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Platelet-derived growth factor-BB enhances MSC-mediated cardioprotection via suppression of miR-320 expression

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1Department of Pharmacology, Harbin Medical University, Harbin, China; 2Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, Indiana; 3Center for Computational Biology and Bioinformatics, Indiana University School of Medicine, Indianapolis, Indiana; and 4Department of Biomedical Engineering, Indiana University Purdue University, Indianapolis, Indiana

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Xu B, Luo Y, Liu Y, Li BY, Wang Y. Platelet-derived growth factor-BB enhances MSC-mediated cardioprotection via suppression of miR-320 expression. Am J Physiol Heart Circ Physiol 308: H980–H989, 2015. First published February 27, 2015; doi:10.1152/ajpheart.00737.2014.—Delivery of bone marrow-derived mesenchymal stem cells (MSCs) to myocardium protects ischemic tissue through the paracrine release of beneficial angiogenic and cytoprotective factors. Platelet-derived growth factor (PDGF)-BB, a potent mitogen of MSCs, is involved in the pathophysiology of ischemic heart disease. However, the role(s) of PDGF in MSC-mediated cardioprotection remains unknown. Here, we found that PDGF treatment of MSCs resulted in rapid activation of both Akt and ERK (central intracellular signal mediators), upregulated VEGF, and induced phosphorylation of the activator protein-1 (AP-1) transcription factor c-Jun. Examination of several microRNA genes having predicted promoter c-Jun-binding sites showed that PDGF treatment resulted in upregulation of miR-16-2 and downregulation of miRs-23b, -27b, and -320b. To examine possible PDGF augmentation of therapeutic potential, we evaluated the effects of PDGF using an ex vivo isolated mouse heart ischemia-reperfusion model. Human MSCs, with or without PDGF preconditioning, were infused into the coronary circulation of isolated mouse hearts. The hearts that received PDGF-treated MSCs exhibited a greater functional recovery compared with naïve MSC-infused hearts, following ischemia-reperfusion injury. This enhanced functional recovery was abolished by overexpression of miR-320, a microRNA we found downregulated by PDGF-activated c-Jun. Overexpression of miR-320 also resulted in upregulation of insulin-like growth factor binding protein (IGFBP) family members, suggesting PDGF “cross talk” with the mitogenic IGF signaling pathway. Collectively, we conclude that PDGF enhances MSC-mediated cardioprotection via a c-Jun/miR-320 signaling mechanism and PDGF MSC preconditioning may be an effective therapeutic strategy for cardiac ischemia.

c-Jun; mesenchymal stem cells; PDGF; hsa-miR-320b

MESENCHYMAL STEM CELLS (MSCs) protect infarcted hearts in part through paracrine release of a broad variety of growth factors, cytokines, and chemokines that facilitate heart repair (16–18, 50, 52). These paracrine factors attenuate ventricular remodeling, facilitate neo-angiogenesis, reduce the infarcted size, and promote cardiomyocyte survival, thus improving heart function after ischemic injury (17, 18, 33, 50). However, many clinical trials have shown that the cardioprotective effects of MSC therapy are modest and of limited duration, in part due to low rates of engraftment, transdifferentiation, cell fusion, and survival (14, 23, 30, 36, 48). Moreover, inflammatory molecules, proapoptotic factors, and a hypoxic environment in the ischemic heart also have detrimental effects on these implanted cells. Accordingly, a number of strategies have been attempted to improve the therapeutic efficacy of MSCs, including improving their in vivo survival rate, ability to promote angiogenesis, homing to the heart, and augmentation of MSC paracrine signaling.

One promising agent for enhancing MSC paracrine function and cardioprotection is platelet-derived growth factor (PDGF)-BB. PDGF is a potent mitogen of cells of mesenchymal origin, including MSCs (38) and is synthesized, stored, and released by many differentiated cell types, such as platelets (8), endothelial cells (7), smooth muscle cells (53), fibroblasts (10), and monocytes (34). PDGF is a dimeric glycoprotein composed of two A chains (-AA), two B chains (-BB), or an A/B heterodimer (-AB). Two types of the PDGF receptor (PDGFR) have been identified: PDGFRα and PDGFRβ (32), and both subtypes are capable of binding PDGF. Upon activation by PDGF, the receptor subunits dimerize and subsequently activate numerous signal transduction pathways, including JNK/c-Jun, PKC, ERK, JAK/STATs, and PI3K/Akt, which play profound roles in regulating cell growth, proliferation, and differentiation (9, 27, 40, 42).

PDGF has also been shown to alter MSC microRNA (miRNA) expression (19). miRNAs are small noncoding RNAs that function in transcriptional and posttranscriptional regulation of gene expression (6) via mRNA cleavage, translational repression and polyadenylation (mRNA degradation by removal of its 3′-poly-A tail) (49). miRNA genes can be found in the introns of protein-coding genes and in both introns and exons of noncoding RNAs (41). Expression of miRNAs is regulated in a spatial, temporal, and physiological manner and may require different mechanism of biogenesis, according to their genomic location (e.g., extronic, intronic, etc.) (41).

The present study focused on assessing the therapeutic efficacy of PDGF-preconditioned MSCs and also on the role of c-Jun, a well-known activator protein-1 (AP-1) transcription factor that is activated by PDGF, in regulating the expression of miRNAs that may modulate MSC paracrine signaling. We hypothesized that PDGF improved MSC-derived cardioprotec-
tion via modulating the expression of cytoprotective paracrine factors and miRNAs. By using an isolated mouse ischemic/reperfusion model, we tested the role of PDGF and hsa-miR-320b whose expression level was suppressed by PDGF. Our investigation provides insight into the intracellular mechanisms that underlie the therapeutic potential of MSCs by demonstrating new ways to “prime” these cells for improved therapeutic application.

MATERIALS AND METHODS

Reagents. PDGF was obtained from Becton-Dickenson (Franklin Lakes, NJ). All other chemicals were obtained from Sigma (St. Louis, MO).

Human MSC culture. MSCs (purchased from Lonza, Walkersville, MD) were tested for purity by flow cytometry and for their ability to differentiate into osteogenic, chondrogenic, and adipogenic lineages (35). MSC purity was validated by positivity for CD105, CD166, CD29, and CD44 and negativity for CD14, CD34, and CD45 (3). MSCs were thawed, and the process was performed according to the manufacturer’s instructions, by plating in tissue culture flasks (Corning, Corning, NY) containing MSC growth medium (Lonza) at 37°C in 5% CO₂–90% humidity. The medium was changed every 3 days. After cells attained 70% confluence, MSCs were transferred to 6- or 12-well plates (Corning) at 5 × 10^4 cells-well⁻¹ ml⁻¹ for experimentation.

Isolated mouse heart perfusion (Langendorff). Hearts were isolated as previously described (25). Mice were anesthetized (sodium pentobarbital, 60 mg/kg ip) and heparinized (500 U ip), and the hearts were then rapidly excised via median sternotomy and placed in 4°C barbital, 60 mg/kg ip) and heparinized (500 U ip), and the hearts were then reperfused for 40 min to create global ischemia, during which the heart was placed in a 37°C degassed organ bath for 25 min. Hearts were then reperfused for 40 min after ischemia. The left ventricular developed pressure (LVDP) and the maximum positive and negative left ventricular end-diastolic pressure (EDP) were calculated using PowerLab software.

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then detected by adding horseradish-conjugated streptavidin and SuperSignal West Pico stable peroxidase solution to the membranes (Pierce). Signal densities were measured and compared using NIH ImageJ software.

Predicting AP-1 binding sites in miRNA promoters. Genomic loci for the promoter regions of human miRNAs were acquired using MпромDB, a curated database that documents the annotations of gene promoters derived from ChIP-seq datasets from either RNA polymerase II or histone marks (21). The promoter sequences were further retrieved from the human reference genome (build hg18), based on the promoter regions acquired using MпромDB. AP-1 binding sites within the miRNA promoter regions were predicted based on the position specific scoring matrix (PSSM) of AP-1 binding proteins, as documented in the TRANSFAC database. Matching scores were then calculated for each genomic locus, as described previously (31). A specific genomic locus was considered to be a candidate AP-1 binding site if its matching score was greater than a cutoff score, determined as the top 1% of loci that were evaluated.

Data analysis. All values were reported as means ± SE. LVDP, ±dp/dt, and EDP values were presented as a percentages of baseline. Statistical differences between the control groups and those obtained under various treatment conditions were determined using a Student’s t-test, one-way, or two-way ANOVA, followed by a Tukey post hoc analysis. Values of P < 0.05 were judged to be statistically significant.

RESULTS

**PDGF increases MSC VEGF production in a dose-dependent manner and rapidly activates intracellular signaling cascades.** MSCs (5 × 10⁴) were plated in 12-well plates overnight and then treated with PDGF at different concentrations (0–100 ng/ml) for 24 h. The supernatants were then collected and measured for VEGF production. As shown in Fig. 1A, PDGF treatment increased VEGF expression in a dose-dependent manner. Based on these results, we chose 10 ng/ml, the minimum dose provoking maximum VEGF release, as a standard treatment dose for our later experiments.

We further examined the mechanism by which PDGF may stimulate MSCs to produce VEGF and other cytoprotective paracrine factors. The cells were stimulated with PDGF (10 ng/ml) for 30 min and then exposed to lysis buffer. Activation of intracellular signaling cascades and transcription factors was examined using a Human Phospho-Kinase Antibody Array Assay Kit (see MATERIALS AND METHODS for details). As shown in Fig. 1B, PDGF increased phosphorylated ERK, JNK, Akt, CREB, and β-catenin after 30 min of treatment.

We also examined the activation of c-Jun, a JNK-dependent transcription factor, following PDGF treatment. As shown in Fig. 1C, c-Jun phosphorylation levels were significantly increased 10 min after PDGF treatment. Interestingly, c-Jun and p-c-Jun were strongly detected in the nucleus but not in the cytosol (Fig. 1C).

To investigate whether PDGF progressively activates intracellular signaling molecules, we next examined its time-dependent activation of ERK and Akt. As shown in Fig. 1D, PDGF increased phosphorylation of both ERK and Akt after 5 min. Interestingly, phosphorylation levels of both signaling molecules decreased 24-h after stimulation.

**Fig. 1.** Platelet-derived growth factor (PDGF) increases the expression of the angiogenic factor VEGF and rapidly stimulates intracellular signaling cascades. A: human mesenchymal stem cells were plated in 12-well plates (5 × 10⁴ cell/well) for 24 h and stimulated by PDGF (10 ng/ml) for another 24 h. Supernatants were collected and the total amounts of VEGF measured. Results are means ± SE; n = 3/group. *P < 0.05 vs. naïve mesenchymal stem cell (MSC) control. B–D: cells were stimulated with PDGF (10 ng/ml) and total cell lysates, cytosolic protein, and nuclear protein collected at different time points. The activation of intracellular kinases was measured by protein kinase arrays (B) and Western blots (C and D). C: control; P: PDGF (10 ng/ml) for 10 min; TBP: TATA-binding protein (pr).
**PDGF improves MSC-mediated cardioprotection following ischemic injury.** To examine whether PDGF improved MSC-mediated cardioprotection, a standard ex vivo Langendorff mouse heart model was used. MSCs were treated with 10 ng/ml PDGF for 48–54 h before collection for experiments. Concurrently, isolated mouse hearts were randomized into three groups that were allowed to equilibrate for 15 min, followed by coronary artery infusion with vehicle, naïve MSCs, or PDGF-pretreated MSCs (5 × 10^5), immediately before ischemia. Hearts were then reperfused for 40 min, and the LVP was monitored constantly. Several indexes of left ventricular performance, namely, LVDP, EDP (indicating the compliance of ventricular muscle), maximum +dP/dt, and −dP/dt were obtained by analyzing LVP data.

Figure 2, A–C, shows representative recording traces of LVP of isolated mouse hearts infused with vehicle, naïve human MSCs, and PDGF-pretreated MSCs. Both naïve and PDGF-pretreated MSCs infused into coronary circulation immediately before global ischemia improved heart functional recovery during the 40-min reperfusion period. The isolated mouse hearts that received PDGF-treated MSCs showed better functional recovery, compared with the naïve MSC group. This was demonstrated via improved LVDP, +dP/dt, and −dP/dt in the experimental groups (naïve and/or PDGF-treated MSCs), compared with the vehicle controls (Fig. 2, D–F). However, EDPs were not different between any of the groups (Fig. 2G), which may suggest the equivalence of injury after ischemia-reperfusion.

**PDGF modulates the expression of c-Jun target miRNAs.** We next tested MSC expression of hsa-miRs-16-2, -23b, -27b, and -320b, predicted c-Jun target genes due to their promoter regions possessing predicted c-Jun binding sites greater than a selected threshold. MSCs (1 × 10^5/2-ml/well) were plated in six-well plates and treated with 10 ng/ml PDGF for 48 h. Total RNA extracts were enriched for small RNA species, and the expression of the aforementioned miRNAs was examined using standard TaqMan assays. Following PDGF treatment, MSC expression of hsa-miR-16-2 was significantly higher than in the vehicle control; in contrast, expression levels of hsa-miR-23b, hsa-miR-27b, and hsa-miR-320b were lower (Fig. 3).

**Overexpression of hsa-miR-320b abolishes enhanced MSC-mediated cardioprotection.** Previously, it was shown that mouse cardiomyocytes release exosomal miR-320 for possible uptake by mouse cardiac endothelial cells (51). Based on that study, and our findings described above, we focused on determining the role of hsa-miR-320b in MSC-mediated cardioprotection and paracrine signaling. Consequently, an hsa-miR-320b mimic was transfected into human MSCs, and successful
decreased LVDP, compared with control miR-transfected MSCs, as demonstrated by less MSC-derived protection and functional recovery, compared with naïve MSCs. MSCs demonstrated similar functional recovery, compared with naïve MSCs, although exogenously added PDGF did increase VEGF secretion from MSCs either transfected with nonspecific control miRNA or miR-320. We also confirmed this observation using ELISA (Fig. 5B). Interestingly, the mRNA levels of both factors (VEGF and SDF-1) were not changed at 48 h after PDGF stimulation (Fig. 5B). Both PDGF and miR-320 overexpression increased tissue inhibitor of matrix metalloproteinase (TIMP-1) expression and the rate of cell proliferation (Fig. 5B). The expression levels of other growth factors (as measured by signal densities) are listed in Table 1.

DISCUSSION

In this study, we demonstrated that 1) PDGF increased MSC VEGF production in a dose-dependent manner; 2) PDGF (10 ng/ml) rapidly (<30 min) activated multiple intracellular signaling factors and transcription factors, including JNK/c-JUN, ERK, Akt, CREB, and β-catenin; 3) PDGF inhibited p38α activity; 4) PDGF stimulated intracellular signaling pathways in a spatial and temporal manner; 5) the primary cellular location of c-Jun, in both its activated and quiescent forms, is in the nucleus; 6) 48-h PDGF pretreatment of human MSCs improved MSC-mediated cardioprotection in an ischemic-reperfusion, isolated mouse heart model; 6) PDGF regulated the expression of c-Jun-targeted miRNAs, namely miR-16-2, -23b, -27b, and -320b; 7) transfection of miR-320b mimic attenuated the cardioprotective effect of PDGF-pretreated MSCs; 8) transfection of miR-320b mimic increased the expression levels of IGFBP-1, -2, -3, and TIMP-1; and 9) PDGF increased the expression of SDF-1 and VEGF in cells, with or without miR-320 transfection.

Both allogeneic and autologous MSCs have been reported to favorably affect patient functional capacity, quality of life, and ventricular remodeling (13, 14, 22). MSCs protect ischemic myocardium via secretion of a cascade of factors with cytotoxic and protective potential. Due to the short experimental period (<2 h), it is unlikely neovascularization would occur during the process. However, we observed that MSCs released angiogenic growth factors, cytokines, and chemokines that can induce the formation of new blood vessels in an in vivo setting. These factors include vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin growth factor 1 (IGF-1), the chemokines KC (keratinocyte chemotactant) and monocyte chemotactant protein-1 (MCP-1), and many others (50, 52). The newly formed capillaries should increase blood perfusion of the injured myocardium and improve wound healing.

Besides inducing angiogenesis, MSCs also released paracrine factors that suppressed the inflammatory response of injured tissue, decreasing fibrosis and stimulating the activity of endogenous endothelial progenitor cells (12). Such secretory effects help to preserve heart tissue, decrease cardiac remodeling, and stimulate endogenous regeneration. While the current study focused on paracrine activity, implanted MSCs have further investigated whether overexpression of miR-320 altered MSC paracrine signaling. The expression of paracrine factors was examined by using a human angiogenic growth factor array. The basal expression of insulin-like growth factor-binding proteins (IGF-BP), -1, -2, and -3 was significantly increased after miR-320 overexpression. Additionally, PDGF treatment further increased IGF-BP, -2, -1, and -3 secretion (Fig. 5A). Overexpression of miR-320, alone, did not change basal or PDGF-stimulated VEGF or stromal cell-derived factor-1 (SDF-1) expression, although exogenously added PDGF did increase VEGF secretion from MSCs either transfected with nonspecific control miRNA or miR-320. We also confirmed this observation using ELISA (Fig. 5B). Interestingly, the mRNA levels of both factors (VEGF and SDF-1) were not changed at 48 h after PDGF stimulation (Fig. 5B). Both PDGF and miR-320 overexpression increased tissue inhibitor of matrix metalloproteinase (TIMP-1) expression and the rate of cell proliferation (Fig. 5B). The expression levels of other growth factors (as measured by signal densities) are listed in Table 1.

![Graph showing fold change in miRNAs](http://example.com)
also been shown to phenotypically resemble cardiac myocytes (46); it would be interesting to examine the effects of PDGF on this differentiation-like process.

Besides the secretion of soluble protein factors, MSCs may also communicate with other cell types in the ischemic heart by cell-derived small circular membrane vesicles, called exosomes and microvesicles (29). Shuttling of intracellular components, such as mRNAs, miRNAs, and proteins via secreted cell membrane vesicles, represents a novel mechanism of cell-to-cell communication (47). Thus transfer of intracellular components from MSCs to other cell types, such as cardiomyocytes, fibroblasts, and cardiac progenitor cells, may reprogram cells in the ischemic myocardium to improve the outcome of acute or chronic heart injury. However, clinical trials have demonstrated that while effective, delivery of MSCs to ischemic myocardium resulted in only modest and short-lived benefits (1, 11). Thus it is critical to elucidate the mechanisms by which MSCs mediate their therapeutic benefits to identify ways in which their functions can be optimized.

Platelet-derived growth factor (PDGF) is a potent mitogen of MSCs (26), modulating MSC function via activation of PDGF receptor (PDGFR)-α and PDGFRβ (45). Upon stimulation, the receptors associate with a number of signaling molecules including MAPKs, JNK, PKB/Akt, and gene-regulating transcription factors such as c-Jun and STAT family members (15, 43). Interestingly, PDGF-induced proliferation was prevented by pretreatment with a JNK inhibitor but not by inhibitors of p38 MAPK or ERK1/2 (26). Overexpression of a dominant-negative form of c-Jun, a downstream transcription factor that is activated by JNK, inhibits PDGF-induced proliferation (55). These observations indicate that JNK signaling plays a key role in PDGF-induced proliferation and migration. In the current study, we also observed that PDGF (10 ng/ml) activated c-Jun in only 10 min. Preconditioning of MSCs with PDGF for 48 h significantly improved MSC-mediated cardioprotection in a mouse model of cardiac ischemia-reperfusion injury (Fig. 2). It is possible that PDGF signaling pathways play a beneficial role in MSC-mediated cardioprotection via c-Jun dependent, transcriptional induction of paracrine factors (Fig. 6).

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In addition to increasing the release of protein factors, PDGF may also stimulate the release of exosomes and microvesicles that deliver miRNAs and mRNAs to cells in the ischemic region via horizontal transfer (54). Such transfer of intracellular genetic material would regulate the proliferation, differentiation, and cell-cycle reentry of resident cardiac progenitor cells, thus restraining tissue injury and leading to tissue self-repair.
(5); 2) suppress ischemic cardiomycyte injury and death; and 3) reprogram fibroblasts and epithelial cells to attenuate ventricular remodeling after ischemic injury (Fig. 6). Indeed, we observed that PDGF modulated the expression of c-Jun-targeted miRNAs, and overexpression of miR-320b, a predicted c-Jun target, abolished the effects of PDGF in improving MSC therapeutic efficacy. Thus PDGF may improve MSC-mediated cardioprotection, at least in part, via downregulation of miR-320b.

Despite our intensive characterization, we failed to detect the paracrine factors suppressed by miR-320. Surprisingly,

Fig. 5. Expression of angiogenic growth factors following PDGF treatment. MSCs were plated at 1 × 10^5 per well in 6-well plates for 24 h before microRNA transfection. After transfection, cells were allowed to recover for 24 h and then treated with PDGF (10 ng/ml) for another 48 h. Supernatants were then collected from each group and analyzed using an angiogenesis array (A) and ELISA (B) assays. MSC expression of VEGF and stromal cell-derived factor-1 (SDF-1) was examined using RT-PCR (B). A, top: representative angiogenic array blots. A, bottom: qualified array signals as pixel densities. B, top: total amounts of VEGF, SDF-1 in the various supernatants and cell numbers at time of supernatant collection. Data are representative of at least 3 independent experiments. B, bottom: MSC expression of VEGF and SDF-1 (with control miR or miR-320 transfection) treated with PDGF. Results are means ± SE; n = 3/group. *P < 0.05 vs. control miR group; ^P < 0.05 vs. miR-320 group.
most paracrine factors we detected were found to be unchanged or be upregulated by miR-320 overexpression. IGFBP-1, -2, and -3, however, were found to be significantly unregulated following miR-320 overexpression. IGFBPs help to lengthen the half-life of circulating IGF-1 and may act to enhance or attenuate IGF-1 signaling (24). A role for PDGF in IGF signaling has been previously suggested by demonstrating PDGF upregulated IGFBP-4 in fetal lung fibroblasts and other IGFs/IGFPs, following hypoxic/ischemic brain injury (4, 39). Whether miR-320 alters PDGF-associated IGF-1 signaling by upregulation of IGFBPs in MSCs warrants future investigation.

It is unlikely that residual PDGF in MSC preparations before infusion caused improved heart function, as observed in the PDGF and PDGF + control miR groups, as the MSC-conferred protective effects were completely abolished in the PDGF + miR-320 group. We also tested whether any molecules were released from MSCs during the PBS washing process. The PBS solution separated from MSCs via brief centrifugation was tested using the Langendorff model. No protective effects were observed in the isolated hearts receiving the PBS solution (data not shown), suggesting that the observed heart protection was from MSCs exclusively and not from PDGF contamination or any other biological molecules released into PBS during cell preparation before infusion.

**Perspectives and Significance**

MSCs hold great promise for treating ischemic heart diseases (2, 37). Several groups have reported that intramyocar-

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Table 1. Signal density on protein array

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<th>Angiogenesis-Related Proteins</th>
<th>Alternative Nomencature</th>
<th>Negative Control miRNA</th>
<th>Negative Control miRNA + PDGF</th>
<th>hsa-miR-320b</th>
<th>hsa-miR-320b + PDGF</th>
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For the protein array, n = 2. miRNA, microRNA; IGFBP, insulin-like growth factor binding protein; MMP-9, matrix metalloproteinase-9; TIMP-1, tissue inhibitor of matrix metalloproteinase.

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**PDGF signal transduction pathway and MSC-mediated cardioprotection**

![Fig. 6. Model of PDGF signal transduction pathway in improving MSC therapeutic efficacy. PDGF binding to its receptor (PDGFR) activates JNK to p-JNK, which then phosphorylates and activates c-Jun. c-Jun then binds to its partner c-Fos to form the activator protein-1 (AP-1) heterodimer that activates specific miRNAs and paracrine factors that stimulate angiogenesis and cardiac repair.](http://ajpheart.physiology.org/...)

*Fig. 6. Model of PDGF signal transduction pathway in improving MSC therapeutic efficacy. PDGF binding to its receptor (PDGFR) activates JNK to p-JNK, which then phosphorylates and activates c-Jun. c-Jun then binds to its partner c-Fos to form the activator protein-1 (AP-1) heterodimer that activates specific miRNAs and paracrine factors that stimulate angiogenesis and cardiac repair.*
dial administration of MSCs reduced inflammation, fibrosis, infarct size, and ventricular remodeling, thus improving cardiac function (17, 18, 28, 44). It is of critical importance to study the precise mechanisms underlying MSC therapeutic effects and use growth factors and molecular interventions to modify MSCs and optimize their ability to repair injured heart tissue. Further experiments, such as investigating the differentiation, long-term survival, microvesicle shedding, and paracrine signaling properties of PDGF-pretreated MSCs to ischemic heart may provide better insight into the mechanism of MSC-mediated cardioprotection and ways to maximize their potential for treating this worldwide-leading cause of mortality.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS
Author contributions: B.X., Y. Luo, and Y.W. performed experiments; B.X., Y. Luo, Y. Liu, and Y.W. analyzed data; B.X., Y. Liu, and Y.W. interpreted results of experiments; B.X. and Y.W. prepared figures; Y. Liu, B.-Y.L., and Y.W. edited and revised manuscript; B.-Y.L., and Y.W. approved final version of manuscript; Y.W. conception and design of research; Y.W. drafted manuscript.

REFERENCES
17. Gneccci M, He H, Liang OD, Melo LG, Morello F, Mu H, Noises X, Zang L, Pratt RE, Ingwall JS, Dzau VJ. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med 11: 367–368, 2005.
27. Karpova AV, Aye MK, Li J, Liu PT, Rhee JM, Kuo WL, Hershenson MB. MEK1 is required for PDGF-induced ERK activation and DNA

Lim SK. Exosome secreted by MSC reduces myocardial ischemia/reper-

Wang J, Homma S, Edwards NM, Itescu S. Neovascularization of
WJ. Impact of intracoronary cell therapy on left ventricular
function in the setting of acute myocardial infarction: a collaborative
systematic review and meta-analysis of controlled clinical trials. J Am

Liu Y, Taylor MW, Edenberg HJ. Model-based identification of cis-

Matsui T, Heidaran M, Miki T, Popescu N, La Rochelle W, Kraus M,
Pierce J, Aaronson S. Isolation of a novel receptor cDNA establishes the

Mirotsou M, Jayawardena TM, Schmeckpeper J, Gnecci M, Dzau
VJ. Paracrine mechanisms of stem cell reparative and regenerative actions

Nagaoka I, Someya A, Iwabuchi K, Yamashita T. Comparative studies
on the platelet-derived growth factor-A and -B gene expression in human

Ng F, Boucher S, Koh K, Sastry KS, Chase L, Lakshmipathy U,
Price WA. PDGF-BB regulates IGF-mediated IGFBP-4 proteolysis

Razmara M, Heldin CH, Lennarsson J. Platelet-derived growth factor-
induced Akt phosphorylation requires mTOR/Rictor and phospholipase
C-gamma1, whereas S6 phosphorylation depends on mTOR/Raptor and

Rodriguez A, Griffiths-Jones S, Ashurst JL, Bradley A. Identification
of mammalian microRNA host genes and transcription units. Genome Res

Sachsenmaier C, Sadowski HB, Cooper JA. STAT activation by the
PDGF receptor requires juxtamembrane phosphorylation sites but not Src

Schmahl J, Raymond CS, Soriano P. PDGF signaling specificity is
mediated through multiple immediate early genes. Nat Genet 39: 52–60,
2007.

Steele A, Steele P. Stem cells for repair of the heart. Curr Opin Pediatr

Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human
mesenchymal stem cells differentiate to a cardiomyocyte phenotype in

Tomasoni S, Longaretti L, Rota C, Morici M, Conti S, Gotti E, Capelli
C, Introna M, Remuzzi G, Benigni A. Transfer of growth factor receptor
mRNA via exosomes unravels the regenerative effect of mesenchymal

Uemura R, Xu M, Ahmad N, Ashraf M. Bone marrow stem cells
prevent left ventricular remodeling of ischemic heart through paracrine

Valencia-Sanchez MA, Liu J, Hannon GJ, Parker R. Control of translation and

Wang M, Tan J, Wang Y, Meldrum KK, Dinarello CA, Meldrum DR.
IL-18 binding protein-expressing mesenchymal stem cells improve myo-
cardial protection after ischemia or infarction. Proc Natl Acad Sci USA

Peng T, Fan GC. Cardiomyocytes mediate anti-angiogenesis in type 2
diabetic rats through the exosomal transfer of miR-320 into endothelial

Wang Y, Aharbanell AM, Herrmann JL, Weil BR, Manukyan MC,
Poynter JA, Meldrum DR. TLR4 inhibits mesenchymal stem cell (MSC)
STAT3 activation and thereby exerts deleterious effects on MSC-mediated

Winkle JA, Gay CG. Regulated expression of PDGF A-chain mRNA in
human saphenous vein smooth muscle cells. Biochem Biophys Res Com-

Xin H, Li Y, Cui Y, Yang JJ, Zhang ZG, Chopp M. Systemic
administration of exosomes released from mesenchymal stem cells
promote functional recovery and neurovascular plasticity after stroke in

Zhan Y, Kim S, Yasumoto H, Namba M, Miyazaki H, Iwao H. Effects
of dominant-negative c-Jun on platelet-derived growth factor-induced
vascular smooth muscle cell proliferation. Arterioscler Thromb Vasc Biol