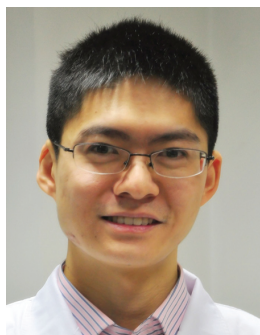


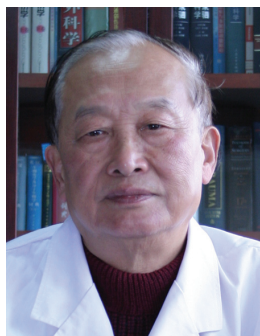
Signaling Pathways Involved in Migration of Mesenchymal Stem Cells

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About the Authors:

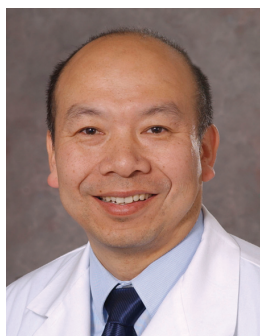
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Dr. Min Zhao was graduated from the Third Military Medical School in 1985, earned Doctoral degree under supervision of Professor Wang Zhengguo in 1991. He worked with Geoff Burnstock at University College London, Colin McCaig and John Forrester at University of Aberdeen, where Min Zhao became a full professor in 2004. In 2007 he moved to School of Medicine, University of California Davis (UC Davis) and became a Professor, with Departments of Dermatology and Ophthalmology. He works on wound healing and regeneration and directional cell and tissue growth.

Abstract

Mesenchymal stem cells (MSCs) hold great promise for wound healing and tissue regeneration. During tissue repair, endogenous bone marrow MSCs or exogenously delivered MSCs migrate to the sites of injury and participate in the repair process. Recent studies identified multiple signaling pathways and molecules that play roles in migration of mesenchymal stem cells. We review briefly some signaling pathways and molecules required for MSC migration. Better understanding of the molecular mechanisms of MSC migration will help to optimize therapeutic strategy to target MSCs at injured tissues, and develop new therapies in regenerative medicine.

Introduction

Mesenchymal stem cells (MSCs) are a subset of nonhematopoietic stem cells, characterized by their ability of self-renewal and differentiation into multiple cell types, including osteoblasts, adipocytes and chondrocytes. MSCs were first described by Friedenstein in 1970s as fibroblast-like, plastic-adherent cells that can be expanded in vitro^[1]. Since then, MSCs have been a research focus and many insights have been gained in understanding MSCs.

MSCs are easy to isolate, with low immunogenicity, multi-differentiation potentials, and lack of ethical controversy. It has been demonstrated that MSCs in the circulation could migrate to the site of tissue damage, such as bone or cartilage fracture^[2], myocardial infarction^[3,4], and ischemic cerebral injury^[5]. Therapeutic values of MSCs have been demonstrated

in animal models of acute lung injury^[6], liver injury^[7], myocardial infarction^[8], diabetes^[9], stroke^[10], limb ischemia^[11], acute renal injury^[12] and sepsis^[13].

In order to participate in repair and regeneration, MSCs have to be mobilized and then migrate to the target sites and integrate with the local tissues. Many studies have been focusing on the mechanisms for MSCs to migrate to injured tissues. Those research have identified some important molecular mechanisms, including chemoattractants, paracrine factors, membrane receptors, and intracellular signaling molecules. We review here signaling pathways that control migration of MSCs, -chemoattractant-receptor axes and intracellular signaling pathways. Extracellular matrix and biophysical factors play important role in guiding migration of MSCs. We will not discuss those signaling mechanisms due to space limit.

Chemotactic factors and membrane receptors

SDF-1–CXCR4 Axis:

Stromal-derived factor 1 (SDF-1), or CXC ligand 12, is a member of a large family of related chemotactic cytokines, called “chemokines”, which was first identified as a lymphocyte and monocyte specific chemo-attractant under both normal and inflammatory conditions^[14]. Subsequently it has been demonstrated that MSCs express CXCR4, the receptor for SDF-1, and therefore SDF-1/CXCR4 axis has been implicated in the migration of MSC in a series of studies^[15-17]. Those studies suggest that SDF-1/CXCR4 axis was required for migration of human bone marrow MSCs and cord blood MSCs. CXCR4 antagonist AMD3100 significantly inhibited chemotaxis of MSCs toward SDF-1^[17, 18]. Rat bone marrow MSCs were shown to migrate towards SDF-1 gradient in a dose-dependent manner^[15, 19]. In a rat model, SDF-1-CXCR4 was shown to mediate homing of transplanted MSCs to injured sites in the brain^[19].

A recent study, however, suggests a role of integrin beta, rather than that for SDF-1/CXCR4 in MSC migration. Ip et al^[20] have shown that in an animal model of myocardial infarction, injected BM-MSCs migrated into the infarcted myocardium in an integrin β 1 dependent manner, whereas blockade of CXCR4 had little effect. The reason may be the expression pattern of CXCR4 on MSCs under different experimental conditions or difference in the nature of the injuries. CXCR4 might present at the surface of a small subset of MSCs^[17, 21]. CXCR4 was usually absent on the surface of culture-expanded MSCs^[22-24]. Further studies are needed to clarify this issue.

CX3CL1-CX3CR1 Axis:

CX3C ligand 1 (CX3CL1, also called fractalkine) and CX3C receptor 1 (CX3CR1) were reported to mediate MSC migration response. Using an in vitro micromultiwell chemotaxis chamber assay, human bone marrow MSCs were found to migrate towards fractalkine gradients, and such response was abrogated using anti-CX3CL1 mAb^[21]. In a rat model of left hypoglossal nerve injury, interaction of fractalkine-CX3CR1 plays an important role in directed migration of transplanted rat MSCs to impaired sites in the brain^[19]. It should be noted that other chemokines and chemokine receptors, including CXCL16-CXCR6, CCL3-CCR1 and CCL19-CCR7, were also reported to participate in MSC migration^[21].

Growth factors and receptors:

Several growth factors and their receptors may be involved in MSC migration. Hepatocyte growth factor (HGF) was upregulated at sites of liver damage [25, 26]. Following myocardial ischemia and reperfusion in a rat model, HGF and its high-affinity receptor c-met were unregulated [27]. In an animal model of myocardium infarction, increased HGF expression was found in the injured heart [28]. Human bone marrow- and circulating blood-derived MSCs expressed functional c-met receptors, and could be strongly attracted by HGF gradients. This chemotactic response could be significantly inhibited using the specific c-met blocking agent K-252a, suggesting that HGF-c-met signaling regulates migration of MSCs [18]. Up-regulation of HGF expression in multiple injured tissues may induce MSCs to migrate to the HGF-rich environment of lesions.

Other growth factors, including platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), epidermal growth factor (EGF) and angiopoietin-1 (Ang-1), were also reported to be chemotactic to MSCs [29-33]. MSCs express receptors for those growth factors at a moderate to high level, including platelet-derived growth factor receptor (PDGF-R), insulin-like growth factor 1 receptor (IGF1-R), epidermal growth factor receptor (EGF-R) and Ang-1 receptor [29]. Thus, multiple growth factor-receptor axes may mediate MSC migration.

LPA and LPA1 Axis:

Lysophosphatidic acid (LPA) is a small bioactive phospholipid, which participates in a variety of physiological and pathophysiological responses, which include wound healing, chemotaxis, tumor cell invasion, metastasis, and cell cycle progression^[34, 35]. It is known that LPA signal in part, through G protein-coupled

receptor LPA1, which was found predominantly expressed in human adipose-derived MSCs (hADSCs) [36]. LPA dose-dependently increased the migration of hADSCs, which was completely inhibited by Ki16425, an antagonist specific for LPA1 [37]. LPA-stimulated migration of human BMSCs was mediated through LPA1-dependent mechanism [38]. Jaganathan et al however, have found in transwell assays that culture-expanded human BMSCs migrated poorly towards LPA [39].

Other chemotactic factors:

Tumor necrosis factor- α (TNF- α) is an important inflammatory cytokine presented at most injury sites with inflammation. TNF- α induces and directs migration of rat bone marrow MSCs in vitro [40]. Extracellular high mobility group box 1 (HMGB1) is a cytokine that plays a role in the processes of inflammation, tissue injury and regeneration. Meng et al [41] showed that HMGB1 could act as a chemoattractant for MSCs in a dose-dependent manner. Monocyte chemotactic protein-1 (MCP-1), a chemokine involved in recruitment and activation of macrophages during inflammation, stimulates MSC migration to ischemia in the rat brain [42], as well as to tumor [43].

Intracellular signaling pathways

Extracellular signals through membrane receptors induce varieties of intracellular signaling pathways, which result in changes in cell motility and migration direction.

PI-3K/AKT signaling pathway:

Phosphoinositide 3-kinase (PI-3k)/ Akt signaling pathway is involved in SDF-1-mediated cell migration of hematopoietic progenitor cells and primary marrow CD34+ cells [44]. Genetically modified BMSC that over-express Snail showed more migration advantages, and disruption of the PI-3k-dependent pathway using specific PI-3k inhibitor, wortmannin, reduced Snail-mediated BMSC migration [45]. SDF-1 α or bFGF-induced MSCs migration was attenuated by PI3k/Akt inhibitor LY294002 or wortmannin [15,46].

MAPK/ERK1/2 signaling pathway:

Mitogen-activated protein/ extracellular signal-regulated kinase 1/2 (MAPK/ERK1/2) signal pathway is involved in the expression of a wide variety of genes controlling migration [47,48]. ERK1/2 may mediate SDF-1-induced cell mobilization [49,50]. Several studies have shown that MAPK/ERK1/2 was involved in MSC migration. Yun et al [51] demonstrated that stable thromboxane A2

(TxA2) mimetic U46619 strongly stimulated migration of human adipose tissue-derived MSCs (hADSCs) through activation of ERK and p38 MAPK. U46619-induced MSC migration was abrogated if the cells were pretreated with the MAPK/ERK kinase (MEK) inhibitor U0126 or the p38 MAPK inhibitor SB202190. Similarly, it has been shown that SDF-1 α -induced rat BMSCs migration was attenuated by ERK inhibitor PD98059 [52], and p38 MAPK inhibition with SB203580 significantly suppressed the chemotaxis effect of TNF- α on rat BMSCs [40]. For human bone marrow MSCs, significant impairment in migration towards SDF-1 and tumor cell-conditioned medium was observed in the presence of MEK inhibitor PD98059 [53]. LPA-induced migration of hADSCs was suppressed by pretreatment of the cells with MEK inhibitor U0126 [37]. However, some other studies suggested minor roles for MAPK/ERK1/2 in MSC migration. Pretreatment of human bone marrow MSCs (BMSCs) with SB203580 (P38 inhibitor), or PD98059 (P42/44 MAPK inhibitor) had little effect on the Snail-mediated MSC migration [45]. Migratory response of rat BMSCs to SDF-1 or TNF- α was not affected by MAPK/ERK inhibitor PD98095 [15,40]. In hematopoietic progenitor cells, primary marrow CD34+ cells, and epithelioid carcinoma cells, PI3K/Akt was required for SDF-1-mediated cell migration, but ERK1/2 were not [44,55].

Wnt3a signaling pathway:

Wnt signaling is involved in the metastasis of many kinds of cancer cells [56,57]. Wnt3a promoted the migration capacity of rat MSCs in transwell migration and wound healing assays through β -catenin nuclear translocation [58]. Wnt3a antibodies significantly reduced migration of MSCs.

Jak/STAT signaling:

PDGF-induced migration of hADSCs was completely blocked by a pretreatment with c-Jun N-terminal kinase (JNK) inhibitor SP600125, but not with MEK inhibitor U0126 and p38 MAPK inhibitor SB202190 [54]. Janus kinase (Jak)/ signal transducer and activator of transcription (STAT) signaling was originally shown to be downstream signaling of interferons during the inflammatory response [59,60]. Jak/STAT pathway activation is required for cell migration in *Drosophila* [61]. MSC migration in response to SDF-1 stimulation resulted in Jak2/STAT3 pathway activation, and inhibition of the pathway using WP1006, a Jak2/STAT3 pathway inhibitor, significantly inhibited MSC migration [53]. Activation of Jak2/STAT3 pathway led to focal adhesion kinase (FAK) and paxillin activation,

which resulted in reorganization of actin filament and cytoskeleton, thus promoting MSC migration.

RhoA-Rho kinase-dependent pathway:

Rho family GTPases play important roles in the regulation of cytoskeleton, by coordinating assembly and activation of actin with actin-binding proteins such as paxillin and α -actinin [62]. Rho GTPases are critical regulators in migration of many cell types, including hematopoietic progenitor cells (HPCs) [63]. Yet, similar to some of the above signaling pathways, Rho GTPases appear to play different roles in MSC migration. Lee et al [37] demonstrated that LPA induced migration of hADSCs required activation of RhoA, and pretreatment of the cells with Y27632, a Rho kinase inhibitor, markedly inhibited the migration response. On the other hand, Jaganathan et al [39] have shown that LPA treatment to human BMSCs activated intracellular Rho and increased actin stress fibers, resulting in poor migration of MSCs towards LPA. Inhibition of Rho resulted in migration of MSCs towards LPA.

SMAD signaling pathway:

Transforming growth factor- β (TGF- β) signaling pathway involves phosphorylation of receptor-regulated SMADs (R-SMADs) by T β RI[64]. Tang et al [65] showed that human BMSCs was induced to migrate towards sites of bone resorption by active TGF- β 1, and this process is mediated through a SMAD signaling pathway. Using pharmacological inhibition and Tgfb1 knockout (Tgfb1-/-) mice, they confirmed that active TGF- β 1 released during osteoclastic bone resorption induced migration of BMSCs through T β RI signaling. Using both short interfering RNAs (siRNAs) and trans-genetic mice model, they showed that SMAD2, SMAD3 and SMAD4, downstream of T β RI, was required for TGF- β 1-induced MSC migration. Thus, TGF- β - T β RI- SMAD pathway was proposed as a novel signaling pathway involved in MSC migration.

Conclusion

Therapeutic potentials of MSCs rely on migration of MSCs from the bone marrow or other residing niches to distant injured tissues, where they participate in repair and regeneration. Recent studies revealed some key molecules and signaling pathways, which could be potential targets for modulation of MSC migration in wound healing, damage repair and regeneration. Those include chemokines and receptors (SDF-1-CXCR4, CX3CL1-CX3CR1), growth factors and their receptors (HGF, PDGF and EGF, IGF and the corresponding

receptors), Lysophosphatidic acid (LPA) and its receptors LPA1, and other chemotactic factors TNF- α , MCP-1. Activation of those membrane receptors leading to activation of multiple intracellular pathways: PI-3K/AKT signaling pathway, MAPK/ERK1/2 signaling pathway, Wnt3a signaling pathway, Jak/STAT signaling, RhoA-Rho kinase-dependent pathway, and SMAD signaling pathway.

Studies showed contrasting results and discrepant conclusion. Different types of MSCs and heterogeneity of MSCs used may account for the discrepancy [66]. Different signaling mechanisms may underlie migration of different types of stem cells [67]. In addition, confluency and passage number of cultured MSCs have been shown to influence migration of MSCs [66, 68, 69]. Heterogeneity between different sources or different passages of MSCs could have significant impact on MSC migration.

Migration of MSCs is controlled by complicated signal networks. Understanding the molecular mechanisms of MSC migration will benefit optimization of stem cell therapies. Regulation of the signaling pathways and extracellular matrix will help to develop strategies to facilitate targeting of transplanted MSCs as well as endogenous MSCs to injured tissues [70, 71].

Acknowledgments

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