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Mesenchymal Stem Cell Paracrine Factors in Vascular Repair and Regeneration

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Abstract

Mesenchymal stem cell therapy show great optimism in the treatment of several diseases. MSCs are attractive candidates for cell therapy because of easy isolation, high expansion potential giving unlimited pool of transplantable cells, low immunogenicity, amenability to ex vivo genetic modification, and multipotency. The stem cells orchestrate the repair process by various mechanisms such as transdifferentiation, cell fusion, microvesicles or exosomes and most importantly by secreting paracrine factors. The MSCs release several angiogenic, mitogenic, anti-apoptotic, anti-inflammatory and anti-oxidative factors that play fundamental role in regulating tissue repair in various vascular and cardiac diseases. The therapeutic release of these factors by the cells can be enhanced by several strategies like genetic modification, physiological and pharmacological preconditioning, improved cell culture and selection methods, and biomaterial based approaches. The current review describes the impact of paracrine factors released by MSCs on vascular repair and regeneration in myocardial infarction, restenosis and peripheral artery disease, and the various strategies adopted to enhance the release of these paracrine factors to enhance organ function.

Keywords

Mesenchymal stem cells; Paracrine factors; Myocardial Infarction; Peripheral Artery Disease; Restenosis

Introduction

Mesenchymal stem cells (MSCs) offer great promise in the treatment of vascular diseases. They are present in various organs, including bone marrow, adipose tissue [1], liver [2], dental pulp [3], amniotic fluid [4] and umbilical cord blood [5]. The International Society of Cellular Therapy defined MSCs as plastic adherent population, expressing cell surface markers such as CD73, CD90, and CD105, and lack expression of other markers including CD45, CD34, CD14, or CD11b, CD79α or CD19 and HLA-DR surface molecules, and showing trilineage differentiation potential to osteocytes, chondrocyte and adipocytes under in vitro conditions [6]. MSCs have also been reported to differentiate into endothelial cells [7,8], haematopoiesis supporting stromal cells [9], cardiomyocytes [10] and even into cells

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of non-mesodermal origin, including hepatocytes [11] and neurons [12]. The potent immunosuppressive functions of MSCs have been demonstrated both in vitro and in vivo [13,14].

MSCs can cause tissue regeneration and repair through several processes: a) transdifferentiation of MSCs into the specific cell type, b) fusion of stem cells with the resident cells, c) through the release of microvesicles or exosomes and most importantly, d) through the release of paracrine factors. Transdifferentiation of MSCs into specific cell type was initially thought to be the principal mechanism underlying their therapeutic action. MSCs were capable of engrafting in the site of injury and differentiating into cardiomyocyte-like cells expressing typical cardiomyocyte markers in a mouse model of myocardial infarction (MI) [10]. Another rare phenomenon affecting cell repair is cell fusion, where MSCs spontaneously fuse with somatic cells in vivo, and the fusion products are capable of tissue-specific function or proliferation depending on the microenvironment [15]. The heterologous cell fusion of adipose derived stem cells to cardiomyocytes promoted cardiomyocyte reprogramming back to a progenitor like state, with the resulting hybrid cells showing early cardiac commitment and proliferation markers [16]. Recent evidence showed that MSC cardiomyocyte fusion includes mitochondrial exchange, which is essential for somatic reprogramming [15]. Another recently reported mechanism of cell repair is through exosomes or microvesicles, which are small, spherical membrane fragments shed from the cell surface or secreted from the endosomal compartment.

MSCs release a significant amount of microvesicles containing mRNA with specific multiple differentiative and functional properties, as well as selected patterns of mature micro RNAs. These nucleic acids can be transferred via microvesicles to recipient cells, inducing functional and phenotypic changes [17]. The extracellular vesicles can act directly through the interaction ligand/receptor or indirectly on angiogenesis by modulating soluble factor production involved in endothelial cell differentiation, proliferation, migration, and adhesion; by reprogramming endothelial mature cells; and by inducing changes in levels, phenotype, and function of endothelial progenitor cells [18]. Extracellular vesicles released by the MSC under hypoxia stimulation were uptaken by endothelial cells and promoted neoangiogenesis in vitro and in vivo [19]. However, reports suggest that frequency of cell engraftment and differentiation either by transdifferentiation or cell fusion, appear too low to explain the significant improvement [20]. Recent studies have shown that the key mechanism by which MSCs enhance tissue function is through its paracrine functions. In the current review we discuss the role of paracrine factors released by MSCs on vascular repair and regeneration in restenosis, peripheral artery disease, and myocardial infarction. We further review the diverse strategies adopted to enhance the release of these paracrine factors to enhance organ function.

Angiogenic MSC Paracrine Factors

The important angiogenic factors secreted by MSCs include vascular endothelial growth factor (VEGF) [21,22], fibroblast growth factor-2 (FGF-2), Angiopoetin-1 (Ang-1) [23], insulin-like growth factor (IGF-1) [24], hepatocyte growth factor (HGF) [22], transforming growth factor (TGF)-β, monocyte chemoattractant protein (MCP-1)[25, 26], interleukin

(IL)-6 [25, 26] and SDF-1α [27]. These paracrine factors function to trophically assist vascular 1 (Ang-1) [23], insulin-like growth factor (IGF-1) [24], hepatocyte growth factor (HGF) [22], transforming growth factor (TGF)-β, monocyte chemoattractant repair and regeneration process at sites of severe tissue ischemia or damage. In addition, MSCs also secrete several anti-apoptotic factors- (VEGF, HGF, IGF-1, staniocalcin-1, transforming growth factor (TGF-β), and granulocyte macrophage derived growth factor (GM-CSF); immunomodulatory factors (inducible nitric oxide (NO), prostaglandin E2 (PGE2), 2,3-dioxygenase, the non-classical major histocompatibility antigen HGF, TGF-β, leukemia inhibitory factor (LIF), and IL-10); factors supporting of tissue stem and progenitor cell proliferation (stem cell growth factor (SCF), LIF, macrophage derived growth factor (M-CSF), stromal cell derived factor-1 (SDF-1) and Ang-1); factors inhibiting fibrosis and scarring in ischemia (HGF, FGF-2, adrenomedullin); and chemoattractants (MCP-1, the macrophage inhibiting protein (MIP-1), chemokine (CC motif) ligand 5 (CCL) 5, IL-8, and SDF-1) (Reviewed by Meirelles Lda et al., 2009 [28]).

VEGF and FGF-2 are most potent inducers of angiogenesis [29]. They regulate many functions of endothelial cells including proliferation, migration, extracellular proteolysis, and tube formation activity [30,31]. Blocking the effects of VEGF and FGF2 in MSC conditioned media partly attenuated the mitogenic effects of the MSC conditioned media on endothelial cells demonstrating the importance of these cytokines in paracrine factor mediated angiogenesis [21]. In another study, bone marrow MSCs contributed angiogenic ligands (FGF2, VEGF, Ang-1) and cytokines (IL-1β and TNF-α), and effectively induced neovascularization in ischemic myocardium [23]. Multiple cytokines secreted by MSCs have additive or synergistic effects on endothelial cell proliferation. VEGF and FGF2 synergistically improved neovascularization in a rabbit hind limb ischemia model [32]. Another important MSC paracrine factor is Ang-1. Ang-1 is a strong inducer of endothelial cell sprouting, which is the first step in both angiogenesis and neovascularization [33]. Ang-1 reduces endothelial permeability and enhances vascular stabilization and maturation [34]. Ang-1 signaling promotes angiogenesis and remodeling of blood vessels through its receptor tyrosine kinase Tie2, expressed on endothelial cells. Coadministration of Ang-1 and VEGF enhanced collateral vascularization in a rabbit ischemic hindlimb model [35].

HGF is a potent angiogenic factor which stimulates endothelial cell motility and growth [36]. HGF gene transfer in a rat MI model resulted in significantly preserved myocardial function, and was associated with significant angiogenesis and a reduction in apoptosis [37]. Tomita et al. [38] demonstrated that HGF stimulated the expression of MMP-1, VEGF, HGF itself, and HGF putative receptor, c-met in human endothelial cells and vascular smooth muscle cells. Upregulation of angiogenesis related genes was largely dependent on the induction of transcription factor ets-1. HGF putative receptor, c-Met, was also identified on murine MSCs. The MSCs successfully achieved MSC commitment towards cardiac phenotype through HGF stimulation [39]. In this study, HGF activity in MSCs was shown to activate the ras-ERK1/2 and p38 MAPKs as well as the PI3K/Akt pathways. Another factor, IGF-1 plays a role in endothelial cell migration and angiogenesis [40], as well as in the activation of endogenous stem cells. The local delivery of both the IGF-1 and HGF in the infarcted heart resulted in the migration of c-kit+ cardiac stem cells toward the injured area and the regeneration of dead portions of the myocardium [41].

TGF-β is another potent angiogenic factor released by MSCs [22]. TGF-β maintains endothelial cell quiescence and induces vessel maturation, promotes basement membrane deposition, and enhances the interactions between endothelial cells and mural cells [42,43]. In addition, TGF-β regulates angiogenesis by influencing the expression and the activities of other angiogenic factors VEGF and PDGF, which are the direct target genes of TGF-β via Smad proteins [44,45]. TGF-β is also reported to promote angiogenesis at least in part via the secretion of the survival factors TGF-α and VEGF, which activate PI3K/Akt and MAPK pathways [25].

MSC secretes significant levels of MCP-1 and IL-6 [26]. Kwon et al [25] found significant levels of these cytokines in human MSC conditioned media and demonstrated their angiogenic activity using specific neutralizing antibodies. MCP-1 modulates angiogenesis by inducing endothelial cell migration and sprouting. In addition, MCP-1 participates in VEGF-mediated angiogenesis and increases vascular permeability [46] and upregulates VEGF expression [47]. MCP-1 is also suggested to induce chemotaxis of endothelial cells [48] or upregulate the expression of hypoxia inducible factor 1α, which in turn induces VEGF-A [47]. Studies by Ma et al. showed that MCP-1 mediates the angiogenic effect of TGF-β by recruiting vascular smooth muscle cells and mesenchymal cells toward endothelial cells thus promoting the maturation of new blood vessels [42]. MCP-1 is a direct gene target of TGF-β, and Smad 3/4 can bind to the MCP-1 promoter and increase its activity. Another paracrine factor, IL-6 is a potent proangiogenic cytokine which stimulates endothelial cell and smooth muscle cell proliferation and migration in vitro, as well as, promotes neovascularization [49]. Shabbir et al. [50] demonstrated that MSC-derived IL-6-type cytokines activate the skeletal muscle JAK-STAT3 axis and increase the levels of VEGF and HGF in the cardiac tissue, when injected into the skeletal muscles of a hamster model of cardiomyopathy.

Another important trophic factor released by MSCs is SDF-1α [27]. SDF-1α is an important chemoattractant for progenitor cells and plays a critical role in endogenous stem cell migration, adhesion, homing, and recruitment from bone marrow. The homing and migration of stem cells is affected through activation of a G protein–coupled receptor, CXCR4 [51]. Moreover, SDF-1 pretreatment can protect the resident cardiomyocytes from apoptosis and improve their survival by paracrine secretion of FGF2 and VEGF. SDF-1/CXCR4 also plays a pivotal role in the biologic and physiologic functions of MSCs [52,53]. Moreover, the local delivery of SDF-1α into injured tissue promotes the recruitment of circulating mesenchymal stromal and progenitor cells to lesions in the heart [54,55].

Vascular repair by MSC paracrine factors in Restenosis

Endothelial denudation and intimal hyperplasia are two of the prime mechanisms leading to restenosis. MSCs are reported to reduce neointimal formation by immunomodulation at the area of injury and by their paracrine effect of stimulation of endogenous stem cells from bone marrow in situ, rather than the direct integration into the endothelial layer (Figure 1). MSCs effectively reduced surgically induced stenosis in rat carotids 30 days following systemic intravenous administration [56].

The lumen area in MSC-treated carotids was 36% greater than in control arteries and inward remodeling was limited in MSC-treated carotids. The injected MSCs caused the reduction in the mRNA expression levels of inflammation related genes IL-1β and MCP-1 after 7 days of MSC treatment. The levels of TGF-β, an immunosuppressive cytokine, increased at injury site in MSC-treated arteries, which could have induced a suppression of local immune response. In this study, MSC did not contribute to endothelial cell recovery and the improvement was mostly attributed to the immunomodulatory and paracrine role of MSCs [56]. A follow up study showed increased expression of VEGF at the injury site and decrease in the serum levels of inflammation related molecules, CXCL1, ICAM-1, L-Selectin, LIX/CXCL5 and CCL5/RANTES, possibly leading to the rapid repair of the injured vessel wall. The immuno-modulatory activity of MSCs was confirmed by the decreased expression of Toll like receptor (TLR)2 and TLR4 in injured carotids [57].

The paracrine effect of MSCs was also observed in another study where MSCs inhibited neointimal hyperplasia in a carotid artery ligation model by decreasing the initial inflammation at the lesion area. There was decreased macrophage infiltration into the ligated artery, as well as, decreased serum levels of MCP-1 and 3, seven days after the administration of MSCs [58]. However, here also there was no indication of MSC incorporation into the lesion. This lead the authors to hypothesize that the effect might also be due to the mobilization of endogenous stem cells from the bone marrow to the peripheral circulation, and the mobilized cells accumulate at the area of vascular injury which may modulate the systemic and local inflammatory responses [58]. Similar results were obtained in a wire injury model of rat femoral artery where the adipose derived MSCs potently and significantly inhibited neointimal formation without being integrated in the endothelial layer. In vitro studies demonstrated the cells release Ang-1, which might have stimulated the migration of endothelial cells in situ and the repair of the endothelial layer in vivo [59]. MSCs also produce NO which is involved in the suppression of T cells as well as in vascular remodeling [60].

Vascular injury can increase the levels of blood VEGF, GM-CSF, SDF-1α, SCF concentrations which can mobilize MSCs from the bone marrow into the circulation [61]. The mobilized cells accumulate at the area of vascular injury which may modulate the systemic and local inflammatory responses. The inflammatory cytokines, tumor necrosis factor (TNF)-α, and IL-6 released at the injury site mobilized endogenous bone-marrow-derived cells which played an important role in the process of neointimal hyperplasia after vascular injury [62]. Another important early target for the paracrine influence of exogenous MSC transfer are the resident stem cells [63]. Pericyte-like cells or MSCs from the adventitia are reported to contribute to restenosis, and these cells are found to be higher in injured arteries [64]. However, whether paracrine factors influence the proliferation or differentiation of these cells war-rants further investigation.

Neovascularization by MSC paracrine factors in Peripheral Artery Disease

Peripheral vascular disease of the lower extremities comprises a clinical spectrum that extends from asymptomatic disease to presentation with critical limb ischemia (CLI) [65]. An impairment in function of endothelium and inhibition of neovascularization are the main

factors for development of peripheral artery disease [66]. Neovascularization is a complex process, involving degradation of the basement membrane by proteases, proliferation and migration of EPCs, lumen formation, basement membrane assembly, recruitment of pericytes or vascular smooth muscle cells, vascular maturation and finally blood flow. These processes are mediated by several growth factors like VEGF, FGF and PDGF [67].

Accordingly, the concept of transplanting MSCs, capable of replenishing the ischemic tissue with new vessels and preventing ischemic tissue damage, was visualized by many researchers as a potent therapeutic modality [68,69]. In the first clinical trial using intramuscular transplantation of bone marrow mononuclear cells in patients with hind limb ischemia, beneficial effects were observed 24th week post cell injection [68]. Kinnaird et al. were the first to demonstrate the potential of stromal cells to augment collateral flow to ischemic tissue through paracrine mechanisms [70]. In this study, MSC injection into the adductor muscles of the ischemic hindlimb improved limb function, reduced the incidence of auto-amputation, and attenuated muscle atrophy and fibrosis. However, the injected MSCs were seen dispersed between muscle fibers and were not seen incorporated into mature collaterals. Injection of MSCs increased adductor muscle levels of FGF2 and VEGF protein compared with controls and colocalization of VEGF and transplanted MSCs were observed. In vitro studies showed the release of VEGF, FGF2, placental growth factor, and MCP-1 in MSC conditioned media. In another study, systemic infusion of bone marrow derived cells enriched for high aldehyde enzyme activity induced transient homing to the ischemic region and the transplanted cells triggered collateral vessel formation or stabilization, resulting in improved perfusion. However, these transplanted cells did not permanently integrate into limb vasculature, suggesting the role of paracrine factors released by the ALDHhi cells in increasing blood vessel density and improving limb perfusion [71].

One of the novel approaches is the perivascular delivery of genetically modified cell encapsulated beads to enhance functional neovascularization in ischemic femoral artery in an immunocompetent mouse model [72]. Glucagon-like peptide-1 (GLP-1) is a major incretin hormone that functions to regulate blood glucose homeostasis, and also has also been demonstrated to exhibit anti-apototic and pro-angiogenic effects. However, the short half-life of GLP-1 in vivo limits its clinical usefulness. MSCs genetically modified with GLP-1 were encapsulated to miniature beads made of a permeable biocompatible alginate which allowed diffusion of inherent paracrine factors like VEGF. The cell viability was ensured by inherent shell to core ratio. Cell beads in the perivascular space enhanced collateralization and neo-angiogenesis through secretion of a variety of paracrine factors that may act directly or indirectly by inducing the upregulation of pro-angiogenic chemokines and downregulation of anti-angiogenic genes. There was a marked increase in genes associated with angiogenesis, such as endoglin, VEGF-A, sphingosine kinase 1, Ang 4, IL-8, and heparanase, in ischemic muscles of microbead-MSC and microbead-MSC-GLP treated mice compared with the control. The expression of several anti-angiogenic genes, such as tissue inhibitor of metalloproteinase 2, tissue inhibitor of metalloproteinase 3, and thrombospondin 2, and proinflammatory genes associated with T lymphocytes induction of MSC apoptosis, including interferon-γ and TNF-α decreased. Investigation of interaction of differentially regulated genes indicated that transplantation of micro bead-MSCs interferes with a molecular network centered on VEGF-A, IL-6, interferon-γ, and TNF-α, leading to

changes in the balance between angiogenic mediators (the chemokines CXCL3 and CCL2/MCP1, the membrane glycoprotein endoglin, and the S1P activator sphingosine kinase 1, stabilizers of vascular growth (Ang 4 and thrombospondin 1), and enzymes implicated in activation (heparanase) or inhibition (tissue inhibitor of metalloproteinases) of extracellular matrix degradation. There was reduced expression of β2-microglobulin, which is a biomarker of peripheral vascular disease after the treatment. This study represents a significant improvement over conventional intramuscular cell therapy, which disperses cells and therapeutic mediators in an unpredictable manner. Encapsulation of human MSCs prevents rapid inactivation of transplanted cells providing a potential means of treating patients with severely debilitating limb ischemia with a single off-the shelf cell product [72].

MSC paracrine factors in Myocardial Infarction

Vascular repair mechanisms constitute an important means to reduce myocardial injury and to improve cardiac function following MI. MSCs are known to secrete soluble paracrine factors that have been postulated to contribute to endogenous cardiomyogenesis and angiogenesis. Proposed mechanisms of paracrine action of MSCs in MI include the enhancement/modulation of neovascularization, cytoprotection, inhibition of fibrosis and stimulation of differentiation of tissue specific stem cells. The cardiovascular repair is modulated by the signaling and growth factors released by MSCs, SDF-1 (SDF-1/CXCL12), VEGF, HGF, IGF-1, FGF-2, hypoxia inducible factor-1 (HIF-1α), Ang-1, MCP-1, IL-1 and 6, placental growth factor, plasminogen activator, TNF-α [73]. MSCs significantly expressed Hypoxia-inducible factor 1-alpha (HIF-1α), and increased the capillary density, angiogenic cytokine levels, Ang-1 and VEGF-A levels in mouse MI model [74]. There was also an increased expression of chemotactic factor granulocyte chemotactic protein-2 (GCP-2), neutrophil-activating protein-2 (NAP-2) as well as the chemokine receptors CCR2, CCR3 and CCR5 demonstrating the chemotactic potential of these cells. In another study, the presence of angiogenic (VEGF, endothelin, and epiregulin), anti-apoptotic (Galectin-3, Smad-5, sRFP-1, and sRFP-4) and anti-remodeling factors (TIMP-1 and TIMP-2) were detected in the tissue seven days following the intracoronary infusion of concentrated MSC-derived growth factors [75].

Genetically modified bone marrow–derived MSCs overexpressing the Akt1 gene, release paracrine factors that exert cytoprotective effects on cardiomyocytes exposed to hypoxia [76]. Concentrated conditioned medium was injected into five different sites in the heart at the infarct border zone 30 min after left coronary artery occlusion in rodent model. A marked antiapoptotic effect was observed after the injection of Akt concentrated medium, yielding a 69% reduction of TUNEL-positive cardiomyocytes as compared with the control concentrated medium group. Several genes coding for VEGF, FGF-2, HGF, IGF-I, secreted by the MSCs and known to exert pro-angiogenic, cardioprotective, and inotropic actions, are upregulated in hypoxic Akt-MSCs, and these factors could mediate the functional improvements observed [77]. Secreted frizzled related protein2 was identified as a major paracrine mediator of Akt-MSCs myocardial survival and reparative effects, and it is known to modulate cell function through wnt signaling [78]. Hypoxia and Akt induced Stem cell Factor (HASF) is another novel factor secreted by MSCs which promotes cardiomyocyte proliferation and also have a cytoprotective effect on ischemia induced cardiomyocyte death.

HASF induces cardiomyocyte proliferation via a phosphoinositide 3-kinase–protein kinase B–cycle-dependent kinase 7 pathway [79]. The cytoprotective effect was regulated through the activation of protein kinase C ε (PKCε) [80].

In a rat model of MI, delivery of MSCs overexpressed for SDF-1α or CXCL12, chemotactic factor for stem cells, enhanced MSC survival and paracrine function in the transplanted area, promoted angiogenesis in the infarcted hearts, and transcriptionally inhibited type I and III collagen, MMP-2, and MMP-9 gene expression. HGF was one of the paracrine factors involved in the process [81]. The adenovirus mediated overexpression of cGMP-dependent protein kinase type 1 α (PKG1 α) transgene, a critical mediator of cGMP signaling in cardiovascular system in MSCs, significantly enhanced their resistance to ischemic stress in the infarcted heart via an antiapoptotic mechanism and a coordinated increase of angiogenesis by paracrine factors in ischemic hearts [82]. mRNA expression of pro-survival and angiogenic factors including HGF, FGF2, SDF-1 and Ang-1, and protein expression of antiapoptosis proteins, pAkt, pGSK3β and Bcl-2 significantly increased before and after oxygen glucose deprivation. The secreted paracrine factors were prominent within 72 h after transplantation and affected the cellular milieu for stem cell retention and survival. At 4 weeks after transplantation, compared to the control group, PKG1α MSCs group showed increased blood vessel density in infarct and periinfarct areas.

Another possible mechanism in which MSC exerts it paracrine effect is through mobilization of endogenous cardiac stem cells (CSCs) to the injured site. MSC-conditioned medium induced increased migration of resident stem cells from human cardiac tissue and PDGF receptor α played an important role in the migration process [83]. The results from this study definitely designate the relevance for future in vivo models examining the migrating behavior of cardiac progenitors after administration of MSC-conditioned medium, and growth factors like PDGF-AA.

MSC paracrine factors can also induce transdifferentiation of MSCs to cardiomyocytes which can enhance cardiac function. Transplantation of MSC engineered with Wnt protein, Wnt11 into the border of ischemic myocardium contributed to cardiac function recovery from acute ischemic injury and protected cardiomyocytes at risk in rat model via paracrine manner. Identification of the factors responsible for MSCWnt11-mediated cardioprotection showed significant increase in TGFβ2. Furthermore, function-blocking antibodies to Wnt11 and TGFβ2 compromised the cardioprotective effect of cardiomyocytes differentiated from MSC-Wnt11 [84]. In vitro studies showed significant increase in the potential trans-differentiation of Wnt11-MSC into cardiac phenotype after co-culture with native cardiomyocytes for 7 days. The differentiation rate was significantly increased from 17.1% in MSCNull to 26.1% in MSCWnt11. A possible mechanism stated was the Wnt11 upregulation of GATA-4 in MSC, which has been documented to upregulate the expression of growth factors, prosurvival factors (eg, Bcl-2), and several heat shock proteins, and cytokines any of these could have mediated the cytoprotective effects [84].

The therapeutic benefits of MSCs could be enhanced in vivo by encapsulating them in selectively permeable biocompatible microbeads before delivery (Figure 2) [51,52]. Similar to the study previously described in peripheral artery desease [72], GLP-1 modified MSC

encapsulated in selectively permeable biocompatible microbeads were used to enhance therapeutic benefits of MSCs in MI [85,86]. Approximately 3500 human MSCs were embedded alginate beads of 600μm diameter. The beads were selectively permeable which allowed passage of oxygen and nutrients to enable MSC survival while also protecting MSCs from host immune response [85]. Approximately 1900 cell beads were infused via a single intracoronary injection to coronary artery branches of a porcine model of early ischemic LV dysfunction. The cell beads provided a prolonged supply of GLP-1 and paracrine stem cell factors, which improved LV function and reduced epicardial infarct size. This was associated with increased angiogenesis and an altered remodeling response. However, an increased initial inflammatory response was observed which could partly be explained by human factors being detected by the host animal, creating a xenogenic response. The use of collagen tissue patches incorporated with MSCs is another effective solution to treat chronic MI. MSC-patch can promote reverse remodeling of the infarcted area through paracrine mechanisms, and a functional myo-cardial improvement [87].

Strategies to enhance the release of paracrine factors by the MSCs

Genetic modification

Genetic modification of MSCs improve survival, metabolic characteristics, contractility, proliferative capacity, or differentiation and also enhance the release of paracrine factors [88]. Heat-shock proteins (Hsps) have been shown to render cardioprotection from stress-induced injury [89]. MSCs adenovirally transduced with Hsp-20 showed enhanced release of growth factors VEGF, FGF-2, and IGF-1 on exposure to oxidative stress [90]. There was an 80% increase in the secretion of VEGF, 55% increase of FGF-2 and a 39% increase of IGF-1 in Hsp20-MSCs compared to that of control GFP-modified MSCs upon H2O2 treatment for 5 hours. Therefore, transplantation of Hsp20-MSCs could provide adequate magnitude and duration of VEGF, FGF-2, and IGF-1 release in the ischemic myocardium, which would provide cardio protective effects and induce revascularization in ischemic heart.

Rat MSCs retrovirally transduced to overexpress the prosurvival gene murine Akt1 (MSC-Akt), were more resistant to apoptosis and improved cardiac function when injected into ischemic myocardium [91]. MSC-Akt improved early repair despite transient engraftment, low levels of cellular fusion, and differentiation. Early paracrine mechanisms mediated by MSCs were responsible for enhancing the survival of existing myocytes and Akt could alter the secretion of various cytokines and growth factors. Analysis of expression profiles of MSC-Akt versus MSC-GFP has observed an enhanced expression of numerous secreted proteins having cardioprotective and/or angiogenic properties especially under hypoxic conditions [92]. VEGF overexpressed MSCs using adenoviral vector contributed to myocardial tissue repair by releasing SDF-1α which then induced mobilization and migration of cardiac stem cells (CSCs) into infarcted areas [27]. The differentiation of CSCs to endothelial cells by the stimulation of VEGF and SDF-1α lead to the enhanced angiogenesis in infarcted myocardium. The newly formed blood vessels and stem cells-differentiated cardiomyocytes worked together to restore the damaged myocardial tissues and improve the function of injured heart.

MSCs transiently transfected with a Notch1 intracellular domain (NICD)-expressing plasmid secreted increased levels of angiogenin, Ang-2, Heparin-binding epidermal growth factor-like growth factor (HB-EGF), and VEGF levels compared to the corresponding parental MSCs [93]. The cell-secreted angiogenic factors promoted several 9 aspects of angiogenesis, which likely contributed to promoting recovery in the injured brain. MSCs lentivirally transduced with GM-CSF produced larger vessels in mouse ischemic limbs when compared to that of MSCs alone, which is an important long-term advantage since larger vessels are more efficient in the reperfusion of ischemic tissues physiologically [94].

Hypoxia

Hypoxia has been shown to enhance paracrine release of angiogenic factors from MSCs which can improve its therapeutic benefit both in vitro and in vivo. Conditioned media from hypoxic MSCs produce higher amounts of antiapoptotic and angiogenic factors, such as IL-6, VEGF, FGF2, IGF-1, or HGF [95–97]. Conditioned media from hypoxic MSCs reduced apoptosis and enhanced tube formation in human endothelial cells when compared to one from normoxic MSCs. The hypoxic MSC-conditioned medium activated the PI3K-Akt pathway in endothelial cells and thereby inhibited hypoxia-induced endothelial apoptosis and increased angiogenesis of endothelial cells in vitro [98].

Hypoxia preconditioned MSCs had a greater capacity to engraft ischemic tissue and induce neoangiogenesis in a murine limb ischemia model. Wnt4 signaling was an important factor in this hypoxia induced tissue regeneration, as well as vessel formation and stabilization [99]. MSC cultured in hypoxia induced expression of cMet, the major receptor for HGF, and enhanced cMet signaling, suggesting a mechanism by which hypoxia enhances the homing and tissue-repairing capacity of MSC [100].

In vitro hypoxic preconditioning enhanced the capacity of MSCs to repair infarcted myocardium. There was increased expression of pro-survival and pro-angiogenic factors including hypoxia-inducible factor 1, Ang-1, VEGF, Flk-1, erythropoietin, Bcl-2, and Bcl-xL in mice MI model following hypoxic preconditioning [101]. In another study, the conditioned media from hypoxic MSCs when tested in a rodent model of MI caused a significant reduction in left ventricular end-diastolic pressure and improvement of cardiac contractility and relaxation compared to non-conditioned medium 19-21 days after medium injection [102].

Preconditioning with TLR-agonists

Adult MSCs express functional Toll-like receptors (TLRs), including TLR (1–9) except TLR8 [103]. MSCs employed in therapy can be potentially exposed to TLR ligands, which may modulate their therapeutic potential in vivo. TLR3 activation augmented the release of trophic factors by MSCs and enhanced their therapeutic potency [104]. MSC-preconditioning using 4 μg/ml TLR3 agonist, Polyinosinic-polycytidylic acid (poly(I:C)) for 24 h, induced IL-6, IL-10, IL-11, LIF, VEGF, SDF1, and HGF without induction of the inflammatory cytokines. Preconditioning of MSCs with LPS (TLR4 agonist) could promote expression of cytokines, such as FGF2, IGF-1, and HGF [105]. TLR 2/6 agonist, Macrophage-activating lipopeptide of 2kDa (MALP-2) could be used to stimulate MSCs in

order to promote angiogenesis in vitro and in vivo [106]. MALP-2 is a bacterial lipopeptide which naturally occurred in mycoplasma species and is recognized by a heterodimer of TLR2 and TLR6 [107]. Conditioned medium from MALP-2-stimulated human MSCs enhanced migration, proliferation and tube formation of endothelial cells in vitro. Among the strongest induced factors identified were VEGF, GM-CSF, MCP-3, MCP-4 and platelet endothelial cell adhesion molecule (PECAM)-1 [106].

Preconditioning with Phospholipids

Lysophosphatidic acid (LPA, 1-acyl-2-lyso-sn-glycero-3-phosphate), as a simple endogenous bioactive phospholipid, is present in most tissues and biological fluids at nM to μM concentrations and has diverse effects on cell function [108,109]. LPA promotes VEGF secretion by human MSCs and this effect is enhanced under hypoxia, making LPA a natural target for stimulating trophic factor secretion and endothelial cell recruitment in ischemic defects [110]. LPA upregulated angiogenic growth factor production by MSCs under two- and three-dimensional in vitro models of serum deprivation and hypoxia (SD/H), and that these factors significantly enhanced endothelial cell migration [110]. Transplantation of LPA treated MSCs increased capillary density in the myocardium and improved myocardial function in the ischemic heart. In vitro experiments showed that LPA-induced VEGF secretion of MSCs occurred at the post-transcriptional levels and was mediated through the classical ER/Golgi-dependent protein secretory route. LPA also increased ORP150, a chaperone in ER, protein expression [108].

Improved cell selection methods

Selective enrichment of immature mesenchymal precursor cells (MPC) in the culture based on their expression of cell surface antigens is another strategy to improve the release of growth factors. Prospective immunoselection of STRO-1+ or STRO-3+ MPCs have reported to be advantageous in maintaining an immature mesenchymal precursor population during ex vivo expansion [111,112]. Enrichment for STRO-1 increased the expression of cardiovascular-relevant cytokines and enhanced trophic activity [111]. STRO-1-MPC exhibited higher expression of CXCL12 and HGF, which are crucial in endothelial tube formation and cardiac cell proliferation. The culture-expanded progeny of STRO-3+ MPCs demonstrated therapeutic potential in the post-MI heart, attributable at least in part, to the secretion of pro-angiogenic and cardioprotective factors [112]. Compared to MSCs conventionally isolated by plastic adherence, STRO-3+ MPCs demonstrated increased proliferative capacity during culture expansion, expressed higher levels of early ‘stem cell’ markers and various pro-angiogenic and cardioprotective cytokines, and exhibited greater trilineage developmental efficiency [112].

Improved cell culture methods

The culturing of hMSCs as three-dimensional cellular aggregates provides a method to concentrate proangiogenic factors secreted by hMSCs and allows for reduction of serum concentration in conditioned medium [113]. Analysis of paracrine factors in medium conditioned by a monolayer of hMSCs and hMSC spheroids demonstrated 5–20 times

increase in the concentrations of VEGF, FGF-2, angiogenin, procathepsin B, IL-11, and bone morphogenic protein 2 in medium conditioned by hMSC spheroids. The conditioned medium by hMSC spheroids were more effective in stimulation of umbilical vein endothelial cell proliferation, migration, and basement membrane invasion than medium conditioned by a monolayer of hMSCs. This medium also promoted endothelial cell survival in vitro.

Biomaterial based approach

The use of instructive biomaterial-based scaffolds with cues to direct increased secretion of paracrine factors is another approach. This method can potentially mitigate the use of preconditioning strategies, as well as, the prolonged in vitro culture required to generate genetically modified cells that over express growth factors. Biocompatible matrices like alginate and fibrin gels have been incorporated with proteins or peptides to promote cell adhesion and to instruct cell phenotype to enhance the release of trophic factors. Conjugation of the proangiogenic tripeptide, Glycine-Histidine-Lysine (GHK), to alginate hydrogels significantly increased MSC trophic factor secretion [114]. The localized presentation of GHK would enhance trophic factor secretion by MSC and that this signal was covalently incorporated into alginate gels to drive production of these factors by entrapped cells [114]. Fibrin gels incorporated with Lysophosphatidic Acid (LPA) were used as carriers of MSCs to enhance revascularization in hindlimb ischemia. Adipose derived MSCs responded to LPA when incorporated to fibrin gels without any preconditioning regimens [110].

The diverse microenvironments for example, the substrate elasticity also influences the secretory profile of MSC-derived trophic factors. IL-8 was up-regulated as much as 90-fold in MSCs cultured for 2 days on hard substrates, whereas levels were consistently low on soft substrates [115]. Tailoring the composition of the scaffold has also an influence on the trophic factor release. Controlling the mass ratio of hydoxyapatite to polymer Poly(Lactide-Co-Glycolide) (PLG) composite scaffold could modulate the trophic factor secretion by human MSCs [116]. Eight weeks after implantation, scaffolds with higher HA:PLG ratios exhibited greater vascularization and more mineralized tissue.

Conclusion

There is mounting evidence that paracrine factors released by the MSCs contribute to the vascular repair and regeneration. The use of MSC conditioned media which contains a cocktail of growth factors and cytokines provides an easy alternative to cell-based therapy. Several concerns regarding the application of MSCs in vasculature, such as the possibility of embolization of MSCs in the small vessels, smooth muscle cell differentiation of the MSCs, chance of pro-fibrotic effect on the vessel wall, and their ability to promote deleterious expansion of adventitial vasa vasorum due to angiogenesis, could be avoided by the application of direct application of stem cell paracrine factors in the vessel wall. The use of preconditioned, genetically engineered and physically modulated MSCs will enhance the release of the growth factors and significantly advance this therapy. However, care should be taken that the MSCs also secrete inflammatory cytokines such as TNF-α and IL-6, which can be deleterious to the tissue. The micro-environment in which the MSC factors are

injected also regulates the activity of these factors. The nature of the MSC secretome from MSCs of different sources and different passage should be extensively characterized before clinical use. Nevertheless, this cell free therapy holds great promise in that the identification of key molecules in the MSC-CM that mediate different aspects of angiogenesis will allow development of efficient drug therapies to treat ischemic diseases in the future.

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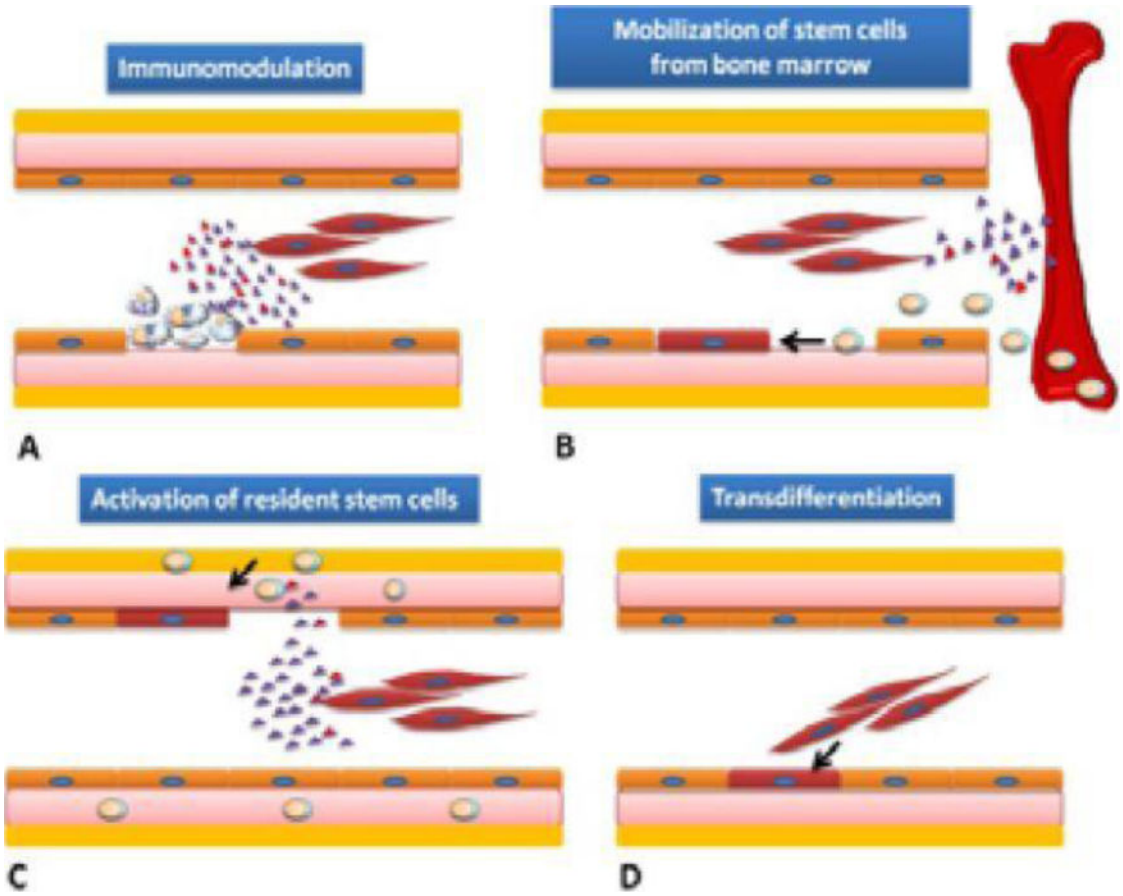

Figure 1.

Mechanisms of vascular repair and regeneration by MSCs during restenosis: A) Immunomodulation by the paracrine factors released at the injury site B) Mobilization of stem cells from the bone marrow by the paracrine factors released by the MSCs C) Activation of resident stem cells in the blood vessel by the paracrine factors released by MSCs D) Transdifferentiation of MSCs to endothelial cells.

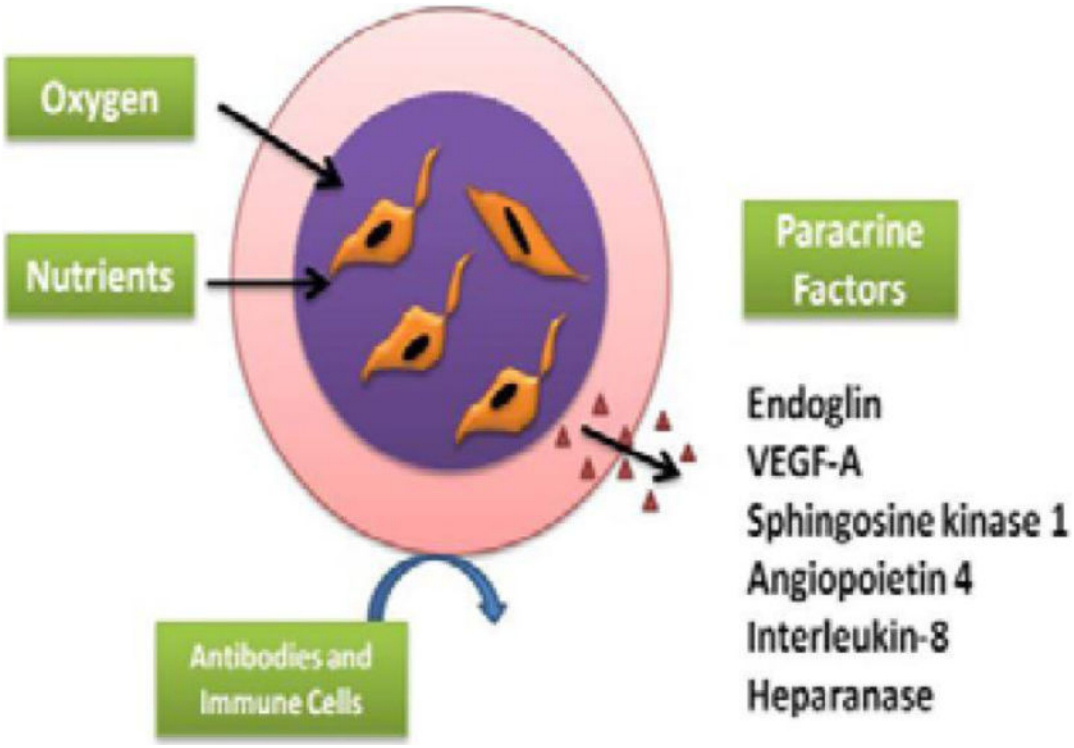

Figure 2.
Microencapsulation approach to enhance therapeutic angiogenesis: