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# The Angiogenic Paracrine Potential of Mesenchymal Stem Cells

*Jafar Rezaie, Morteza Heidarzadeh, Mehdi Hassanpour, Hassan Amini, Elhameh Shokrollahi, Mahdi Ahmadi and Reza Rahbarghazi*

## Abstract

Tissue engineering and regenerative medicine are branches of biomedical sciences that facilitate the use of cells and biocompatible scaffolds in favor of tissue restoration. In this regard, restoration and maintenance of angiogenesis and blood supplementation could be an effective strategy for injured tissue removal, accelerating healing rate, and successful transplantation of cells and scaffolds into target sites. It has been elucidated that mesenchymal stem cells have the potency to promote angiogenesis via paracrine activity and trans-differentiation into the endothelial lineage. In this chapter, we highlighted the paracrine property of mesenchymal stem cells to modulate angiogenesis in the target tissues.

**Keywords:** mesenchymal stem cells, angiogenesis, paracrine activity, exosomes

## 1. Introduction

Angiogenesis, termed as neovascularization, is defined as de novo vascularization from the pre-existing vascular network and activated in response to numerous pathological and physiological stimuli, playing critical roles during development and tissue repair [1]. Recent advances in the field of stem cell research, notably MSCs, have opened new horizons to human medicine in the promotion of angiogenesis and restoration and salvage of ischemic tissues [2]. MSCs actively participate in angiogenesis via direct differentiation, cell contact interaction with endothelial lineage, and releasing pro-angiogenic factors via a paracrine manner [3]. Due to the low survival and differentiation rate of MSCs posttransplantation into ischemic microenvironment, it is proposed that the paracrine activity is the principal mechanism for the therapeutic outcome [4]. It has been well-established that stem cell-secreted growth factors are responsible for, at least in part, therapeutic effects. As a matter of fact, MSC-derived secretome is thought to be a suitable alternative therapeutic modality to MSCs posttransplantation. At present, the underlying mechanisms by which MSC secretomes contribute to tissue healing and angiogenesis are not fully addressed and many efforts are needed to fill knowledge gaps by experimental animal research and clinical trials prior to application to human medicine [5, 6]. Paracrine factors could increase the blood supplement of damaged tissues via the activation and recruitment of resident/circulating stem cells and progenitor cells [7, 8]. Several experiments detected the pro-angiogenic

capacity of MSCs isolated from different sources [9, 10]. **Table 1** ELISA and liquid-chip assays of cytokine content of umbilical cord MSCs revealed several angiogenesis factors, including interleukin-8 (IL-8), insulin-like growth factor 1 (IGF-1), and vascular endothelial growth factor (VEGF) compared to mature cell types such as fibroblasts. These pro-angiogenic factors are able to form vascular networks and increase the migration of endothelial lineage in vitro [51]. In addition to the secretion of angiogenic factors by MSCs, it was revealed that various factors existing in secretome could activate the angiogenic behavior in endothelial cells (ECs). For instance, equine peripheral blood MSC angiocrine was found to stimulate endothelial functional behavior by the induction of VEGF-A signaling pathway via several factors such as endothelin-1, IL-8, platelet-derived growth factor-AA (PDGF-AA), and IGF-2 [52]. Due to the variety of factors released by MSCs such as VEGF, monocyte chemoattractant protein-1 (MCP-1), and IL-6, an increased angiogenesis rate was observed in the mouse model of hindlimb ischemia, and even the combination of VEGF, MCP-1, and IL-6 could be served as a commercial cocktail for the promotion of angiogenesis either in vivo or in vitro [53]. In addition to the existence of the pro-angiogenic factor in MSC secretome, some authorities, however, showed the anti-angiogenic properties of these cells (**Table 2**) [67]. In some circumstances, the dual effect of a distinct factor was proved related to angiogenesis status. For example, in VEGF-free condition, the attachment of angiopoietin-2 (Ang-2) to receptor tyrosine kinase (RTK), namely Tie-2, promotes vascular destabilization and regression by reduction of pericyte-EC interaction, while in normal condition Ang-2 could increase EC migration and tip cell formation required for neovascularization [68]. Commensurate with these comments, one could hypothesize that the dynamic balance of MSC secretome, cell source, purity, and preconditioning could predetermine the pro- and/or anti-angiogenic property of MSCs [67].

By modulating distinct signaling pathway/s inside the MSCs, cell bioactivity would be induced in favor of neovascularization. For instance, it was shown that the activation of sonic hedgehog (Shh) factor in Wharton's jelly-derived MSCs (WJ-MSCs) induced the production of pro-angiogenic factors such as angiogenin, angiopoietin-1, activin A, matrix metalloproteinase-9 (MMP-9), granulocyte-macrophage colony-stimulating factor, and urokinase-type plasminogen activator, indicating WJ-MSCs an ideal cell source for the induction of vascularization [69]. An experiment conducted by Matluobi et al. showed an enhanced vascular formation capacity of human MSCs after treatment with carvacrol evaluated by chicken chorioallantoic membrane angiogenesis assay. The carvacrol-treated MSCs tended to trans-differentiate into endothelial lineage by the expression of VWF and VE-cadherin [70]. MSCs have the ability to adapt themselves with environmental condition increasing regenerative potential in different conditions [71]. Maintaining the MSC cross talk with other cells is required for cell hemostasis, stemness feature, and regenerative potential in the distinct niche. For example, the normal bioactivity of Hox gene, *Abdominal-B*, seems to be essential in *Drosophila* cystic stem cells to obtain multipotentiality [72].

Regarding issues related to isolation protocols and stem cell proliferation rate, a careful selection is essential for high-throughput results. Vizoso et al. demonstrated large-scale secretome production and release of a vast array of bioactive factors in human uterine cervical stem cells with considerable advantages over MSCs from other tissues for research and clinical application [73].

The emergence of some conditions could change the trans-differentiation capacity of MSCs into distinct phenotypes. In the case of the vicious cycle of abnormal placental development in intrauterine growth restriction, placental mesenchymal stromal cells lose angiogenic potential while acquiring adipogenic capacity which is coincided with a metabolic shift from aerobic to anaerobic state [71]. It seems that

<b>Factor</b>	<b>Amniotic fluid/ bone marrow MSCs</b>	<b>Function</b>
Angiogenin	+/+	A pancreatic ribonuclease, known as ribonuclease 5, which induces vascularization [11]
Angiopoietin-1	+/+	Activates TEK/TIE2 receptor; promotes angiogenic processes, endothelial cell survival, migration, proliferation, and stabilization; and during embryogenesis has a role in heart development [12]
Angiopoietin-2	+/-	Binds to TEK/TIE2, in the presence of VEGF and Ang-2 and promotes neovascularization [13, 14]
Angiopoietin-4	+/+	Binds to TEK/TIE2, modulating ANGPT1 signaling, can induce tyrosine phosphorylation of TEK/TIE2, and promotes endothelial cell survival, migration, and angiogenesis [15]
Amphiregulin	+/-	An EGF-like ligand that binds to the EGFR, enhanced lymphangiogenesis, and stimulates the growth of normal epithelial cells [16]
Artemin	+/-	Binds for the GFR-alpha-3-RET and GFR-alpha-1-RET receptor and promotes angiogenesis [17]
Tissue factor	+/-	Stimulates PDGF receptor signaling pathway, angiogenesis, endothelial cell migration, chemotaxis and proliferation, and coagulation factor III/CD142; improves transcription of VEGF; and reduces transcription of the thrombospondins [18]
CXCL16	+/+	Encourages a chemotactic response, pro-angiogenic [19]
DPPIV	+/-	A membrane-bound oligopeptidase acting on and modulating the pro-angiogenic chemokine CXCL12 [20]
Epidermal growth factor	+/-	Encourages the growth of epithelial tissues, is anti-apoptotic, induces lymphangiogenesis, and improves MSC survival [21]
EG-VEGF	+/-	Also called Prokineticin 1. Binds to PROKR1 and PROKR2, pro-angiogenic [22]
Endothelin-1	+/+	Derived from the endothelium with vasoconstrictor and angiogenic effects, prolymphoangiogenic [23]
Endoglin	+/-	Also called CD 105. Modulates TGF-β1 and β3 responses, vascular development, and angiogenic effects [24]
FGF-7	+/+	Has positive effects on cell proliferation, migration and division, chemotaxis, and arteriogenesis [25]
Acidic FGF/FGF-1	+/-	Binds to for FGFR1 and integrins and induces angiogenesis [26]
Basic FGF/FGF-2	+/-	Ligand for FGFR1, FGFR2, FGFR3, and FGFR4, Vascular regeneration; role in cell migration and proliferation involved in angiogenesis, stimulates arteriogenesis [27]
FGF-4	+/-	Has positive effects in MSC proliferation, pro-angiogenic [28]
GDNF	+/-	Has positive effects in angiogenesis [29]
GM-CSF	+/-	Has positive effects in angiogenesis [30]
Heparanase	+/+	Has positive effects in angiogenesis [31]
Heparin binding-EGF	+/+	Has positive effects in angiogenesis [32]
Hepatocyte growth factor	+/-	Has positive effects in angiogenesis [33]
HIF-1α	+/+	Functions as a master transcriptional regulator of the adaptive response to hypoxia and influences cell metabolism, cell survival, and angiogenesis [34]

Factor	Amniotic fluid/ bone marrow MSCs	Function
IL-1 $\beta$	+/-	Has positive effects in angiogenesis and lymphangiogenesis [35]
IL-6	+/-	A potent pro-angiogenic cytokine which stimulates endothelial cell and smooth muscle cell proliferation and migration and promotes neovascularization [36]
IL-8	+/-	Has a role of pro-angiogenic factor [37]
Leptin	+/-	Stimulates vessel formation [38]
MCP-1	+/-	CCL2. Induces stabilization of new vessels [39]
MIP-1 $\alpha$	+/-	CCL3. Induces vessel formation
MMP-8	+/-	Known as collagenase 2. Breaks collagen types I, II, and III and has positive effects on angiogenesis [40]
MMP-9	+/-	Called as gelatinase B. Breaks both collagens and gelatins and has positive effects on angiogenesis [41]
NRG1- $\beta$ 1	+/-	Promotes angiogenesis and arteriogenesis [42]
Pentraxin-3 (PTX3)	+/+	Has a role of a pro-angiogenic agent [43]
PD-ECGF	+/-	Stimulates angiogenesis [44]
PDGF-AA	+/+	Has positive effects on MSC proliferation and stimulates angiogenesis [45]
PDGF-AB/ PDGF-BB	+/-	Induces neovascularization and arteriogenesis [27]
Persefin	+/-	Induces angiogenesis [3]
PlGF	+/+	Has a role of a pro-angiogenic factor [46]
Prolactin	+/-	Has a role of a pro-angiogenic factor in intact form [47]
Sphingosine kinase 1	+/+	Promotes angiogenesis [48]
SDF-1 $\alpha$	-/+	An important chemotactic factor for progenitor cells. Stimulates stem cell migration, adhesion, and homing [3]
TGF- $\beta$ 1	+/-	Promotes angiogenesis at least in part via the secretion of the survival factors TGF- $\alpha$ and VEGF [3]
uPA	+/+	Promotes endothelial cell proliferation and migration and has positive effects in vascular network formation [49]
VEGF	+/+	Promotes angiogenesis [50]
VEGF-C	+/-	Promotes lymphangiogenesis [50]

**Table 1.**

*Comparison of angiogenic paracrine factors secreted by MSCs from amniotic fluid and bone marrow.*

external environmental influence could alter the therapeutic potency of MSCs by rendering epigenetic marks associated with cell differentiation capacity [74]. In support of this claim, Rezaie and co-workers found a decrease of angiogenic human MSC potential after exposure to diabetic sera. The diabetic MSCs showed a declined migration capacity by suppressing the transcription of MMP-2, MMP-9, and CXCR-4 and aborted the secretion of Ang-1, Ang-2, and VEGF [75]. The expression of CXC chemokine receptors such as CXCR-1, CXCR-2, and CXCR-4 was found to accelerate and direct MSC migration in response to the chemokine gradients. A blockade of CXCR chemokine such as CXCL6 had potential to abrogate cardiac stem cell migration and motility [76].

Factor	Amniotic fluid/ bone marrow (MSCs)	Function
Angiopoietin-2	+/-	Binds to TEK/TIE2 and induces endothelial cell apoptosis in the absence of VEGF [54, 55]
Angiostatin	+/-	Angiogenic inhibitor. Acts as an inhibitor of endothelial cell proliferation and migration [56]
Endostatin	+/-	Acts as inhibitor of endothelial cell proliferation and migration and angiogenesis and induces endothelial apoptosis [56]
TGF- $\beta$ 1	+/-	Angiogenic inhibitor [57]
Platelet factor 4 (PF4)	+/-	Angiogenic inhibitor [58]
Serpin B5	+/-	Maspin. A member of the serine protease inhibitor family and negative regulator of angiogenesis [59]
Serpin E1	+/+	Serine protease inhibitor; inhibition of angiogenesis; inhibitor of uPA; preserves the vascular integrity [60]
Serpin F1	+/+	Serine protease inhibitor, inhibition of angiogenesis [61]
TIMP-1	+/+	Angiogenesis inhibitor [62]
TIMP-4	+/+	Angiogenesis inhibitor [63]
Thrombospondin-1	+/+	Anti-angiogenic. Inhibits endothelial cell proliferation [64]
Thrombospondin-2	+/-	Anti-angiogenic. Inhibits endothelial cell migration and tubule formation [65]
Vasohibin	+/-	A negative feedback regulator of angiogenesis [66]

**Table 2.**  
*Comparison of anti-angiogenic paracrine factors secreted by MSCs from the amniotic fluid and bone marrow.*

At present, the combination of cell and tissue engineering techniques increased the restoration potential of a distinct cell type after transplantation [77]. In most of these approaches, the maintenance of cell-to-cell interaction in 3D microenvironment could increase survival signaling pathway and organotypic plasticity of cells. For instance, it seems that cell encapsulation by the mixture of alginate-gelatin promotes angiocrine cues and vascular network formation [77]. The introduction of MSC-alginate microbeads to ischemic hindlimb mouse model promoted arterial collaterals after the occlusion of the femoral artery by the modulation of VEGF-A signaling pathway [78]. A side-by-side comparison of MSCs expanded in 2D, and alginate microbeads revealed enhanced angiogenic and chemotactic activity in cutaneous healing [79].

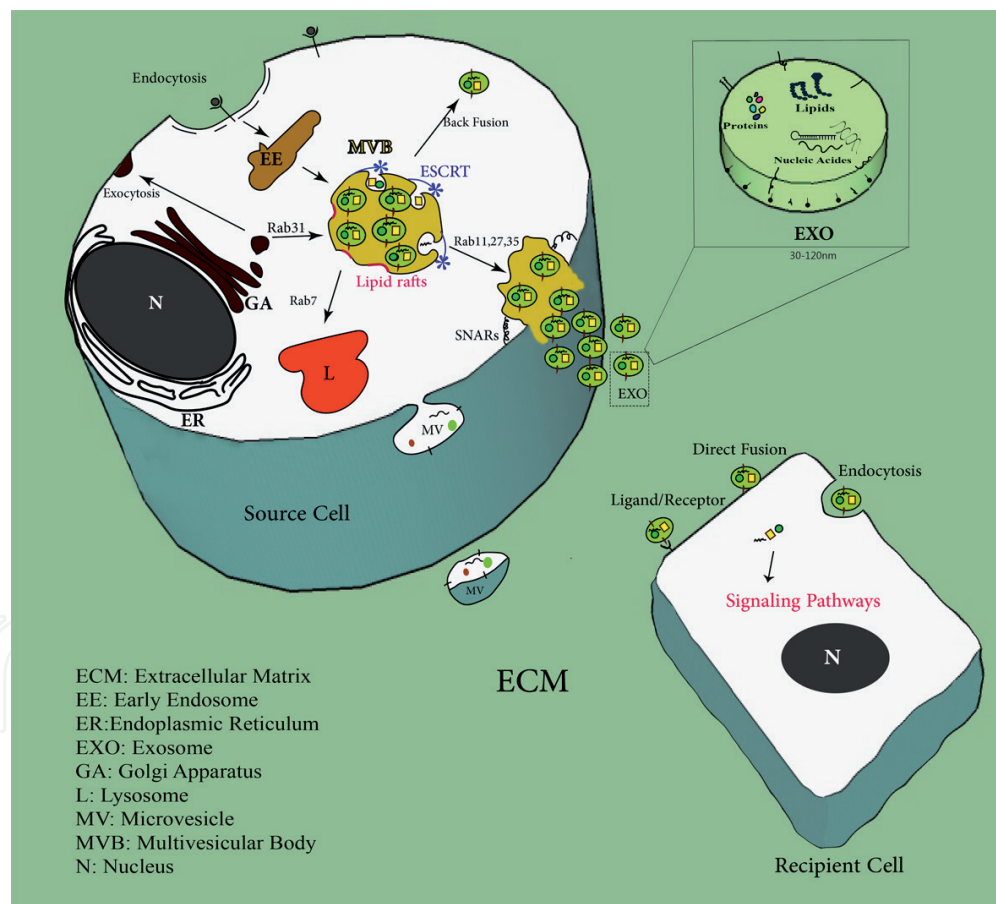
## 2. Modulation of angiogenesis by exosomes

Regarding paracrine activity, MSC exosomes transfer various bioactive molecules, microRNAs, and protein factors with the ability to modulate angiogenesis behavior in the target cells.

### 2.1 Exosomes biogenesis

Exosomes are a subtype of extracellular vesicles (EVs, 40–200 nm) found in bio-fluids and released from all cell types. They maintain cell-to-cell communication through shuttling diverse biomolecules [80–82]. The first intracellular step

in exosome biogenesis involves the invagination of the membrane of the multivesicular body (MVB) to form membrane-bound vesicles in MVB lumens that are identified as intraluminal vesicles (ILVs) (**Figure 1**) [83, 84]. Various factors and signaling pathways have been considered in biogenesis, trafficking, and abscission of exosomes [85]. Of note, endosomal sorting complexes required for transport (ESCRT) machinery with four complexes, ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III, participate in exosome formation and packing cargo incorporation with different accessory proteins (**Figure 1**) [81, 85, 86]. Noteworthy, the formation of MVBs in the absence of the ESCRT machinery is aborted. In this condition, oligo-dendroglial cell ceramide is a key molecule to induce inward budding of the limiting membrane of MVBs [83, 87]. After MVB formation, intracellular trafficking of vesicle systems was orchestrated by Rab-GTPase family proteins [81]. As shown in **Figure 1**, several Rab proteins specifically contribute to the transfer of vesicles in definitive pathways. Along with these factors, soluble NSF attachment protein receptor (SNARE) has been suggested to control the fusion of MVBs with the plasma membrane (**Figure 1**) [88]. At the intracellular level, three possible fates are considered to involve MVBs such as secretory, lysosomal, and back fusion pathways. Once secreted, exosomes can be received by neighboring cells by three possible



**Figure 1.**

*Biogenesis, structure, and uptake of exosomes. Exosomes are produced during the invagination process of MVB's membrane. ESCRT machinery and ESCRT-independent mechanisms (lipid rafts/tetraspanin) contribute to form exosomes and sort several molecules including proteins, miRNA, mRNA, DNA strands, and lipids into their lumen or limiting membrane of exosomes. Exosome cargoes are collected from materials received by endocytic pathway, Golgi apparatus, and cytoplasm. Rab-GTPase family proteins regulate intercellular trafficking and docking of MVBs. In the secretory pathway, MVBs actively fuse with the plasma membrane to release exosomes into the extracellular space. In alternative pathways, MVB could prefer binding to the lysosome or directly fuse back to the plasma membrane. Once secreted, exosomes enroll several mechanisms to arrive at the target cell: (I) enter through internalization process; (II) bind through receptor-ligand interactions, (III) direct fusion with the plasma membrane of the target cell. Exosomes are able to affect the biological processes of the target cells.*

mechanisms: (i) internalization, (ii) direct fusion, and (iii) receptor-ligand interaction. Exosomal uptake results in triggering signaling pathways reprogramming fate, proliferation, survival, and morphology of recipient cells (**Figure 1**) [89, 90].

## 2.2 Pro- and anti-angiogenic capacity of exosomes

It was shown that a significant portion of MSC angio-activity drives from their potency to release exosomes that can affect the function of ECs, either by increasing the production of pro-angiogenic factors or decreasing the production of anti-angiogenic factors [91]. The fact that MSC exosomes promote angiogenesis, by delivering mediators such as miRNAs, protein factors to distinct cells, was confirmed in various *in vivo* experimental studies [89, 92, 93].

### 2.2.1 miRNAs

It seems that exosomal cargo such as cytokines and miRNAs could be easily transferred to recipient cells. Increasing evidence indicates that exosomal pro-angiogenic miRNAs (miRNA-125a, miRNA-30b, miRNA-30c, miRNA-424, miRNA-150, and let-7f) are important regulators of angiogenesis in the target sites [89, 94–96]. Data suggest that exosomal miR-150 is a key contributor to the pro-angiogenic activity of MSC exosomes following ischemic injuries [89, 96, 97]. In contrast, anti-angiogenic function on tumor cells was reported by a research group guided by Lee et al. They demonstrated the anti-angiogenic function of MSC exosomes on breast cancer cells governed by delivering miR-16 to suppress VEGF factor [91]. In a recent study conducted by Chen et al., they declared that exosome can be used as therapeutic transfer vesicles to carry miRNAs and genetic molecules to modulate VEGF content and control untamed angiogenesis in rheumatoid arthritis [98]. Based on the literature, the expression of VEGF, endothelial marker CD31, and matrix metalloproteinases-14 (MMP-14) activity is induced in patients with rheumatoid arthritis. The application of MSC-derived exosomes containing miRNA-150-5p (Exo-150) clearly decreased transcription of VEGF and MMP-14 in synovial fluid. Consistent with these changes, the pro-inflammatory response was blunted by decreasing IL-1 $\beta$ , transforming growth factor- $\beta$  (TGF- $\beta$ ) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) content in synovial fluid. This study has shown that MSC-derived Exo-150 can be used as bio-shuttle and magic bullet for inhibiting an exacerbated angiogenesis via the modulation of angiogenesis-related factors. However, some contradictory facts exist regarding the sole application of exosomes in the context of tumor cells.

### 2.2.2 Exosomal pro- and anti-angiogenic factors

MSCs can secrete signal transducer and activator of transcription-3 (STAT3) mRNAs via exosomes that augment the transcription of hepatocyte growth factor (HGF), IL-6, and VEGF, promoting proliferation and migration of ECs [99]. In this context, MSC exosomes abundantly are enriched with VEGF factor that increases neovascularization through the Wnt4/ $\beta$ -catenin pathway in epithelial cells [100, 101]. The pro-angiogenic propriety of MSC exosomes has been previously shown in myocardial ischemia/reperfusion injury experiments following acute myocardial infarction [102–104]. In contrast, MSC exosomes may contain abundant anti-angiogenic factors that could regulate tumor angiogenesis rate. Lee et al. showed that exosomes from MSCs significantly downregulated the expression of VEGF in breast cancer cells, leading to the abortion of angiogenesis [91]. However, there are contradicting results. For example, human bone marrow MSC



exosomes promoted VEGF synthesis in colonic and gastric carcinomas through the activation of extracellular signal-regulated kinase1/kinase2 (ERK1/ERK2) and p38 MAPK pathways [105]. Taken together, these issues show a fact that exosomes from various MSC types can mediate physiological and pathological angiogenesis and could be considered as a suitable bio-shuttle for establishing promising therapeutic approaches in an individual with cancers and ischemic pathologies. The feasibility of exosome uptake by recipient cells, make these cell products for introducing in clinical approaches. Xue and colleagues investigated the effects of cord blood and adipose-derived MSC exosomes on human EC angiogenesis capacity under hypoxic and normal conditions [106, 107]. They noted the potency of isolated exosomes in triggering angiogenesis rate especially under the hypoxic condition compared to exosome counterpart originated from normal milieu. Based on their data, the transcription level of genes related to angiogenesis such as angiopoietin-1 (Ang-1) and VEGF receptor-2 (also termed FLK-1) was induced significantly after exposure to exosomes collected from hypoxic MSCs rather than that of normal cells. Following the induction of Ang-1 and FLK-1, the status of some downstream effectors would be turned to an activated form. For instance, it was found that protein kinase A (PKA) is indirectly triggered after the activation of genes *Ang-1* and *VEGFR-2*. Along with changes, the transcription level of angiogenesis inhibitory gene like *Vash1* is completely suppressed. The inhibitory angiogenesis potential of MSCs was investigated on cancer cells or progenitors residing inside tumor mass. Both anti-inflammatory and pro-angiogenesis property of MSC-derived exosomes were shown in cardiovascular disease [92, 97]. In addition to the promotion of cell surface receptors, exosomes could augment the synthesis of VEGF factor in targeted cells. Doeppner et al. also previously demonstrated that MSC-derived exosomes initiated healing processes after the onset of neurological diseases by increasing angiogenesis and blood supply which led to the neurological recovery and neurogenesis [108]. Other experiments added notion on the potency of exosomes to reduce neuroinflammation in traumatic brain injury [109]. However, some contradictory facts exist regarding the sole application of exosomes in the context of tumor cells. The superior stimulatory effect of MSC-derived exosomes on tumor angiogenesis was also addressed by different authors [110]. For example, Zhu et al. demonstrated the vasculogenic role of MSC exosomes after addition to human gastric carcinoma (SGC-7901) and colon cancer (SW480) cell lines [105, 111]. They found that the normal status of signaling effectors such as phosphorylated ERK1/ERK2, Bcl-2, and VEGF proteins; alpha-smooth muscle actin ( $\alpha$ -SMA); CXCR-4; and mouse double minute 2 homolog (MDM2) mRNA was modulated in the favor of angiogenesis in a mouse cancer model. In addition to the direct fast action on recipient cells, it is reasonable to hypothesize that exosomes are able to dictate pro-/anti-angiogenesis pattern in distinct cells by provoking specific signaling pathways and effectors such as ERK1/ERK2 and p38 MAPK kinase routes.

The engagement of factors such as AKT, STAT3, Wnt/ $\beta$ -catenin, and ERK happens following cutaneous wound regeneration treated with MSC exosomes. Proteomic analysis revealed that the protein content of growth factors IL-6, stromal cell-derived factor-1 $\alpha$  (SDF-1 $\alpha$ ), IGF-1 $\alpha$ , STAT3, and HGF contributed to cell proliferation, migration, and angiogenesis, improving reepithelialization in wound sites [112]. The modulation of Wnt/ $\beta$ -catenin pathway targeting Wnt4 diminishes the number of cells with apoptotic changes with the levels of pro-angiogenic factors such as IL-6 and IL-8, granulocyte-colony-stimulating factor (G-CSF), PDGF-BB, MCP-1, and VEGF are increased. In response to treatment with exosomes, phosphorylation of glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ) as a main negative regulator of Wnt signaling pathway is initiated, resulting in the progression of a cell from phase G1 to S and cutaneous cell proliferation [113]. An enhanced angiogenesis rate

with reduced cardiomyocyte apoptosis was reported following the administration of MSCs to infarct area. After being exposed to the ischemic/hypoxic condition, MSCs were programmed to secrete exosomes. Under these conditions, GATA-4 is induced which prevents cell apoptosis, reducing the infarct size. Meanwhile, the level of an anti-apoptotic agent such as miR-19a and miR-22 was increased in the target sites [114]. In another experiment conducted by Teng et al., it was shown that MSC-derived exosomes harboring miRNA-132 efficiently are delivered to human umbilical vein ECs (HUVECs). Therefore, it could be pointed out that MSCs could dictate prominent changes in the target cells. They also declared that endothelial Ras signaling pathway effectors are modulated by recipient cells after direct interaction of this miRNA with *RASA1* gene. Ras group genes have a basic role in controlling cell proliferation and differentiation [107]. Along with these statements, the bona fide effects of MSC exosomes need to be precisely addressed by a plethora of various experiments.

In the context of tumor niche, both anti- and pro-tumorigenic features was found after the treatment of cancer cells with MSCs exosomes. The migration and proliferation of tumor cells were tightly regulated by exosome factors by the modulation of PDGFR, C-Met, and EGFR signaling. Ex vivo modulation showed this fact that MSC exosomes could activate or phosphorylate intracellular kinase domain of relevant receptors, thereby triggering Akt, PKC/PKB, and MAP signaling pathways, leading to proliferation and migration of gastric tumor cells [115]. Exosomes released by human bone marrow MSCs augmented VEGF in colonic carcinoma and gastric carcinoma tumor cells through the activation of ERK1/ERK2 and p38 MAPK pathways [105]. This hypocrisy generates doubts on the definite therapeutic effect of exosomes from MSC source in various niches. In an experiment, the lack of cell response was approved in dormant-like tumor-initiating cells [116]. The differences in tumor cells to MSC secretome may relate to the divergence of factors and dynamic growth of target cells inside tumor niche [116]. In light of various genetic and proteomic reservoir, the target signaling and possible side effects of exosome treatment are required to be investigated in relation to specific distinct signaling pathway. It seems that exosome therapy is at the beginning step, and the type and source of cells have a superior role in the orientation of target cell behavior. A more deep understanding of the regulatory signaling pathways and precise inquiry in profiling of components transferred by exosomes is required to enroll and engineer the exosomes for therapeutic angiogenesis or targeted therapy

### **3. The application of MSCs and secretome in ischemic cardiac disease**

Cardiovascular diseases remain the leading cause of mortality and morbidity in worldwide. Various investigators have continued to assess a large number of cell types injected through several routes to promote cardiac repair in patients with cardiovascular diseases in both the preclinical and clinical stages. Clinical studies have largely been focused on the administration of MSCs [117, 118]. For instance, intracoronary injection of bone marrow MSCs led to an improved function of the left ventricle in subjects with acute myocardial infarction [119]. Mechanisms of action of MSCs administrated to the injured myocardium include accelerating angiogenesis process, diminished fibrosis, and regulation of immune response [102, 120]. Both in vitro and in vivo investigations have confirmed the trans-differentiating capacity of MSCs to effective cardiomyocytes in injured cardiac tissue [50]. In addition, documents revealed that MSCs from different sources release greater amounts of angiogenic factors (HGF, VEGF, and other growth factors), cell migration chemokine (SDF-1 $\alpha$ ), immune-signaling elements (IL-6, IL-8, and

MCP-1) TGF- $\beta$ , neurotrophic factors (brain-derived neurotrophic factor (BDNF)), nitric oxide (NO), and improved cardiac restoration after injury [121].

Exosomes from MSCs exposed to hypoxia and FBS-free condition enhanced neovascularization in the injured heart [92, 122–124]. In a preclinical study, intramyocardial transplantation of exosome secreted from MSCs significantly improves blood flow rate and reduced infarct zone in the rat model [125]. Approximately, the entire small and large animal model of CVD preclinical investigations along with high-quality phase 0, I, II, and III clinical trials and meta-analysis studies vigorously confirmed that MSC therapy has the effective effects in developing angiogenic networks in ischemic regions [126, 127].

Ongoing researches on preconditioning and genetic manipulations of MSCs are needed to enhance angiocrine capacity governed by growth factors, microvesicles, microRNAs, long noncoding RNAs (lncRNAs), etc. [128, 129]. Finding the route of cell delivery, the optimum dose, the excellent cell source, and transplantation time are factors that still require to be addressed so as to achieve the aim of comprehensive cardiac regeneration.

## **4. Angiogenesis assays**

Both in vitro and in vivo angiogenesis assays are commonly used to investigate pro- and anti-angiogenic potential of stem cells and different cell types.

### **4.1 In vitro analyses**

#### *4.1.1 Proliferation and survival assays*

Monitoring the proliferation of ECs is needed to develop microvascular units. Different survival and proliferation assays based on DNA synthesis or metabolic status are applicable. These assays could also predetermine the anti-angiogenic property of a specific compound in the context of tumor biology.

#### *4.1.2 Migration assays*

This method shows the migration in response to diverse factors, ability to digest basal membrane, and healing capacity of MSCs which is done by various assays as follows: Boyden chamber assay, Transwell® inserts, agarose assay, wound-healing assay, Teflon fence assay, phagokinetic track, etc. [130].

#### *4.1.3 Tube formation (tubulogenesis) assay*

This system is done in the 2D and 3D milieu and able to monitor alignment and juxtacrine connection of cells after plating on a specific substrate such as Matrigel, Fibrin, etc. Plated cells acquire phenotype to form capillary-like structures and lumen which are applicable to in vivo condition and evaluated in terms of tube area and number per microscopic field [130, 131].

#### *4.1.4 Aortic ring assay*

In this assay, the aorta from mouse or rats was removed and placed on collagen or fibrin matrix in serum-free condition. The angiogenic potential is determined by EC sprouting, polarized cells, and outgrowth appearance to the periphery [132].

## **4.2 In vivo analyses**

### *4.2.1 Corneal angiogenesis assay*

The cornea is considered as avascular tissue with unique properties for monitoring the angiogenesis and done in the model of mouse, rat, and rabbit. In the procedure of corneal angiogenesis, the candidate biomaterials and polymer with putative pro- and anti-angiogenic factors were transplanted into the stromal pouch created by surgical approach. The penetration and ingrowth of nascent vessels into the avascular area is monitored by the time [133].

### *4.2.2 Chicken chorioallantoic membrane angiogenesis assay*

This assay is performed on embryonated eggs by using polymer pellets and silastic rings containing target molecules on the surface of the chorioallantoic membrane. After the completion of distinct time, the number and dilation of blood vessels from avian source to the implants were quantified [70].

### *4.2.3 Matrigel plug assay*

It is a choice of in vivo angiogenesis assay following administration of gelatinous protein mixture termed Matrigel into subcutaneous space. The target molecules could be administrated with Matrigel at the site of injection and systemically to the circulation system. To precisely elucidate the formation of de novo capillaries, fluorochrome agent could be administrated into the systemic circulation [130].

## **5. Conclusion**

It is anticipated that MSC secretome and angiocrine could be used as an off-the-shelf alternative therapy to modulate angiogenesis/vascularization in distinct tissues. Considering both pro- and anti-angiogenic capacity, a big question remains to the identification of safety and efficacy of MSC secretome under specific conditions. Based on the data from different experiments, the angiogenic paracrine potential of MSCs is currently under investigation, and results of preclinical and translational studies, if confirmatory of previous basic experiments, could lead to human medicine for angiogenic modulation of tissues. The discovery of the signaling pathways that mastermind the paracrine pro- and anti-angiogenic potential of MSCs enables us to find appropriate policies for modulating angiogenic switch on/off in in vivo condition.

## **Acknowledgements**

All authors declared that there is no conflict of interest regarding the content of the current chapter.

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