. Thus, the identification of the MSCs' *in vivo* origin is vitally important for validating results obtained *in vitro*. In recent years, many studies have been performed to identify the *in vivo* origin of MSCs. Here, we summarized the newest reports on the *in vivo* origins of MSCs.

Pericytes

It has been proposed by Farrington-Rock *et al.* and Crisan *et al.* that the ancestor of MSC is natively associated with the blood vessel wall and, more precisely, belongs to a subset of perivascular cells.[14,15] This could possibly explain why MSCs could be found in almost all organs, where blood vessels exist. Human perivascular cells, more specifically pericytes sorted from multiple human tissues, could be cultured for long term and give rise to plastic-adhesive, multipotent stromal cells that exhibit the typical characteristics of MSCs. Pericytes isolated from different organs share a profile of CD markers such as CD146+, CD56−, CD34− and CD45−.[14] Pericytes encircle endothelial cells of capillaries

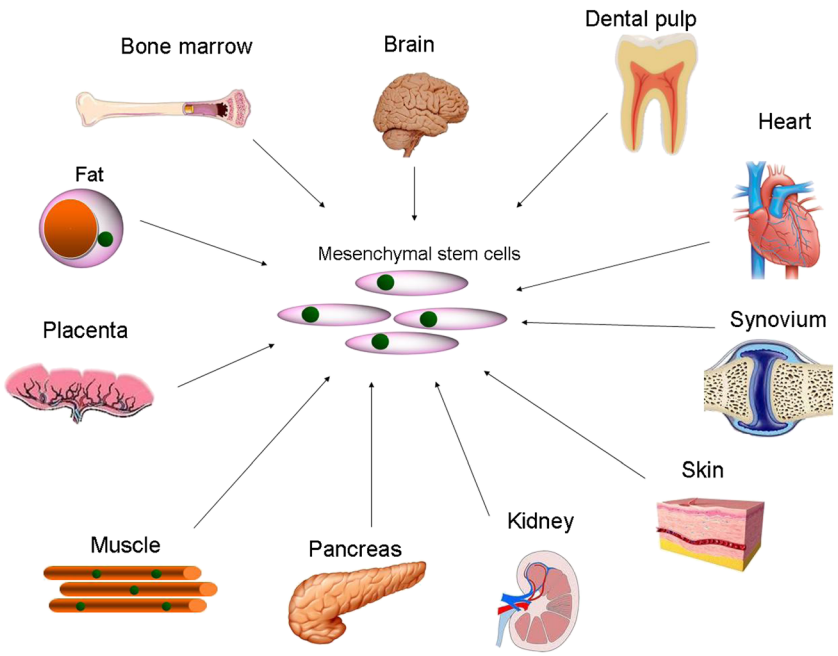

Figure 1. Schematics of MSCs origins from multiple organs

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and microvessels and express the adhesion molecule CD146 and the PDGFR-β but lack endothelial and hematopoietic markers such as CD34, CD31, von Willebrand factor, ligand for Ulex europaeus 1 and CD45, respectively. The proteoglycan NG2 is a pericyte marker exclusively associated with the arterial system. Besides its expression in smooth muscle cells, smooth muscle actin (α-SMA) is also detected in subsets of pericytes.

Adventitial cells

In addition to human pericytes, which encircle capillaries and microvessels, there are more cells around the blood vessels that give rise to MSCs in cultures. Corselli *et al*. reported that a group of CD34+, CD31−, CD146− and CD45− cells reside in the tunica adventitia in the outmost layer of blood vessels.[16] These cells intrinsically expressed MSC markers identical to BM-derived MSCs and gave rise to multipotent stromal cells in culture flasks. Despite common characteristics of MSCs, adventitial cells exhibit different phenotypes in cultures when compared with pericytes. However, adventitial cells may acquire pericyte-like phenotypes in the presence of growth factors involved in vascular remodelling. On the basis of the hypothesis of Corselli *et al*.,[16] MSCs have two separate perivascular origins: pericytes in capillaries and microvessels and adventitial cells around larger vessels.

Endothelial cell

Besides pericytes and adventitial cells, vascular endothelial cells were proposed to be another origin of MSCs *in vivo*. It was recently reported that vascular endothelial cells may be transformed into multipotent cells with characteristics of MSCs.[17] This conversion is mediated by an activin-like kinase-2 (ALK2) receptor-dependent mechanism. Medici *et al*. reported that constant expression of exogenous ALK2 in endothelial cells induced endothelial–mesenchymal conversion and made endothelial cell acquire some features of MSCs.[17] Their results also indicate that treatment of endothelial cells with transforming growth factor-β2 or bone morphogenetic protein-4 caused the transition from endothelial to mesenchymal in an ALK2-dependent manner. It was shown that the converted endothelial cells were able to differentiate into osteoblasts, chondrocytes or adipocytes.

Non-prevascular origin of MSCs

So far, most of the published studies support the perivascular origin of MSCs *in vivo*. However, some reports clearly indicate that stem-like stromal cells can also be isolated from tissues that have completely no vasculature. Mark Erwin *et al*. reported that a group of stem cells or progenitors can be isolated from non-chondrodystrophic canine intervertebral disc.[18] Their data suggested that nucleus pulposus progenitor cells could differentiate into chondrogenic, adipogenic and neurogenic lineages *in vitro* and neurogenic lineage *in vivo*.[18] Another example is chondrogenic progenitor cells (CPCs) isolated from human articular cartilage of late stage osteoarthritis. Koelling *et al*. reported the multilineage differentiations of CPCs *in vitro*.[19] Under specific culture conditions, CPCs may differentiate into adipocytes, osteoblasts and chondrocytes. Other characteristics of MSCs such as *in vitro* expansion can also be found in CPCs. Interestingly, they also claimed that healthy chondrocytes did not differentiate and died soon after the start of the test. It looks like that these MSC-like features of cells in avascular tissue might be associated with certain pathological conditions. However, more studies need to be carried out for further investigations.

INTERACTIONS BETWEEN PARENCHYMAL TISSUES AND MSCS IN TISSUE REGENERATION AND CANCER METASTASIS

What common functions may be shared by MSC present in such functionally distinct organs such as the brain, muscle or kidney? Indeed, perivascular cells are functionally and structurally associated with the endothelium and are involved in the universal programmes of tissue remodelling and neovascularization in normal and pathological conditions. However. more recently, it became clear that MSC produce a number of growth factors that may support parenchymal cells of different organs and tissues. This 'supportive function' discussed in the current review is a revised point of view on the 'epithelial–stromal' interaction that have been extensively studied for several decades. It becomes more and more clear that supportive functions of MSC are much broader than we used to think and now can be projected to many non-epithelial organs and tissues (Table 1).

The microenvironment in different organ systems where parenchymal cells naturally reside is referred to as a parenchymal cell niche, representing functional and structural units that spatiotemporally regulate cell division and differentiation. This microenvironment provides all necessary signalling molecules, extracellular matrices, intercellular interactions and chemical requirements to maintain tissue homeostasis and is also crucial for the tissue-specific stem/progenitor cell fate.[43,44] Control of these niches is emerging as a key role, played by MSC in a broad array of tissues. Therefore, MSCs are now considered not only as precursors for certain lineages but also as regulatory cells involved in the maintenance of the homeostasis of parenchymal cells in normal and pathological conditions. The exact mechanism of this regulatory action of MSCs is still highly debatable and controversial.[45,20,46] It is proposed that these interactions are mediated via paracrine signalling, cell fusion, cell–cell interaction or differentiation to specific cell types in the different organs as discussed in detail below.

In recent years, MSCs have been proposed to play as trophic mediators in tissue repair. The term 'trophic mediators' initially refers to bioactive factors produced by nerve terminals, which are non-neurotransmitters.[47] In relation to MSCs, the term trophic was first used to describe the process in which MSCs secrete factors that stimulate nearby cells to release functional molecules.[48] Later, the term was related to the effect of the factors produced by MSC on viability, proliferation and matrix production of local cells. This hypothesis leads

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Table 1. Summary of interactions between parenchymal and mesenchymal cells

Parenchymal cell	Supportive mesenchymal cell	Mechanism	Effect on parenchymal cells	Ref.
Cardiac cells (swine)	BM-MSCs fractioned (Ficoll gradient and attachment to plastic-based selection)	Cell to cell interaction: gap junction through conexin 43.	Stimulation of CSC proliferation	20
Cardiac fibroblasts (rat)	BM-MSCs (attachment to plastic-based selection. $CD29^+$ and $CD90^+$)	Regulation of cardiac fibroblast proliferation and transcriptional downregulation of types I and III collagen syntheses.	Reduction of fibrosis	21
Endothelial cells (human)	BM-MSCs (attachment to plastic-based selection)	Paracrine release of VEGF.	Proangiogenic	22
Cardiomyocytes (mouse)	BM-MSCs (attachment to plastic-based selection and $Sca1^+$, retroviral modification, Akt-MSCs)	Paracrine release of VEGF, FGF2, HGF and thymosin β4 (TB4).	Inhibition of hypoxia induced apoptosis and increased spontaneous contractility.	23
Cardiomyocytes (rat)	BM-MSCs (Ficoll gradient, attachment to plastic-based selection and retroviral modification, Akt-MSC)	Paracrine release of Sfrp2 resulting in increased cellular β-catenin and upregulation Bcl2 and Birc1b.	Antiapoptotic	24
Cardiomocytes (rat)	ASCs (attachment to plastic-based selection)	Paracrine release of VEGF and insulin-like growth factor-1	Antiapoptotic and pro-angiogenic	25
Hepatocytes (rat)	BM-MSCs (attachment to plastic-based selection)	Cell to cell interaction through LRP and paracrine release IL-6.	Prosurvival Improved metabolic capacity.	26,27
Hepatocytes (rat)	BM-MSCs (attachment to plastic-based selection)	Signalling through Notch/Jagged1 pathway.	Stimulation of proliferation	28
Hepatocytes (rat)	BM-MSCs (attachment to plastic-based selection)	Not known	Prosurvival, proliferation	29
Lung epithelial cancer cells (human)	BM-MSCs (attachment to plastic-based selection)	Upregulation of stanniocalcin-1 expression	Anti-apoptotic	30
Kaposi's sarcoma cell line (human)	BM-MSCs (commercial cell line)	Activation of Akt pathway through E-cadherin	Anti-tumorigenic	31
Hepatoma cell lines (human)	Foetal dermal derived MSCs (Not available)	Downregulation of Bcl-2	Anti-proliferative	32
Brest cancer cell lines (human)	BM-MSCs (Histopaque gradient and attachment to plastic-based selection)	Paracrine release of CCL5	Enhanced motility, invasion and metastasis.	33
Primary neural cells (mouse)	BM-MSCs (attachment to plastic-based selection)	Activation of STAT3 and Akt pathway proposed	Neuroprotective	34
Clonal pheochromocytoma cell line (rat)	AMCs (not available)	Upregulation of X-chrosome-linked inhibitor of apoptosis factor through PI3-K/Akt activation	Anti-apoptotic	35
Cortical astrocytes cell line (mouse)	BM-MSCs (attachment to plastic-based selection)	Downregulation of glial fibrillary acidic protein through downregulation of IL-6/IL-6R/gp130 pathway	Reduced astrocytic activation	36
Dorsal root ganglion neurons (rat)	BM-MSC (not available)	Direct and indirect inhibition of metalloproteinases	Anti-apoptotic	37
Hematopoietic progenitor cells. ($cd34^+$ $cd133^+$, human)	Murine foetal liver stromal cell line (AFTO24) and human BM stromal cells (attachment to plastic-based selection).	β1-Integrin-dependent mechanism.	Stimulation of proliferation and self-renewal of HPC	38
Ucb mononuclear cells (human)	BM-MSCs (Percoll gradient)	Not known	Anti-apoptotic effect and inhibition of differentiation	39
$Cd133^+$ hematopoietic stem cells (human)	BM-MSCs ($CDw90^+$, $CD105^+$, $CD166^+$, $CD73^+$, and $CD34^-$ and CD45)	Not known	Stimulation of proliferation, migratory potential	40
$Cd34^+$ cells (human)	BM-MSCs (Attachment to plastic-based selection)	Not known	Stimulation of proliferation	41
Chondrocytes (human)	BM-MSCs and ASCs; (Attachment to plastic-based selection)	Paracrine release of soluble factors	Stimulation of chondrocyte proliferation and matrix formation	42

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to a shift of opinion in the way MSCs function in tissue repair. Traditionally, MSCs were believed to repair damaged tissue by differentiating into tissue-specific cells and replacing lost tissue;[49] nowadays, the trophic role of the MSC in tissue repair is considered more important.[50]

MSCs in cardiac tissue

The presence of MSCs in the heart supports the concept that these cells may be involved in the regulation of cardiomyocyte molecular homeostasis. The unique properties of MSCs (easily isolated and amplified from different tissues, immunologically tolerated as an allogeneic transplant and their multilineage potential) have led to their intense investigation as a cell-based therapeutic strategy for cardiac repair. Their mechanisms of action in cardiac repair are likely to be multifaceted, and the data accumulated to date in large animal models and humans have shown that MSC therapy for cardiac disease is safe and provides substantial improvements in cardiac structure and function.[51–53,23] Because the frequency of MSC engraftment and differentiation in the heart is very low compared with the functional recovery observed after cell transplantation, this observation has raised a question whether MSC engraftment and differentiation is the predominant mechanism of action. Because MSCs are known to secrete a wide range of cytokines and growth factors that can suppress the immune system, inhibit fibrosis and apoptosis, attenuate pathological ventricular remodelling, enhance angiogenesis and contribute to endogenous cardiomyogenesis,[54,55,21] paracrine secretion seems to be the predominant mechanism. Most likely, paracrine mediators are expressed/released in a temporal and spatial manner, exerting their effect depending on the microenvironment they are being released (e.g. post-injury and physiological). In addition, these released factors may have autocrine actions on the biology of MSCs themselves. For example is what has been reported that autocrine release of leukaemia inhibitory factor (LIF) is responsible for maintaining the multipotent programme of MSCs.[56]

Strong support of paracrine mechanism for cardiac repair comes from experimental studies showing the effect the conditioned medium collected from MSC cultures exerts on different parenchymal cell types. Paracrine mechanisms mediated by MSCs are also capable of enhancing the survival of existing myocytes. Conditioned medium from hypoxic Akt-modified MSCs (Akt-MSCs) prove to markedly inhibit hypoxia-induced apoptosis and triggered vigorous spontaneous contraction of adult rat cardiomyocytes *in vitro*.[23] Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), hepatocyte growth factor (HGF) and thymosin β4 (TB4) are among the factors responsible for this protective effects. It has also been shown that Akt-MSCs can promote cardiomyocyte survival by increasing cellular β-catenin and upregulating anti-apoptotic genes such as Bcl2 and Birc1b through paracrine release of secreted frizzled related protein 2.[24] Ohnishi *et al.*[21] showed that the effect of paracrine effects on MSCs can be extended to cardiac fibroblasts. By culturing cardiac fibroblast in the presence of MSC conditioned medium, the authors demonstrated that MSC exerted paracrine anti-fibrotic effects at least in part through regulation of cardiac fibroblast proliferation and transcriptional downregulation of types I and III collagen syntheses.

Recent studies document that also direct interactions between MSCs and progenitor cell populations can promote their proliferation and differentiation *in vitro*. Hatzistergos *et al*. demonstrated that MSCs have the ability to stimulate proliferation of endogenous c-kit$^+$ cardiac stem/progenitor cells and enhance cardiomyocyte cell cycling.[20] It has also been described that the co-culture with MSCs significantly improves functional cardiac differentiation *in vitro*.[57] Evidence from murine stem cell differentiation into cardiac lineage suggests that the JAK2/STAT3 pathway is essential for the initial stages of cardiomyogenesis and that STAT3 is able to regulate the expression of GATA4, TBX5 and NKX 2·5 genes (by binding to their promoters), all of which are essential for cardiac development.[58,59] Even though it still remains unclear what triggers the activation of JAK2/STAT3 pathway, it is possible that cytokines [such as LIF or interleukin-6 (IL-6)] released by the adjacent MSCs during cardiac development are responsible for this activation.

Neovascularization is one of the biological processes positively influenced by MSCs in a paracrine fashion. Nitric oxide, VEGF, bFGF, HGF and angiopoietin (Ang-1) are produced by MSC and have been demonstrated to be directly involved in angiogenesis and arteriogenesis. It has been shown that MSCs can express pro-angiogenic factors and that the release of these factors may play an important role in determining the increase in angiogenesis.[45] For example, conditioned medium collected from MSC promotes proliferation and migration of endothelial cells and vascular smooth cells in a dose-dependent manner *in vitro*.[54,22] These and other results showed that MSCs can improve neovascularization through pro-angiogenic and pro-arteriogenic paracrine effects.

MSCs in hepatic tissue

Numerous studies have shown a broad range of biological interactions between hepatocytes and MSCs. Hepatocyte morphology could be sustained much longer in co-cultures with MSCs when compared with hepatocytes cultured alone.[26] Corlu *et al*. demonstrated that the liver-regulating protein (LRP; a membrane-associated protein) is in part responsible for this support. LRP is expressed by BMMSCs, hepatocytes, liver epithelial cells, stellate cells, Kupffer cells and similar cell types such as those in the thymus and spleen.[60] Co-cultures of rat hepatocytes and rat liver epithelial cells with BM-MSCs showed that LRP is essential for maintaining the mature hepatocyte phenotype and that direct cell contact is required for this effect. Mizuguchi *et al*. have proposed another interesting mechanism for the interaction between hepatocytes and MSCs.[28] However, the gene encoding LRP has not been identified yet, and more studies have to be carried out to further dissect this mechanism. They found that in co-cultures of hepatocytes and BM-MSCs, one of the Notch ligands, Jagged1, was induced at both mRNA and protein levels and that

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only BM-MSCs adjacent to the proliferating hepatocytes expressed Jagged1.[28]

Bone marrow-derived MSCs can also help hepatocytes to maintain their metabolic activity. Isoda *et al*. demonstrated that when hepatocytes were cultured with BMMSCs, they kept the albumin and ammonia metabolic capacity at higher levels than in controls.[27] On the contrary with the previously stated, this effect appeared to be independent of cell–cell contact because both conditioned medium from BM-MSCs and transwell co-cultures showed the same results as direct co-cultures. The same authors also showed that IL-6 is one of the cytokines responsible of this effect, as urea production was significantly improved in the presence of this cytokine when compared with untreated monocultures, or cultures treated with antibodies against IL6.

Finally, there is evidence showing that BM-MSCs may facilitate the survival, proliferation and differentiation of primary rat hepatocytes by providing the appropriate environment through extracellular matrix deposition. The production of type I collagen by BM-MSC-derived fibroblasts show to promote appearance of bile canaliculi forming hepatocytes,[29] but the mechanism underlying this effect remains to be uncovered. Hence, it seems that MSCs can exert its effect on hepatocytes not only through soluble molecules or cell to cell contact but also by providing insoluble signals such as extracellular matrix deposition.

MSCs and blood cells

Hematopoietic stem cells (HSCs) represent one of the best-defined adult stem cells in mammals. Using multicolor flow cytometry, HSCs can be identified and enriched by their surface antigen profile. These cells have the ability to reconstitute the entire hematopoietic system. HSCs reside in the BM, proximal to the BM stromal cells, and this is considered to be their niche in a broad sense.[61] This niche comprises non-haematopoietic cells such as fibroblasts, reticular cells, endothelial cells, adipocytes and osteoblasts that provide soluble factors and extracellular matrix to support the expansion and activity of HSCs,[62,63] but the exact location and the definition of the HSC niche still remains controversial. Previous studies have shown HSCs both located near the endosteal region (closer to the osteoblasts)[64,65] or in perivascular regions near the reticular cells that express high levels of chemokine C-X-C motif ligand 12 (CXCL12).[66] Studies published by Bianco *et al*. demonstrate that a $CD146^+$ population of stromal cells isolated from the BM demonstrates all characteristics typical for MSC.[67] These $CD146^+$ cells, if injected to experimental animals, generate all components of fully functional BM supporting the development of hematopoietic progenitors.

Not only BM derived but also placental MSCs support the expansion of hematopoietic progenitors. It has been demonstrated that stromal cell lines generated from human placenta and expressing a pericyte-like immunophenotype ($CD146^+$ and $NG2^+$) are able to support the expansion of human cord blood $CD34^+$ hematopoietic progenitors *in vitro*. This study suggests that the human placenta is not only a potent hematopoietic niche but also a potentially useful source of cells for hematologic clinical applications and human regenerative medicine.[68]

Numerous groups have been trying to reveal the functional role specific of stromal cell types in the HSC niche. Méndez-Ferrer *et al*. published an elegant study in which they were able to identify a stromal $Nestin^+$ population with full MSC potential that was spatially associated with HSCs and adrenergic nerve fibres, and highly expressed HSC maintenance genes (including CXCL12, stem cell factor, Ang-1, interleukin-7, vascular cell adhesion molecule 1 and osteopontin). Their data suggest that MSC $nestin^+$–HSC pairing conform a structurally unique niche in the BM.[69] It remains unclear whether the CD146 population described by Bianco *et al*. is identical to the $Nestin^+$ cells described by Méndez-Ferrer *et al*. Omatsu *et al*. recently proposed CXCL12-abundant reticular cells as key components of HSC niche, being involved in the proliferation of HSCs and lymphoid and erythroid progenitors as well as the maintenance of HSCs in an undifferentiated status.[70] Again, it remains to be determined whether this population is different from the previously reported cell subsets.

Although HSCs had been widely studied in the past and HSC transplantation has become a well-established treatment for hematologic malignancies and non-malignant disorders, the expansion of HSCs *in vitro* is difficult to achieve because of concomitant differentiation and gradual loss of stemness.[71]

Even though many studies have demonstrated that HSCs can be expanded in cytokine-driven cultures during the past years, many groups have reported the interaction between HSCs and MSCs as an important issue for maintaining HSCs *in vitro*. Most likely, the lack of proper signals that *in vivo* would be provided by stem cell niche is actually lacking in the current *in vitro* expansion systems.[72] Little is known about the signalling intermediates that direct HSCs' self-renewal; however, it was shown that STAT3 may be one of the regulators of hematopoietic regeneration.[73,74] What triggers STAT3 activation in HSC is not clear, but it is likely that cytokines such as LIF, IL-6 or interleukin-3 produced by MSCs present in the hematopoietic niche are responsible for such activation.

Mesenchymal stromal cells have been proven to have some beneficial effects on *ex vivo* expansion of $CD34^+$ peripheral blood progenitor cells (PBSC) while decreasing their allostimulatory capacity. Although high levels of IL-6 on its own have been proven to be sufficient to significantly improve PBSC expansion, direct contact between MSCs and PBSCs is required to achieve maximal expansion, indicating that paracrine release of soluble factor is unlikely to be the only mechanism that may explain the positive effect of MSC on HPC expansion and that cell to cell contact is also playing a role.[41] Alake *et al*. demonstrated that adhesion and direct cell to cell contact with an MSC feeder layer supports *ex vivo* expansion, migratory potential and stemness of $CD133^+$ HPC,[40] but the mechanisms by which MSCs exert their effects have not been revealed. In line with the previously stated, a paper published by Gottschling *et al*. demonstrated that MSCs are able to

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induce self-renewing divisions of HPC. Using MSCs as *in vitro* surrogate niche, they monitored the division history and subsequent functional fate of individually plated $CD34^+/133^+$ cells in the absence or presence of an anti-β1 integrin-blocking antibody by time-lapse microscopy and subsequent long-term culture-initiating cell assays.[38] The results showed that the initial self-renewing cell divisions of HPC are regulated by a β1-integrin-dependent mechanism.

MSCs in neural tissue

The positive effect of MSCs on survival of neural cells has been widely reported. Different mechanisms of action have been described depending on the origins and type of neural cells. ASCs have been reported to secrete high levels of angiogenic and antiapoptotic growth factors such as granulocyte macrophage-colony stimulating factor, glial-derived neurotrophic factor, nerve growth factor, VEGF, HGF, bFGF, TGF-β, brain-derived neurotrophic factor and insulin-like growth factor-1. These factors have been proven to be neuroprotective, reducing apoptosis of neural cells under pathological circumstances.[75] Zhao *et al*. reported that one of the underlying mechanism of brain-derived neurotrophic factor and HGF neuroprotection is due to the upregulation of X-chrosome-linked inhibitor of apoptosis factor through the activation of the PI3-K/Akt pathway.[76] In accordance with the previous work, human and murine BM-derived MSCs recued cortical neurons from apoptotic cell death in an *in vitro* model of cerebral ischaemia and that this neuroprotective effect is mediated by paracrine factors, such as IAP-1. Although the mechanism was not described, the authors proposed that MSC-derived soluble factors either directly or indirectly activate STAT3 and Akt-dependent anti-apoptotic pathways in neurons.[34]

Bone marrow-derived MSCs showed beneficial effects on astrocytes as well. Gao *et al*. demonstrated in an *in vitro* model that ischaemia-induced astrocytic activation could be reduced by the presence of stromal cells.[36] BM-MSCs downregulated glial fibrillary acidic protein expression in astrocytes without affecting their morphological integrity and proliferation rate and that its effect may derive from the downregulation of the IL-6/IL-6R/gp130 pathway. This and other findings from the same group suggest that BM-MSCs may suppress the detrimental element of astrogliosis while enhancing the beneficial role of reactive astrocytes in assisting neuroregeneration.[36] Finally, it has been shown that MSCs can prolong the life of neurons by inhibiting photolytic enzymes such as metalloproteinases.[37]

MSCs and cancer

Mesenchymal stromal cells are known to home at different kinds of tumours, including gliomas[77] and breast,[78,33,79] colon,[80] ovarian[81] and lung carcinomas, among many other primary and metastatic tumours,[82,83] from a large variety of administration routes. This suggests that the capacity of integrating into tumours is an intrinsic property of these cells, and this is consistent with the hypothesis that integration of exogenously delivered MSCs during tumour formation is a recapitulation of the natural recruitment of endogenous, circulating MSCs to aid tissue repair and remodelling.[77,84] One of the possible explanations for the tropism of MSCs for tumours may be explained, at least in part, by the release of soluble tumour-derived factors such as stromal cell-derived factor-1 (SDF-1), tumour necrosis factor-α, chemokines, interleukins and tumour cell-specific receptors expression by the tumorigenic cells. Nakamizo *et al*. demonstrated that although human gliomas express EGF, PDGF, VEGF and FGF as well as the chemokine SDF-1a, there is a selectivity of the MSC response to these factors. Whereas FGF and VEGF had little effect on MSC migration, PDGF, EGF and SDF-1a enhanced MSC tropism.[77] Indeed, MSCs are known to express EGF and PDGF receptors on their surface.[85]

What is the role of MSCs once they arrive at the tumour is a matter of debate. Many reports indicated that MSCs display tumour-supporting roles on arrival to the tumour microenvironment, such as inhibition of apoptosis,[33,30] proliferation[79] and angiogenesis. In this context, Karnoub *et al*. demonstrated that human MSCs are particularly important to enhance the metastatic ability of human tumour xenografts. All of four human breast cancer cell lines tested showed increased metastasis potential in the presence of MSCs. Although the exact mechanism remains unclear, it appears that breast cancer cells stimulate secretion of chemokine CCL5 from MSCs, which then acts in a paracrine fashion on the cancer cells to enhance their motility, invasion and metastasis.[33]

On the other hand, many reports showed that MSCs can also have anti-tumorigenic effects, as shown in Khakoo *et al*. They described that MSCs can exert potent anti-tumorigenic effects in a model of Kaposi's sarcoma by activating the *Akt* pathway in a mechanism that is in part dependent of E-cadherin.[31] Recently, Qiao *et al*. also demonstrated that human MSCs could inhibit the proliferation and colony-forming ability of human cancer cell lines, possibly through the downregulation of anti-apoptotic Bcl-2.[32]

MSCs in cartilage regeneration

Wu *et al*. reported that pellet co-culture of chondrocytes and MSCs benefited cartilage matrix formation and that cartilage matrix genes were mainly expressed by chondrocytes.[42] In addition, the authors have also showed that the ratio of MSCs decreased dramatically because of massive cell death of MSCs by apoptosis. Proliferation of chondrocyte increased by either co-culturing with MSCs or culturing in conditioned medium of MSCs. These findings were confirmed by an independent study performed by Acharya *et al*..[86] These two studies together demonstrated a new mechanism of cellular interactions in co-culture of MSCs and chondrocytes, in which MSCs play as trophic mediators to stimulate chondrocyte proliferation and cartilage matrix deposition rather than actively differentiate into chondrocytes. Another study from the same group showed that these trophic effects are independent of culture conditions and origins of MSCs.[87]

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CONCLUSIONS AND FUTURE PERSPECTIVE

Growing body of literature indicates that biological functions of MSCs are not limited to their ability to differentiate into several mesenchymal lineages. It becomes more and more clear that MSC play a fundamental role in controlling tissue homeostasis and niches in which different parenchymal cells reside and function. However, the exact mechanism through which MSCs exert their effect in different organs still remains unclear. It is likely that secreted and non-secreted factors released or present in the membranes of MSCs can support organ-specific cells and can stimulate regeneration.

Mechanisms proposed so far vary depending on the tissue under study, but it has mainly been reported that MSCs can act by releasing paracrine factors (e.g. nitric oxide, VEGF, bFGF, HGF, IGH, stem cell factor, IL-6, LIF, interleukin-7, vascular cell adhesion molecule 1 and osteopontin) and extracellular matrix proteins (e.g. laminins, nidogens and fibronectin) or through cell to cell contact (gap junctions, conexins, LRP, Jagged1–Notch signalling and integrins).[88] It is likely that a combination of secreted factors and cell to cell contact is necessary for the MSCs to perform at their maximum potential. Future studies will characterize the molecular mechanisms through which perivascular MSCs are able to regulate homeostasis of parenchymal cells located in different organs and tissues.

CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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