Platelet-derived growth factor-D promotes fibrogenesis of cardiac fibroblasts

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Zhao T, Zhao W, Chen Y, Li VS, Meng W, Sun Y. Platelet-derived growth factor-D promotes fibrogenesis of cardiac fibroblasts. Am J Physiol Heart Circ Physiol 304: H1719–H1726, 2013. First published April 12, 2013; doi:10.1152/ajpheart.00130.2013.—Platelet-derived growth factor (PDGF)-D is a newly recognized member of the PDGF family with its role just now being understood. Our previous study shows that PDGF-D and its receptors (PDGFR-β) are significantly increased in the infarcted heart, where PDGFR-β is primarily expressed by fibroblasts, indicating the involvement of PDGF-D in the development of cardiac fibrosis. In continuing with these findings, the current study explored the molecular basis of PDGF-D on fibrogenesis. Rat cardiac fibroblasts were isolated and treated with PDGF-D (200 ng/ml medium). The potential regulation of PDGF-D on fibroblast growth, phenotype change, collagen turnover, and the transforming growth factor (TGF)-β pathway were explored. We found: 1) PDGF-D significantly elevated cardiac fibroblast proliferation, myofibroblast (myoFb) differentiation, and type I collagen secretion; 2) matrix metalloproteinase (MMP)-1, MMP-2, and MMP-9 protein levels were significantly increased in PDGF-D-treated cells, which were coincident with increased expressions of tissue inhibitor of metalloproteinase (TIMP)-1 and TIMP-2; 3) PDGF-D significantly enhanced TGF-β1 synthesis, which was eliminated by TGF-β blockade with small-interfering RNA (siRNA); 4) the stimulatory role of PDGF-D on fibroblast proliferation and collagen synthesis was abolished by TGF-β blockade; and 5) TGF-β siRNA treatment significantly suppressed PDGF-D synthesis in fibroblasts. These observations indicate that PDGF-D promotes fibrogenesis through multiple mechanisms. Coelevations of TIMPs and MMPs counterbalance collagen degradation. The profibrogenic role of PDGF-D is mediated through activation of the TGF-β1 pathway. TGF-β1 exerts positive feedback on PDGF-D synthesis. These findings suggest the potential therapeutic effect of PDGFR-β blockade on interstitial fibrosis in the infarcted heart.

Cardiac fibroblasts; proliferation; collagen turnover; transforming growth factor-β1

PLATELET-DERIVED GROWTH FACTOR (PDGF) is one of the numerous growth factors that regulate cell growth and division. In particular, it plays a significant role in blood vessel formation (angiogenesis) (1, 5, 11, 18, 33). The PDGF family is composed of four different polypeptide chains: the traditional PDGF-A and PDGF-B and the more recently discovered PDGF-C and PDGF-D. The biologically active PDGF protein forms disulfide-bonded dimers, including four homodimers (PDGF-AA, PDGF-BB, PDGF-CC, and PDGF-DD) and one heterodimer (PDGF-AB). PDGF isoforms exert their biological activities by activating two structurally related tyrosine kinase receptors, PDGFR-α and PDGFR-β, that activate overlapping signal transduction pathways, including phosphatidylinositol 3-kinase, Ras-MAPK, Src family kinases, and phospholipase Cγ (10, 28).

The functions of PDGF-A and PDGF-B have been well documented and characterized. PDGF-D, however, is the latest member recognized in the PDGF family, whose role is only beginning to be understood. The cellular actions of PDGF-D are mediated by platelet-derived growth factor receptor (PDGFR)-β (14). In normal processes, PDGF-D has been linked to important functions both in embryogenesis and in adult tissues (22). The growing body of literature strongly suggests that PDGF-D may function as a key player in the development and progression of cancers by regulating the processes of cell proliferation, differentiation, apoptosis, migration, invasion, angiogenesis, and metastasis (3, 15, 30, 32). In addition, PDGF-D and PDGFR-β expressions are increased in the region of renal fibrosis, indicating the role of PDGF-D on the development of tissue fibrosis (4).

Following myocardial infarction (MI), fibrogenesis (scar formation) occurring at the site of myocyte loss preserves structural integrity and is essential to the heart’s recovery. Cells responsible for fibrous tissue formation at the site of MI are myofibroblasts (myoFb), i.e., phenotypically transformed fibroblasts. myoFb are not present in the normal heart but appear in the infarcted myocardium. Cells that account for the appearance of myoFb are primarily interstitial fibroblasts. Our recent study has shown that the expression of PDGF-D is significantly increased in the infarcted myocardium, where cells responsible for the expression of PDGF-D are primarily myoFb and endothelial cells, indicating that PDGF-D is involved in cardiac repair by promoting scar formation and angiogenesis (37).

Cardiac remodeling, characterized as interstitial fibrosis, is often developed in the noninfarcted myocardium in the late stage of MI, particularly in hearts with large infarctions, contributing to ventricular dysfunction. Our previous study has shown that PDGF-D expression is elevated in the noninfarcted myocardium in the late stage of MI, and cells expressing PDGF-D are primarily interstitial fibroblasts (37). This finding suggests that PDGF-D contributes to the development of interstitial fibrosis in the infarcted heart.

Our previous study has further revealed that the expression of PDGFR-β is increased in the infarcted heart, colocalized with the elevated expression of PDGF-D. These observations have demonstrated that PDGF-D plays a role in cardiac fibrous tissue formation in an autocrine/paracrine manner. The molecular basis of PDGF-D on cardiac fibrogenic response, however, remains to be elucidated. In the current study, we sought to determine the underlying mechanisms by which PDGF-D stimulates cardiac fibrogenesis. The potential influences of PDGF-D on fibroblast growth, migration, phenotype change, collagen synthesis, and degradation were explored.
Transforming growth factor (TGF)-β1 is a central profibrotic factor that promotes fibroblast proliferation and extracellular matrix production in both physiological and pathological situations, including the infarcted heart (6, 26). There is evidence for a TGF-β-dependent autocrine PDGF-A/PDGFRα signaling loop in sclerodermal skin and lung fibroblasts, suggesting a cross talk between TGF-β and the PDGF-A pathway (29). It has been reported that PDGF-A stimulates the expression of TGF-β receptors in human dermal fibroblasts (8). In the current study, we also tested the possible interaction between PDGF-D and TGF-β.

MATERIALS AND METHODS

Cell culture. Cardiac fibroblasts were isolated from the left ventricle of 6-wk-old male Sprague-Dawley rats (23). Briefly, rats were anesthetized with ketamine/xylazine (87/13 mg/kg ip), and hearts were excised, washed in PBS, and cut into 1-mm³ pieces. The tissue was digested at 37°C in a digestion medium containing a mixture of collagenase B (115 mg/100 ml; Worthington, Lakewood, NJ) and trypsin (50 mg/100 ml; Sigma, St. Louis, MO) for 10 min with constant shaking. Cells from the third to tenth digestions were pooled, and cells were pelleted down. The pellet was resuspended in 5 ml DMEM 10% FBS, seeded into 60-mm dishes, and kept at 37°C in a CO₂ incubator for a preplating period of 150 min. After incubation, unattached cells were discarded, and attached cells were washed and grown in the plating medium. Fibroblasts were confirmed by immunohistochemical vimentin staining (Sigma). Cultures were maintained at 37°C in a humidified 95% air and 5% CO₂ atmosphere. The dose and time response of PDGF-D (0, 10, 50, and 200 ng/ml medium) on collagen production was first determined.

This study was approved by the University of Tennessee Health Science Center Animal Care and Use Committee. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health.

Morphological analysis. The second-passage cells were grown to confluence, then quiesced for 24 h in serum-deprived medium. Cells were then incubated with or without PDGF-D (200 ng/ml medium) for 24 h. Cell morphology was examined under an inverted microscope using phase-contrast illumination.

Cell proliferation assay. Fibroblast proliferation was assessed using bromodeoxyuridine (BrdU) cell proliferation assay kits (Cell Signaling, Danvers, MA), performed according to the manufacturer’s instructions. The third-passage cells were seeded in 96-well plates. After being quiescent for 24 h, the cells were exposed to PDGF-D (200 ng/ml) for 20 h. BrdU (10 μM) was then added, and cells were further incubated for 4 h. At the endpoint of the incubation, the medium was withdrawn, 100 μl/well of fixing solution was added, and the plates were incubated at room temperature for 30 min. Afterward, the solution was aspirated, and the wells were rinsed three times with a washing solution and eventually dried on a paper towel. Anti-BrdU monoclonal antibodies were then added (100 μl/well), and the wells were incubated for 1 h at room temperature. The medium was removed, and the wells were rinsed three times with a washing solution. Horseradish peroxidase (HRP)-conjugated anti-mouse IgG was added, and the plate was incubated at room temperature for 30 min. After three washes, 100 μl of 3,3′,5,5′-tetramethylbenzidine substrate was added to each well, followed by 100 μl of stop solution for 30 min. The absorbance was read at 450 nm in a microplate reader (7). Eight different wells were counted in each group.

Cell migration assay. Fibroblast migration was measured with a modified Boyden’s chamber assay (21). The cell culture inserts containing membranes with 8.0 μm pore size were placed in a 24-well tissue culture plate (ECM508; Millipore, Billerica, MA). The first-passage fibroblasts were quiesced by incubating with serum-free DMEM for 24 h. The cells were then trypsinized, resuspended in DMEM, and seeded into the upper chamber at 1 × 10⁵ cells/well. The lower chamber contains DMEM with 200 ng/ml PDGF-D as a chemoattractant. After incubation for 6 h at 37°C, cells/media were removed from the top side of the insert by pipetting out the remaining cell suspension. The inserts were placed in clean wells containing 400 μl of cell stain for 20 min. The inserts were then rinsed in water, and cotton-tipped swabs were used to remove nonmigratory cells form the interior of the insert. The inserts were transferred to a clean well containing 200 μl of extraction buffer for 15 min. Dye mixture (100 μl) was transferred to a 96-well plate, and optical density was measured at 560 nm.

Western blotting. The effect of PDGF-D on expressions of α-smooth muscle actin (SMA, a marker of myoFb) in cell lysate and type I collagen, matrix metalloproteinase (MMP)-1, MMP-2, MMP-9, tissue inhibitor of metalloproteinase (TIMP)-1, and TIMP-2 proteins in culture medium were assessed by Western blot. The third-passage cells were plated in six-well plates and grown to subconfluence, then quiesced for 24 h in serum-deprived medium. Cells were then incubated with or without PDGF-D (200 ng/ml) for 24 h. Media were concentrated by centrifugation for 30 min in Amicon ultra centrifugal filters (Millipore). Proteins from cells and medium were loaded on gel, subjected to SDS-PAGE (10% polyacrylamide gel), and transferred onto nitrocellulose membranes using a Bio-Rad Mini Trans Blot electrophoretic transfer unit. Membranes were blocked for non-specific protein with 5% nonfat dry milk in TBS and then probed overnight at 4°C with primary antibodies against α-SMA (Sigma), type I collagen, MMP-1, MMP-2, MMP-9, TIMP-1, or TIMP-2 (Millipore). Membranes were then washed three times (10 min/wash) with TBS with 0.05% Tween 20 to remove unbound antibodies, and then further incubated with appropriate HRP-conjugated secondary antibody (1:2,000). Membranes were developed by a chemoluminescence reagent kit (Amersham Pharmacia Biotech, Piscataway, NJ) according to the manufacturer’s protocol. The amount of protein detected was assessed by means of quantitative densitometry analysis with a computer image analyzing system (36). Six different samples were tested in each group.

RT-PCR. TGF-β1 and PDGF-D gene expressions were assessed by RT-PCR. Total RNA was extracted from cells using the Trizol Reagent (Life Technologies, Carlsbad, CA). The RNA was treated with DNase using the TURBO DNA-free kit (Ambion, Austin, TX) and purified with the RNAeasy Mini Kit (Qiagen USA, Valencia, CA). The purification, concentration, and integrity of the RNA were examined with a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE) and Agilent Bioanalyzer (Agilent Technologies, Foster City, CA). cDNA was prepared from total RNA using a High-Capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA). The gene-specific probe and primer sets for TGF-β1 were designed using Universal ProbeLibrary Assay Design software (https://www.roche-applied-science.com). TGF-β1 and PDGF-D mRNA levels were detected and analyzed on a LightCycler 480 System (Roche, Indianapolis, IN) under the following cycling conditions: 1 cycle at 95°C for 5 min, 45 cycles at 95°C for 10 s, 60°C for 30 s, and 72°C for 10 s. The PCR mix contained 0.2 μl of 10 μM primers, 0.1 μl of 10 μM Universal library probe, 5 μl of LC 480 master mix (2X), 2 μl of cDNA template, and RNase-free water to 10 μl. TATA box-binding protein was selected as an endogenous quantity control. Fold change was used to compare the difference between groups (36). Six samples were tested in each group.

ELISA. The effect of PDGF-D on TGF-β1 production in cardiac fibroblasts was determined by ELISA. Supernatants were collected for TGF-β1 detection with a commercial ELISA kit (R&D, Minneapolis, MN). Briefly, TGF-β1 standards, positive control, and 50 μl activated samples were added to the 96-well plate coated with TGF-β1 antibody. The plate was incubated for 2 h at room temperature and then washed by wash buffer (400 μl) four times. TGF-β1 conjugate (100
μl) was then added to the plate. After 2 h of incubation, the plate was washed again four times as above. Substrate solution (100 μl) was added to the plate, followed by the stop solution (100 μl). The optical density of each well was measured within 30 min using a microplate reader set to 450 nm with a correction of 540 nm. Eight samples were included in each group.

TGF-β1 blockade. TGF-β1 blockade with TGF-β1 small-interfering RNA (siRNA) was used to detect whether the regulation of PDGF-D on fibrogenesis is via TGF-β1. At a confluence of 50%, fibroblasts were quiesced for 24 h with serum-free DMEM. TGF-β1 siRNA was introduced to the cells through Lipofectamine RNAiMAX reagent (Invitrogen, Grand Island, NY). The transfection was performed as described by the manufacturer’s manual. The final concentration of siRNA was 30 nM (13). At 6 h after the treatment, PDGF-D was administered to the cells with a final concentration of 200 ng/ml. Cells and medium were collected after 18 h of treatment. Untreated cells and PDGF-D treatment alone served as normal and PDGF-D controls, respectively. Proliferation assay and mRNA and protein expression were analyzed as described above.

Statistical analysis. Statistical analysis of cell proliferation, RT-PCR, Western blot, and ELISA data were performed using analysis of variance or Student’s t-test. Values are expressed as means ± SE, with P < 0.05 considered significant. Multiple group comparisons among controls and each group were made by Scheffe’s F-test.

RESULTS

First we determined the dose and time for PDGF-D treatment of fibroblasts. Cells were treated with PDGF-D with 10, 50, and 200 ng for 24 h. Collagen synthesis demonstrated a dose-dependent pattern (Fig. 1A), with cells receiving 200 ng of PDGF-D treatment inducing the greatest collagen expression. Cells were also given PDGF-D (200 ng) for 4, 12 and 24 h, which revealed a time-dependent pattern of collagen production (Fig. 1B). Cells treated with PDGF-D for 24 h generated the most collagen compared with other time points. Based on these findings, the following data were collected from cells treated with 200 ng of PDGF-D for 24 h.

**PDGF-D stimulates fibroblast-to-myofb differentiation.** myofb differentiation from fibroblasts is a critical component of cardiac repair following MI. myofb are activated fibroblasts and contain the characteristics of both fibroblasts and smooth muscle cells. The hallmark of myofb is the expression of α-SMA. To investigate the possible role of PDGF-D in myofb differentiation, we analyzed levels of α-SMA using Western blot. We found that fibroblasts treated with PDGF-D expressed significantly higher levels of α-SMA compared with untreated fibroblasts (Fig. 2A).

Myofb are irregular in shape, which is morphologically different from the normal spindle-shaped fibroblasts (Fig. 2B). The current study showed that, compared with untreated fibroblasts, PDGF-D treatment led to morphological changes, resulting in irregularly shaped cells characteristic of myofb (Fig. 2C). These data imply that PDGF-D promotes fibroblast differentiation to myofb.

**PDGF-D increases fibroblast proliferation.** The potential role of PDGF-D on fibroblast proliferation was examined by the BrdU cell proliferation assay. After 24 h of PDGF-D treatment, fibroblast proliferation was increased several times compared with untreated control cells (Fig. 3). The data indicate that PDGF-D plays a stimulatory role in fibroblast proliferation.

**PDGF-D stimulates fibroblast migration.** The potential role of PDGF-D on fibroblast migration was examined by Boyden’s chamber assay. After 24 h of PDGF-D treatment, fibroblast migration was significantly increased compared with untreated control cells (Fig. 3).

**PDGF-D promotes type I collagen synthesis.** Collagen production is the key function of fibroblasts. Collagens are secreted proteins released into the interstitial space of tissue. In cultured fibroblasts, collagen is secreted into the culture medium. Type I collagen is the major collagen isoform. Via Western blot, we observed low levels of type I collagen in the medium of the control cells. PDGF-D treatment significantly increased type I collagen content in the culture medium compared with the control group (Fig. 3). This observation suggests that PDGF-D promotes type I collagen synthesis.

**PDGF-D upregulates MMP-1, MMP-2, and MMP-9 expressions.** MMP-1 is an interstitial collagenase, which breaks down type I, II, and III collagen. MMP-2 and MMP-9 are gelatinases, which degrade type IV and V collagen. MMPs are secreted proteins, and Western blot detection revealed that MMP-1, MMP-2, and MMP-9 levels were very low in
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PDGF-D elevate TGF-β1 expression. TGF-β1 is a profibrogenic mediator produced by several types of cells, including fibroblasts. Detected by RT-PCR, low levels of TGF-β1 were detected in both control and PDGF-D-treated fibroblasts. However, PDGF-D treatment significantly increased TGF-β1 expression compared to control cells. This indicates that PDGF-D stimulates the expression of TGF-β1 in fibroblasts.

PDGF-D promotes TIMP-1 and TIMP-2 expressions. TIMPs are glycoprotein peptidases involved in inhibition of extracellular matrix degradation. TIMPs are also secreted proteins that are released in the tissue interstitial space from cells and in the medium in culture. Western blot detection showed that fibroblasts in the control group produced very low levels of TIMP-1 and TIMP-2. PDGF-D treatment significantly increased TIMP-1 and TIMP-2 levels in the medium compared with that of the control group (Fig. 5). The observation indicates that PDGF-D upregulates TIMP synthesis in fibroblasts.

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mRNA were detected in untreated fibroblasts. TGF-β1 mRNA was significantly increased in cells treated with PDGF-D compared with control cells (Fig. 6).

TGF-β1 is a secreted protein. Total TGF-β1 protein level in the culture medium was measured by ELISA. In the control cells, TGF-β1 protein level was barely detectable in the medium. However, in the PDGF-D-treated group, TGF-β1 protein level was significantly higher than the control cells (Fig. 6). These observations indicate that PDGF-D stimulates TGF-β1 synthesis and release by fibroblasts.

Effect of TGF-β blockade on PDGF-D-induced fibrogenesis.

To determine whether the role of PDGF-D on fibrogenesis is via activation of the TGF-β pathway, we blocked TGF-β1 synthesis with TGF-β1 siRNA. TGF-β1 siRNA treatment significantly eliminated PDGF-D-induced TGF-β synthesis at both mRNA and protein levels (Fig. 5). TGF-β1 siRNA treat-
ment further blocked PDGF-D-induced fibroblast proliferation and type I collagen synthesis (Fig. 6).

**Effect of TGF-β blockade on PDGF-D expression.** To determine the potential regulation of TGF-β1 on PDGF-D synthesis, fibroblasts were treated with TGF-β1 siRNA. Compared with untreated controls, PDGF-D treatment significantly decreased PDGF-D gene expression. Additionally, TGF-β1 siRNA further suppressed PDGF-D gene expression (Fig. 7).

**DISCUSSION**

The key aspects relevant to cardiac fibrosis include fibroblast proliferation, migration, differentiation, and extracellular matrix (ECM) turnover. The current study explored the molecular mechanisms of PDGF-D-induced fibrogenesis. First, we detected whether PDGF-D plays a role in cardiac fibroblast growth, migration, and phenotype changes. Our data have shown that PDGF-D treatment markedly increased cardiac fibroblast proliferation. This observation indicates that PDGF-D accelerates cardiac fibroblast growth, promoting fibrogenesis. PDGF-D is involved in the growth of multiple cell types. The regulatory role of other PDGF isoforms on cell proliferation has been well recognized. Numerous studies have shown that PDGF-A and PDGF-B stimulate cell proliferation in the various pathological conditions and promote tissue remodeling, healing, and uncontrolled angiogenesis in cancer (1, 27, 31, 34, 35). Conversely, downregulation of PDGF-D inhibits endothelial cell proliferation and suppresses angiogenesis (21, 22, 33).

Cell migration is a central process in the maintenance of tissue structure during embryonic development, wound healing, and immune responses. Cells often migrate in response to specific external signals. PDGF-A and -B have been shown to stimulate fibroblast migration (21). The current study further reveals that PDGF-D significantly increases cardiac fibroblast migration. Thus, PDGF-D has overlapping function with other PDGF isoforms in stimulating fibroblast proliferation and migration.
PDGF-D has been reported to play a role in cell differentiation in certain pathological conditions. It induces osteoclast differentiation in intraosseous tumors (12, 24). In addition, PDGF-D has been shown to play important roles in the acquisition of the epithelial-mesenchymal transition phenotype of cancer cells (16, 25). In the repairing heart, fibroblasts undergo phenotype transformation and differentiate into myoFb (25). These cells are responsible for fibrous tissue formation at the site of MI and facilitate cardiac repair. Our previous study has shown that myoFb expressed PDGF-D and PDGFR-β in the infarcted myocardium (37). In the current study, we explored the potential role of PDGF-D on fibroblast phenotype change. Our data have shown that fibroblasts receiving PDGF-D expressed α-SMA and developed the morphological features of myoFb. These results indicate that PDGF-D advances myoFb appearance, thus facilitating fibrogenesis.

Second, we detected whether PDGF-D regulates the function of fibroblasts. The key role of fibroblasts is to produce collagen, the major component of fibrous tissue. So far, 29 types of collagen have been identified and described. Over 90% of the collagen in the body, however, is type I collagen. Type I collagen is also the main element of cardiac fibrosis (25). Our current study has revealed that PDGF-D significantly elevates type I collagen secretion by cardiac fibroblasts, indicating the stimulatory role of PDGF-D on type I collagen synthesis. The previous reports have shown that other PDGF isoforms also promote collagen synthesis in the pathological conditions, including liver and kidney fibrosis (2, 9). Thus, PDGF-D has an equivalent effect with other PDGF isoforms in stimulating collagen synthesis, contributing to fibrous tissue formation.

Fibrous tissue accumulation relies on the balance between collagen synthesis and degradation. Elevated collagen synthesis and/or suppressed collagen degradation lead to tissue fibrosis. We determined the potential regulation of PDGF-D on collagen degradation. MMPs play a central role on collagen degradation. MMPs are secreted proteins that are produced by various types of cells, including fibroblasts (20). Our data have shown that PDGF-D treatment significantly elevated MMP-1, MMP-2, and MMP-9 expression and increases MMP-1, MMP-2, and MMP-9 production in fibroblasts.

However, after secretion into the interstitial space, MMP activities are controlled by TIMPs. TIMPs function as an important regulatory brake on MMP activity by inhibition of the active species, thereby suppressing collagen degradation (19). TIMPs comprise a family of four subtypes and are produced in various cells, including fibroblasts. The current study has shown that PDGF-D treatment significantly increased TIMP-1 and TIMP-2 synthesis in fibroblasts, which despite increased MMP production, suppresses MMP activity and overall limits collagen degradation.

Third, we tested the possible interaction between PDGF-D and TGF-β. PDGF-D treatment significantly elevates TGF-β1 expression in both gene and protein levels in cultured fibroblasts, indicating that PDGF-D enhances TGF-β synthesis. Our study further shows that TGF-β1 blockade suppressed PDGF-D synthesis in fibroblasts, suggesting that TGF-β1 promotes PDGF-D expression in fibroblasts.

Taken together, PDGF-D upregulates fibroblast proliferation, myoFb differentiation, and collagen synthesis by induction of the TGF-β1 signaling pathway. Cardiac repair/remodeling appear in both the infarcted and noninfarcted myocardium (17, 24). Our previous study has shown that PDGF-D and PDGFR-β are elevated in the infarcted heart, temporally and spatially coincident with fibrosis. Findings from the current study indicate that PDGF-D promotes scar formation in the infarcted myocardium, which facilitates cardiac repair and recovery. PDGF-D is also involved in the development of interstitial fibrosis in the noninfarcted myocardium. Further studies are required to determine whether blockade of PDGFR in the late stage of MI suppresses cardiac fibrosis and improves ventricular function. Clinical data are needed to properly evaluate the efficacy of anti-PDGF-D-based antifibrotic treatment in the infarcted heart.

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**DISCLOSURES**

The contents of this work are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

**AUTHOR CONTRIBUTIONS**

Author contributions: T.Z., W.Z., Y.C., V.L., and W.M. performed experiments; T.Z., W.Z., and Y.C. analyzed data; T.Z., W.Z., and Y.S. interpreted results of experiments; T.Z., W.Z., and Y.S. prepared figures; T.Z., W.Z., and Y.S. approved final version of manuscript; Y.S. conception and design of research; Y.S. drafted manuscript.

![Fig. 7. TGF-β stimulates PDGF-D synthesis. Compared with controls, PDGF-D treatment significantly decreased PDGF-D gene expression. Cotreatment of TGF-β1 siRNA further suppressed PDGF-D expression in fibroblasts. *P < 0.05 vs. controls (CTL).](image-url)
REFERENCES


