Arteriosclerosis, Thrombosis, and Vascular Biology

BASIC SCIENCES

Diabetes Impaired Ischemia-Induced PDGF (Platelet-Derived Growth Factor) Signaling Actions and Vessel Formation Through the Activation of Scr Homology 2-Containing Phosphatase-1

Clément Mercier,* Tristan Brazeau,* Jérémy Lamoureux, Elizabeth Boisvert, Stéphanie Robillard, Valérie Breton, Martin Paré, Andréanne Guay, Farah Lizotte, Marc-Antoine Despatis, Pedro Geraldes

OBJECTIVE: Critical limb ischemia is a major complication of diabetes characterized by insufficient collateral vessel development and proper growth factor signaling unresponsiveness. Although mainly deactivated by hypoxia, phosphatases are important players in the deregulation of proangiogenetic pathways. Previously, SHP-1 (Scr homology 2-containing phosphatase-1) was found to be associated with the downregulation of growth factor actions in the diabetic muscle. Thus, we aimed to gain further understanding of the impact of SHP-1 on smooth muscle cell (SMC) function under hypoxic and diabetic conditions.

APPROACH AND RESULTS: Despite being inactivated under hypoxic conditions, high glucose level exposure sustained SHP-1 phosphatase activity in SMC and increased its interaction with PDGFR (platelet-derived growth factor receptor)-β, thus reducing PDGF proangiogenic actions. Overexpression of an inactive form of SHP-1 fully restored PDGF-induced proliferation, migration, and signaling pathways in SMC exposed to high glucose and hypoxia. Nondiabetic and diabetic mice with deletion of SHP-1 specifically in SMC were generated. Ligation of the femoral artery was performed, and blood flow was measured for 4 weeks. Blood flow reperfusion, vascular density and maturation, and limb survival were all improved while vascular apoptosis was attenuated in diabetic SMC-specific SHP-1 null mice as compared to diabetic mice.

CONCLUSIONS: Diabetes and high glucose level exposure maintained SHP-1 activity preventing hypoxia-induced PDGF actions in SMC. Specific deletion of SHP-1 in SMC partially restored blood flow reperfusion in the diabetic ischemic limb. Therefore, local modulation of SHP-1 activity in SMC could represent a potential therapeutic avenue to improve the proangiogenic properties of SMC under ischemia and diabetes.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: hypoxia ■ ischemia ■ muscle cells ■ peripheral arterial disease ■ platelet-derived growth factor

Diabetic peripheral arterial disease (PAD) is a source of high morbidity and cost, and amputation is one of the most feared complications among patients.[1] In addition, the outcomes after surgical revascularization are often worse in patients with diabetes, which are 10 to 16 times more susceptible to undergo major amputation.[2] Peripheral vascular pathology is characterized by atherosclerosis-induced occluded vessels, which reduces distal blood flow reperfusion. Collateral vessel formation, a normal response to occlusion of a large artery, is seriously impaired in patients with diabetes rendering the tissue downstream more susceptible to critical ischemia.[3] Several abnormalities in the angiogenic response to ischemia have been documented in the

Correspondence to: Pedro Geraldes, PhD, Université de Sherbrooke, 3001 12e Ave Nord, Sherbrooke, Québec, Canada J1H 5N4. Email pedro.geraldes@usherbrooke.ca
*C. Mercier and T. Brazeau contributed equally.
The Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/ATVBAHA.121.316638.
For Sources of Funding and Disclosures, see page 2481.

Arterioscler Thromb Vasc Biol is available at www.ahajournals.org/journal/atvb

Nonstandard Abbreviations and Acronyms

eNOS	endothelial nitric oxide synthase
ERK	extracellular signal-regulated kinase
FBS	fetal bovine serum
FGF2	fibroblast growth factor 2
Flk-1	fetal liver kinase 1
GFP	green fluorescent protein
HG	high glucose
HIF-1α	hypoxia-inducible factor-1α
KDR	kinase insert domain
NG	normal glucose
PDGFR	platelet-derived growth factor receptor
PTP	protein tyrosine phosphatase
SHP-1	Scr homology 2-containing phosphatase-1
SMC	smooth muscle cell
VEGF	vascular endothelial growth factor

diabetic state involving complex interactions of multiple growth factors and vascular cells.[4] Interestingly, hypoxia and oxidants, 2 major inducers of proangiogenic factors, are elevated in diabetes. However, despite the presence of hypoxia and oxidative stress, expression of angiogenic factors remains reduced in the peripheral limbs.[5–7] Although extensive animal studies with VEGF (vascular endothelial growth factor) have been done to improve collateral vessel formation and vascular healing in a diabetic state, local treatment by gene therapy of VEGF in the settings of diabetes did not provide significant benefits in terms of clinical outcomes as it has been shown in the absence of diabetes.[8,9]

Besides VEGF, it is well recognized that the expression of PDGF (platelet-derived growth factor) is important in the formation of collateral vessels in response to ischemia.[10] PDGF is critical for vascular smooth muscle cell (SMC) and pericyte recruitment to a variety of vascular beds, including the heart, lung, kidney, retina, brain, adipose tissue, and skeletal muscle.[11] Insufficient contribution of PDGF provokes naked, leaky, and fragile nascent capillaries that can easily rupture resulting in vessel regression.[12] Mice lacking either PDGF-B or PDGFR (PDGF receptor)-β have demonstrated a deep reduction of pericyte/SMC blood vessel coverage leading to extensive leakage of the vasculature, hemorrhage, and edema formation.[13,14] These experiments suggest that PDGF is essential to stabilize newly formed blood vessels and to prevent vessel regression. We have reported that hyperglycemia-induced deregulation of PDGF signaling, and to a lesser extent its expression, was associated with pericyte loss and poor collateral vessel formation following ischemia in diabetic mice.[7,15,16] Since proangiogenic signaling is regulated by a fine balance between tyrosine phosphorylation and dephosphorylation of tyrosine kinase receptors, any slight alteration in the equilibrium between protein tyrosine kinase and PTP (protein tyrosine phosphatase) activity will trigger abnormalities in the angiogenic response to ischemia. PTPs have progressively emerged as potent players in growth factor unresponsiveness in vascular cells leading to the progression of several vascular complications of diabetes.[17] Previous studies have reported that hyperglycemia-induced modification of PTP expression or activity has been shown to deregulate the insulin, PDGFR-β, and VEGFR2 pathways contributing to nephropathy,[18] retinopathy,[16] or PAD.[19]

Among PTPs, SHP-1 (Scr homology 2-containing phosphatase-1) plays an important role in the regulation of an array of mechanisms that are involved in the homeostasis and development of various processes. Diabetes was found to upregulate SHP-1 expression and activity resulting in retinal perivascular cell apoptosis.[16] In the context of PAD, we have reported that SHP-1 markedly decreased VEGFR2 activation in endothelial cells.[19] Although SHP-1 expression was found to be increased in muscle of diabetic mice,[7] the definite proof that enhanced SHP-1 activity in the vascular SMC lineage contributes to poor angiogenic response to ischemia causing diabetic PAD remains unresolved.

Highlights

- The specific deletion of SHP-1 (Scr homology 2-containing phosphatase-1) in smooth muscle cell improves blood flow reperfusion and vessel density in the diabetic ischemic limb.
- Hyperglycemia sustained SHP-1 phosphatase activity and interaction with PDGFR (platelet-derived growth factor receptor)-β in hypoxic smooth muscle cell.
- PDGF signaling and proangiogenic actions are restored by the inactivation of SHP-1 in smooth muscle cell exposed to high glucose and hypoxia.

METHODS

The authors declare that all supporting data are available within the article (and its Data Supplement).

Reagents and Antibodies

Primary antibodies for immunoblotting were purchased from commercial sources: actin (horseradish peroxidase; I-19), GAPDH horseradish peroxidase (V18), SHP-1 (C19), PDGF-β (N30), and Bcl-xL (H-62) from Santa Cruz Biotechnology Inc (Dallas, TX); protein kinase B (Akt), phospho-Akt (D9E), phospho-PDGFR-β (Y1009), PDGFR-β, phospho-ERK (extracellular signal-regulated kinase), ERK and secondary antibody of anti-rabbit and anti-mouse

peroxidase-conjugated from Cell Signaling (Danvers, MA); anti-α smooth muscle actin from Abcam (Toronto, ON); and anti-CD31 from BD Bioscience (Mississauga, ON). Secondary antibodies for immunofluorescence Alexa-488 conjugated anti-rabbit IgG and Alexa-594 conjugated anti-rat were purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Fetal bovine serum (FBS), PBS, penicillin-streptomycin, endothelial cell growth supplement, and DMEM low glucose (31600-034) were obtained from Invitrogen (Burlington, ON). All other reagents used, including streptozotocin, PDGF-BB, ethylenediaminetetraacetic acid, BSA, d-glucose, d-mannitol, leupeptin, phenylmethylsulfonyl fluoride, aprotinin, heparin, and Na_3VO_4 were purchased from Sigma-Aldrich (St Louis, MO).

Cells Culture and Adenoviral Infection

Vascular SMCs were isolated from freshly harvested male bovine aortas that were obtained from a local abattoir (only male bovines were available at the local abattoir). Cells from passages between 2 and 7 were then trypsinized and cultured in DMEM 5% FBS, 1% penicillin-streptomycin. Cells were exposed to DMEM 0.1% FBS containing normal glucose (NG; 5.6 mmol/L + 19.4 mmol/L mannitol to adjust osmotic pressure) or high glucose (HG; 25 mmol/L) levels up to 48 hours. To mimic the ischemia environment, cells were placed into the hypoxic incubator (1% O_2) for the last 16 hours and then stimulated with PDGF-BB (2 ng/mL) for 5 minutes. Adenoviral vectors containing the dominant-negative (Ad) form of SHP-1 (Ad-dnSHP-1) or GFP (green fluorescent protein; Ad-GFP) as control were used to infect SMC as we have previously reported.[20,21]

Proliferation Assay

Vascular SMCs were seeded in a 96-well plate at 3000 cells per well for 4 hours. Cells were then exposed to NG or HG concentrations for 48 hours with or without PDGF-BB (10 ng/mL) for another 24 hours and placed into the hypoxic incubator for the last 16 hours. Cells were then fixed in 4% paraformaldehyde for 5 minutes, rinsed twice in PBS, and stained with DAPI (4′,6-diamidino-2-phenylindole; Sigma-Aldrich) at 0.001 mg/mL for 10 minutes. Cells were counted using the NIS-Elements software of Nikon Eclipse Ti microscope and reported as the number of cells/mm^2 for analysis.

Migration Assay

Vascular SMCs were seeded at 20 000 cells per chamber of Ibidi's culture-insert placed into an 8-well chamber slide (Ibidi) for 4 hours. Cells were then exposed to NG or HG for 48 hours. Following this exposure, each insert was removed, and the wells were filled with 1 mL of either NG or HG for another 24 hours, with or without PDGF-BB (10 ng/mL). During the last 16 hours of exposure, SMCs were placed into the hypoxic incubator (1% O_2). Cell migration was evaluated using a Live Cell Imaging chamber adapted to Nikon Eclipse Ti microscope in which images were taken immediately after removing the insert and at the end of the experiment (16 hours later). Analysis was performed with ImageJ software by measuring the difference of occupied surface immediately after insert removal and following 16 hours of PDGF treatment in NG or HG conditions.

Immunoprecipitation and Phosphatase Assay

Immunoprecipitation of SHP-1 was performed as previously described.[20] Phosphatase activity of SHP-1 was assessed following its immunoprecipitation by using the Tyrosine phosphatase assay system (V2471, Promega, Madison, WI) according to the manufacturer's instructions as we previously described.[18]

Animal and Experimental Design

The SM22-rtTA inducible male mice were purchased from The Jackson Laboratories (JAX stock no. 006875; FVB/N-Tg(Tagln-rtTA)E1Jwst/J) and were crossbreed for 8 generations with female C57BL6J (JAX stock no. 000664). Mice were then bred with the (tetO)7-Cre (JAX stock no. 006234) and the *Ptpn6*$^{flox/flox}$ mice (JAX stock no. 008336; B6.129P2-Ptpn6tm1Rsky/J). Male mice were rendered diabetic by intraperitoneal streptozotocin injection (50 mg/kg in 0.05 mol/L citrate buffer, pH 4.5; Sigma) on 5 consecutive days after overnight fasting at 7 weeks of age; control mice were injected with citrate buffer. Only male animals were used in our study since it is known that female animals are resistant to STZ-induced diabetes. After 2 months of diabetes, mice were fed with doxycycline (625 mg/kg; standard laboratory diet; Dox, Envigo, TD.08541) for a period of 2 weeks prior femoral artery ligation. Bodyweight was evaluated, and blood glucose was measured by Glucometer (Contour, Bayer Inc). Indirect calorimetry study was performed using Promethion High-Definition Room Calorimetry System (GA3, Sable Systems. Las Vegas, NV). Animals were acclimated to cages for 2 days followed by 5 days of data acquisition under standard laboratory diet to record the basal metabolic parameters. A 12-hour light/dark cycle (6:00–18:00) was maintained throughout the experiment. Data acquisition and instrument control were coordinated by MetaScreen version 2.3.0, and the raw data were processed using ExpeData version 1.8.4 (Sable Systems). All experiments were conducted in accordance with the Canadian Council of Animal Care and University of Sherbrooke and the NIH Guide for the Care and Use of Laboratory Animals.

Assessment of SMC-Specific SHP-1 Deletion

Pulmonary endothelial cells were extracted from the lungs of each group of mice. Lungs were sliced into 1 to 2 mm pieces and incubated at 37 °C for 1 hour in 0.2% collagenase type 1 followed by tissue homogenization and passage through a 40 µm cell strainer. Following centrifugation, pellets were resuspended in 1 mL DMEM/0.1% BSA and incubated with CD31-conjugated Dynabeads for 30 minutes at 4 °C. Beads were washed with DMEM/0.1% BSA using a magnetic support and resuspended in growth medium (DMEM 10% FBS, 1% penicillin-streptomycin, endothelial cell growth supplement 50 µg/mL, and heparin 1 mg/mL). Cells were plated in 100 mm dishes coated with type 1 collagen 0.1 mg/mL in 0.02N acetic acid. Peritoneal macrophages were harvested with cold PBS from mice 4 days after intraperitoneal injection of 2 mL of 3% thioglycolate broth. Following centrifugation, cell pellets were resuspended and plated in 1 mL DMEM/10% FBS. Mouse heart and aorta were harvested upon euthanasia, and a fraction was lysed in immunoprecipitation buffer before immunoblot analyses.

Hindlimb Ischemia Model

Blood flow was assessed in nondiabetic and 18-week-old diabetic mice with or without the specific deletion of SHP-1 in SMCs (nondiabetic mice; diabetic mice; SMC-SHP-1$^{+/+}$, SMC-SHP-1$^{-/-}$). Animals were anesthetized by inhalation of Isoflurane USP (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) at a concentration of 5% (initiation) and then maintained at 1% to 2% during the whole surgical procedure (≈20 minutes). To reproduce PAD, ischemia of one lower leg was induced by ligation of the femoral artery as we previously described.[7,19]

Laser Doppler Perfusion Imaging and Physical Examination

Hindlimb blood flow was measured using a laser Doppler perfusion imaging (PIMIII) system (Perimed Inc). Consecutive perfusion measurements were obtained by scanning the region of interest (hindlimb and foot) of anesthetized animals. Measurements were performed preartery and postartery ligation, and additionally on postoperative days 7, 14, 21, and 28. To account for variables that affect blood flow temporally, the results at any given time were expressed as a ratio against simultaneously obtained perfusion measurements of the right (ligated) and left (nonligated) limb. The euthanasia was performed following laser Doppler perfusion imaging at day 28 by exsanguination via the left ventricle under deep anesthesia (Isoflurane USP, inhalation at a concentration of 5%). Tissue necrosis was scored to assess mice that had to be euthanized during the course of the experiment due to necrosis/loss of toes.

Histopathology

Right and left adductor muscles from nondiabetic mice; diabetic mice; SMC-SHP-1$^{+/+}$, SMC-SHP-1$^{-/-}$ mice were harvested for pathological examination, and sections were fixed in 4% paraformaldehyde (VWR Canada) for 18 hours and then transferred to 70% ethanol. Paraformaldehyde-fixed tissues were embedded in paraffin and 4 µm sections were stained with hematoxylin & eosin or used for immunofluorescence. The entire tissue sample of the muscle fiber structure of 6 mice per group was visualized under a light microscope on a Nikon Eclipse Ti.

Immunofluorescence and TUNEL

Cross-sections of adductor muscles of each group were blocked at room temperature for an hour with 10% goat serum and then exposed overnight to primary antibodies (CD31 [1:50] and α-smooth muscle actin, 1:200) followed by 1 hour incubation with secondary antibody dilute 1:400. Vessels with a diameter <10 µm and ranging from 10 to 30 µm were separately counted and considered as capillaries and arterioles, respectively. Vascular density was normalized by muscle fiber density. Apoptotic cells were detected using the TACS 2 Tdt-Fluor in situ apoptosis detection kit (Trevigen, Gaithersburg, MD) according to the manufacturer's instructions, and as we previously described.[19]

Immunoblot Analysis

Adductor muscles or cells were lysed in RIPA buffer containing protease inhibitors (1 mmol/L phenylmethylsulfonyl fluoride, 2 µg/mL aprotinin, 10 µg/mL leupeptin, 1 mmol/l Na_3VO_4, and 1 mmol/L NaF). Protein amounts were measured with DC kit (BioRad). Primary antibodies were incubated overnight at 1:1000 in 5% skim milk or in 5% BSA for phospho-PDGFR. Antigens were detected using anti-rabbit or mouse horseradish peroxidase-conjugated antibody 1:10 000 (or 1:2000 for phospho-PDGFR) and detected with Luminata forte western horseradish peroxidase substrate (Millipore, Etobicoke, ON). Protein content quantification was performed using computer-assisted densitometry with ImageLab imaging software (Chemidoc, BioRad).

Quantitative Polymerase Chain Reaction Analysis

Real-time polymerase chain reaction was performed to evaluate mRNA expressions of genes of interest in ischemic adductor muscles as we previously described.[7,19] Polymerase chain reaction primers are listed in Table I in the Data Supplement. GAPDH expression was used for normalization.

Human Tissue Study

Following written consent, muscle tissue was obtained from patients with or without diabetes at the time of the lower limb amputation. SHP-1 mRNA and protein levels were measured from the gastrocnemius muscle tissue of nondiabetic and diabetic patients by quantitative polymerase chain reaction and immunoblot assay as described above. The study was approved by the Research Ethics Board of the Centre Intégré Universitaire de Santé et de Services Sociaux de l'Estrie - Centre Hospitalier Universitaire de Sherbrooke and was conducted in accordance with the Declaration of Helsinki.

Statistics

The data were shown as mean±SD or SEM (blood flow measurements only) for each group. Statistical analysis was performed by unpaired 1-way (Figures 1, 2, 3, and 4B, 4C, 4D, 4E, and 4F) and 2-way (Figures 5 and 6A through 6D) ANOVA followed by Tukey test correction for multiple comparisons. Statistical analysis was performed by unpaired Student *t* test and Mann-Whitney *U* test for Figure 2D and Figure 6E and 6F, respectively. Data in each group were checked for normal distribution using D'Agostino and Pearson normality test based on α=0.05. All results were considered statistically significant at $P<0.05$.

RESULTS

High Glucose Level Exposure Prevented Hypoxia-Induced PDGF Action by Maintaining SHP-1 Phosphatase Activity

During the process of angiogenesis, pericytes and SMC are later required to proliferate and migrate to surround and stabilize the newly formed blood vessels. Hypoxia is also known to enhance PDGF-induced SMC proliferation and migration.[22] Therefore, we have investigated PDGF-BB-induced SMC angiogenic properties using cultured vascular SMC exposed to NG and HG levels

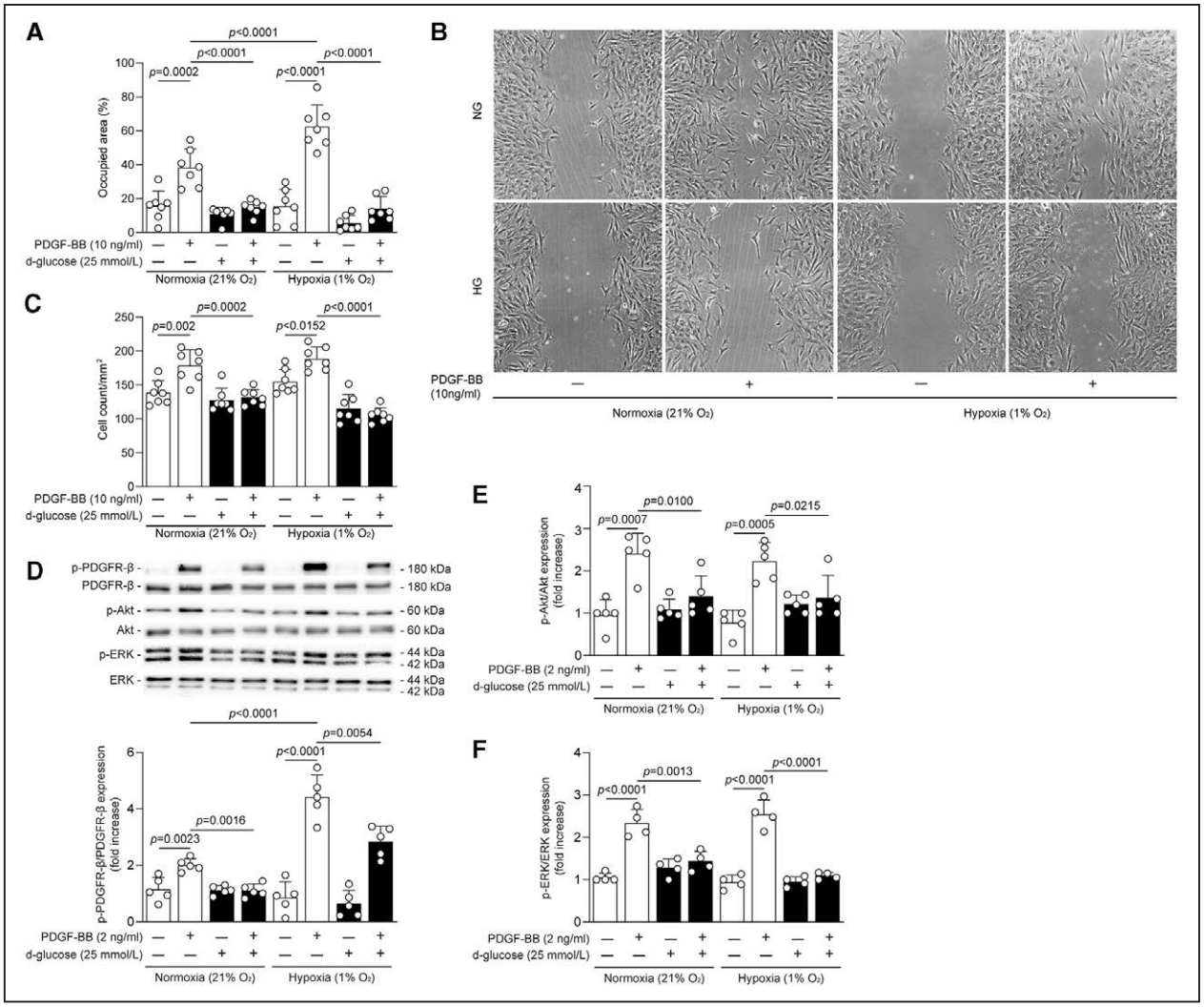

Figure 1. Combination of high glucose level and hypoxia exposure prevented PDGF (platelet-derived growth factor)-BB-induced smooth muscle cell (SMC) migration, proliferation, and signaling.
SMCs were incubated with normal glucose (NG; 5.6 mmol/L + 19.4 mmol/L of mannitol; white bars) or high glucose (HG; 25 mmol/L; black bars) for 48 h and then stimulated with PDGF-BB for another 24 h (**A**–**C**) or 5 min (**D**–**F**). SMCs were exposed to normoxia (21% O_2) or hypoxia condition (1% O_2) for the last 16 h of treatment. **A**, Representative images of the cell migration assay using the Ibidi's insert. **B**, The percentage of the surface area occupied by the SMC was quantified. **C**, Cells were fixed and stained DAPI (4′,6-diamidino-2-phenylindole). SMC were then counted using the NIS-Elements software of Nikon eclipse Ti microscope. **D**, Protein expression of PDGFR-β, Akt, and ERK (extracellular signal-regulated kinase) phosphorylation. **D**–**F**, The densitometry quantitation was measured. Results are shown as mean±SD of 7 (**A**–**C**), 5 (**D**), 5 (**E**), and 4 (**F**) biologically independent cell experiments. Two-way ANOVA with Tukey post hoc test.

under normoxic and hypoxic conditions. During the last 24 hours, PDGF-BB was added into the media, and vascular SMCs were placed in an normoxic (21%) or hypoxic chamber (1% O_2). Vascular SMC migration was significantly increased by 145% under normoxia (P=0.0002) and by 3-fold under hypoxia (P<0.0001) following PDGF-BB stimulation, an effect that was inhibited completely in cells exposed to HG concentrations (Figure 1A and 1B). Concurrently, treatment with PDGF-BB enhanced vascular SMC proliferation by 29% in normoxia (P=0.002) and by 30% in hypoxia condition (P=0.0152), which was totally blunted by HG level exposure (Figure 1C). Mechanistically, binding of PDGF-BB to its receptor, PDGFR-β, promotes Akt and ERK activation to subsequently trigger cell migration and proliferation. In accordance with migration and proliferation data, PDGF-induced phosphorylation of PDGFR-β, Akt, and ERK was significantly reduced in vascular SMC exposed to HG levels as compared to NG conditions (Figure 1D through 1F). Interestingly, the phosphorylation of PDGFR-β was markedly enhanced under hypoxia as compared to normoxia condition (P<0.0001, Figure 1D). In normoxia, PTP, such as SHP-1, is known to downregulate the PDGFR-β signaling pathway.[23] However, in hypoxic condition, PTP is usually downregulated.[22] In relation with these latest studies, PDGF-induced PDGFR-β phosphorylation and

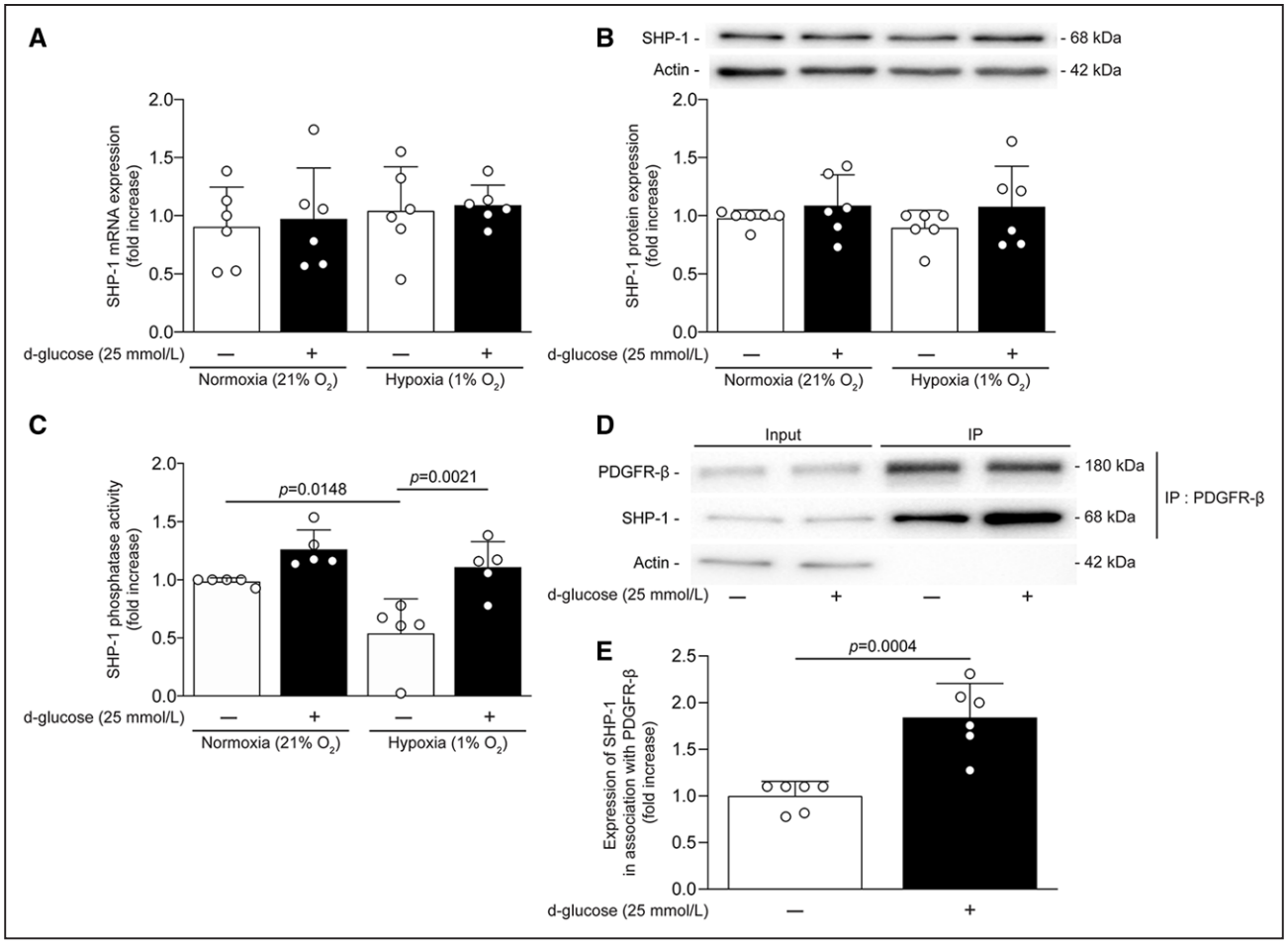

Figure 2. High glucose concentration exposure sustained SHP-1 (Scr homology 2-containing phosphatase-1) phosphatase activity and binding interaction with PDGFR (platelet-derived growth factor receptor)-β in hypoxic smooth muscle cell (SMC). SMCs were treated in normal glucose (NG; 5.6 mmol/L + 19.4 mmol/L of mannitol; white bars) or high glucose (HG; 25 mmol/L; black bars) for 48 h and exposed or not to hypoxia (1% O_2) for the last 16 h of treatment. SHP-1 (**A**) mRNA and (**B**) protein expression was measured. **A**, GAPDH gene was used for mRNA normalization and (**B**) the densitometry was quantified. **C**, Phosphatase activity of SHP-1. **D**, Coimmunoprecipitation assay of PDGFR-β followed by SHP-1 immunoblot analysis. Input was 1% (5 µg) of the total amount of immunoprecipitated lysate (500 µg). The densitometry quantitation was measured. Results are shown as mean±SD of 5 (**C**) and 6 (**A**, **B**, and **D**) biologically independent cell experiments. **C**, One-way ANOVA with Tukey post hoc test. **D**, Unpaired Student *t* test.

subsequent SMC migration were significantly increased by 2-fold and 1.5-fold in hypoxic SMC as compared to normoxic cells, respectively ($P<0.0001$; Figure IA, IB, and ID in the Data Supplement). Our results indicated that both high glucose levels and hypoxia exposure did not influence SHP-1 mRNA and protein expression (Figure 2A and 2B). However, whereas hypoxia reduced SHP-1 phosphatase activity by 45% ($P=0.0148$) in NG conditions, exposure to HG concentrations under hypoxia exhibited a 2.05-fold increase ($P=0.0021$) in SHP-1 phosphatase activity as compared to NG conditions (Figure 2C). Coimmunoprecipitation assays were performed to demonstrate if SHP-1 can interact with PDGFR-β. Our results demonstrated that SHP-1 remained capable of binding PDGFR-β during hypoxia, an effect that was significantly increased in HG conditions as compared to NG concentrations (Figure 2D and 2E and Figure I in the Data Supplement). Taken together, these data support the notion that despite being exposed to hypoxia, HG levels increased SHP-1 activity and interaction with PDGFR-β, consequently impaired PDGF actions in vascular SMC.

Inhibition of SHP-1 Restored PDGF Signaling Actions on Vascular SMC Migration and Proliferation

To investigate the precise involvement of SHP-1 in vascular SMC function during hypoxia, cells were transfected with an adenoviral vector of the dominant-negative form of SHP-1. In GFP-overexpressed cells, exposure to HG levels reduced PDGF-BB actions under hypoxia on vascular SMC migration by 64% (Figure 3A and 3B) and proliferation by 100% (Figure 3C) as compared to NG conditions. Interestingly, overexpression of the dominant-negative form of SHP-1 (Ad-dnSHP-1)

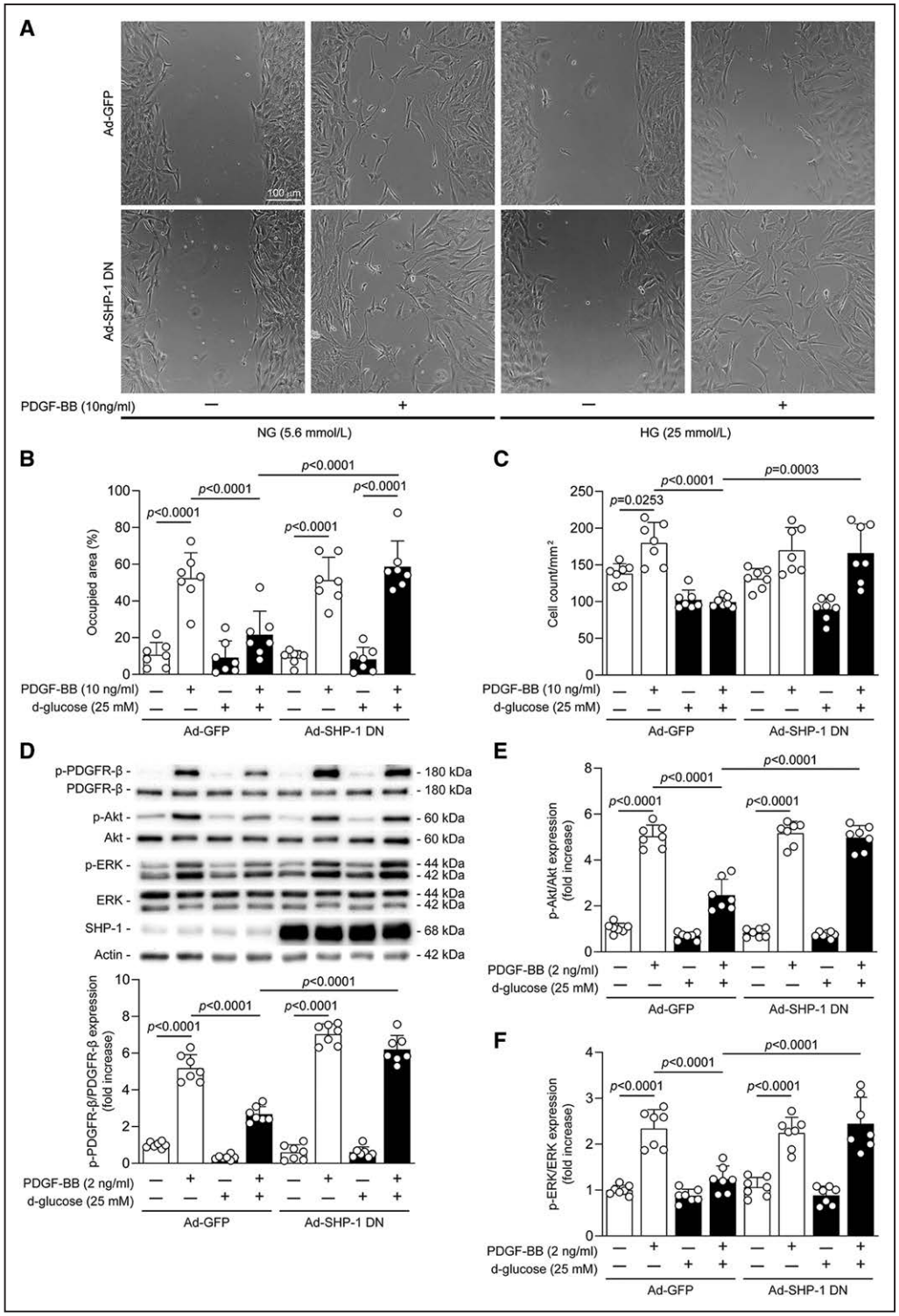

Figure 3. Inhibition of SHP-1 (Scr homology 2-containing phosphatase-1) prevented reduction of PDGF (platelet-derived growth factor) signaling and actions caused by high glucose level and hypoxia exposure in smooth muscle cell (SMC). SMCs were infected with either Ad-GFP (green fluorescent protein) or Ad-dnSHP-1 before being exposed to either normal glucose (NG; 5.6 mmol/L + 19.4 mmol/L of mannitol; white bars) or high glucose (HG; 25 mmol/L; black bars) for 48 h and then stimulated with PDGF-BB for another 24 h (**A**–**C**) or 5 min (**D**–**F**). SMCs were exposed to hypoxia condition (1% O_2) for the last 16 h of treatment. **A**, Representative images of the cell migration assay using the Ibidi's insert. **B**, The percentage of the surface area occupied by the SMC was quantified. **C**, Cells were fixed and stained DAPI (4′,6-diamidino-2-phenylindole). SMCs were then counted using the NIS-Elements software of Nikon Eclipse Ti microscope. **D**, Protein expression of PDGFR-β, Akt, and ERK (extracellular signal-regulated kinase) phosphorylation. **D**–**F**, The densitometry quantitation was measured. Results are shown as mean±SD of 7 biologically independent cell experiments. Two-way ANOVA with Tukey post hoc test.

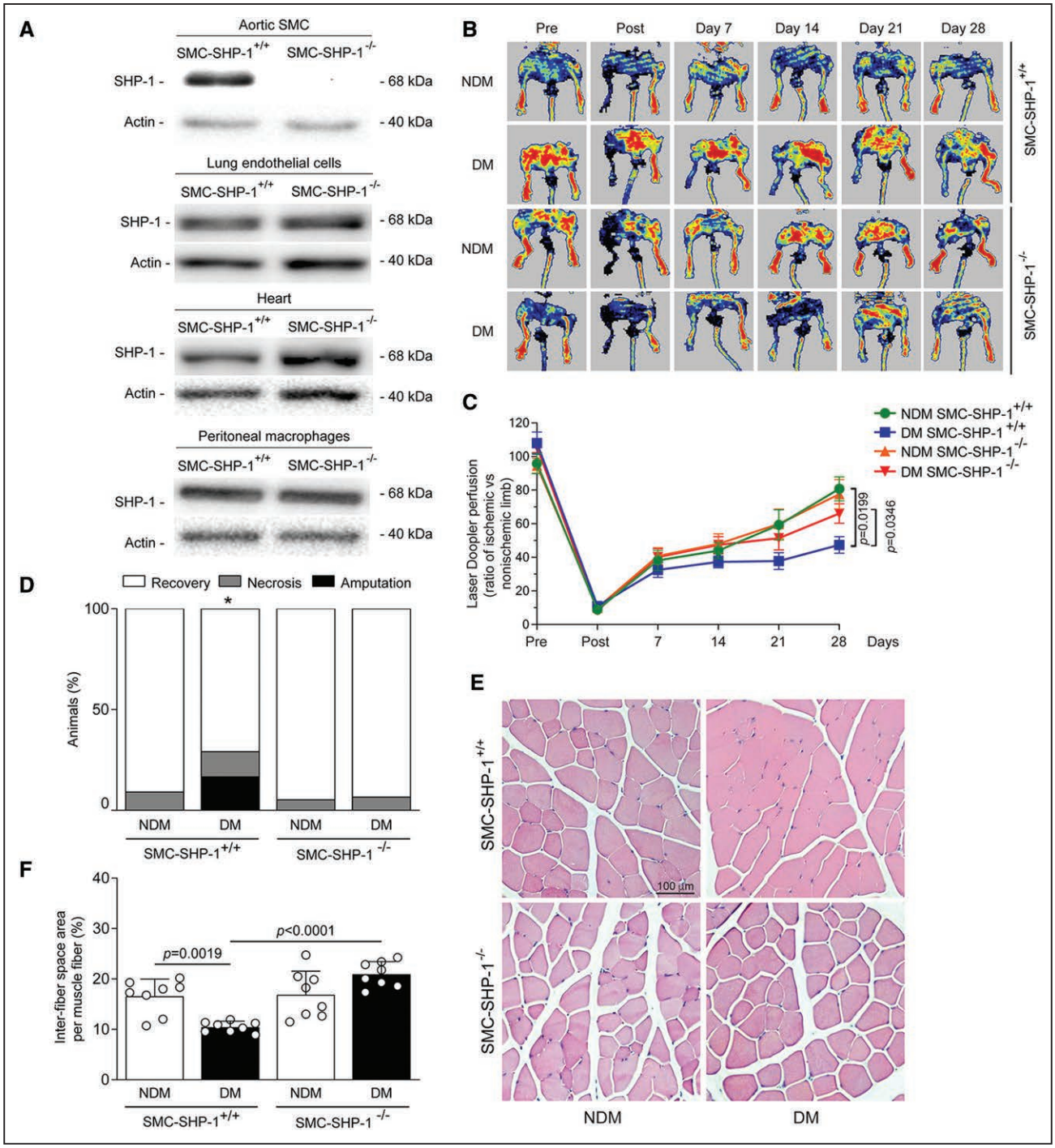

Figure 4. Ablation of SHP-1 (Scr homology 2-containing phosphatase-1) in smooth muscle cell (SMC) improved blood flow reperfusion and maintained muscle structure following critical limb ischemia in diabetes.

A, SHP-1 protein expression in isolated aorta, pulmonary endothelial cells, heart, and peritoneal macrophages from SMC-SHP-1$^{+/+}$ and SMC-SHP-1$^{-/-}$ mice. **B**, Laser Doppler imaging and (**C**) reperfusion analysis of nondiabetic (NDM) and diabetic (DM) SMC-SHP-1$^{+/+}$ and SMC-SHP-1$^{-/-}$ mice. **D**, Morphological and observational analysis of toe necrosis and amputation postsurgery. **E**, Structural analysis of the ischemic muscles stained with hematoxylin and eosin (H&E) and (**F**) quantification of the space between muscle fibers of the ischemic adductor muscles of NDM (white bars) and DM (black bars) SMC-SHP-1$^{+/+}$ and SMC-SHP-1$^{-/-}$ mice. Results are shown as mean±SEM of 12–15 mice per group (**B** and **C**) and as mean±SD of muscle fibers of 8 mice per group (**E** and **F**). One-way ANOVA with Tukey post hoc test.

fully restored vascular SMC migration by 125% (Figure 3A and 3B) and proliferation by 79% (Figure 3C) as compared to GFP-overexpressed SMC in HG concentrations. In line with our migration and proliferation data, the overexpression of the dominant-negative form of SHP-1 in vascular SMC exposed to HG levels and hypoxia reestablished PDGF-induced phosphorylation of PDGFR-β by 98%, Akt by 80%, and ERK by 70% which

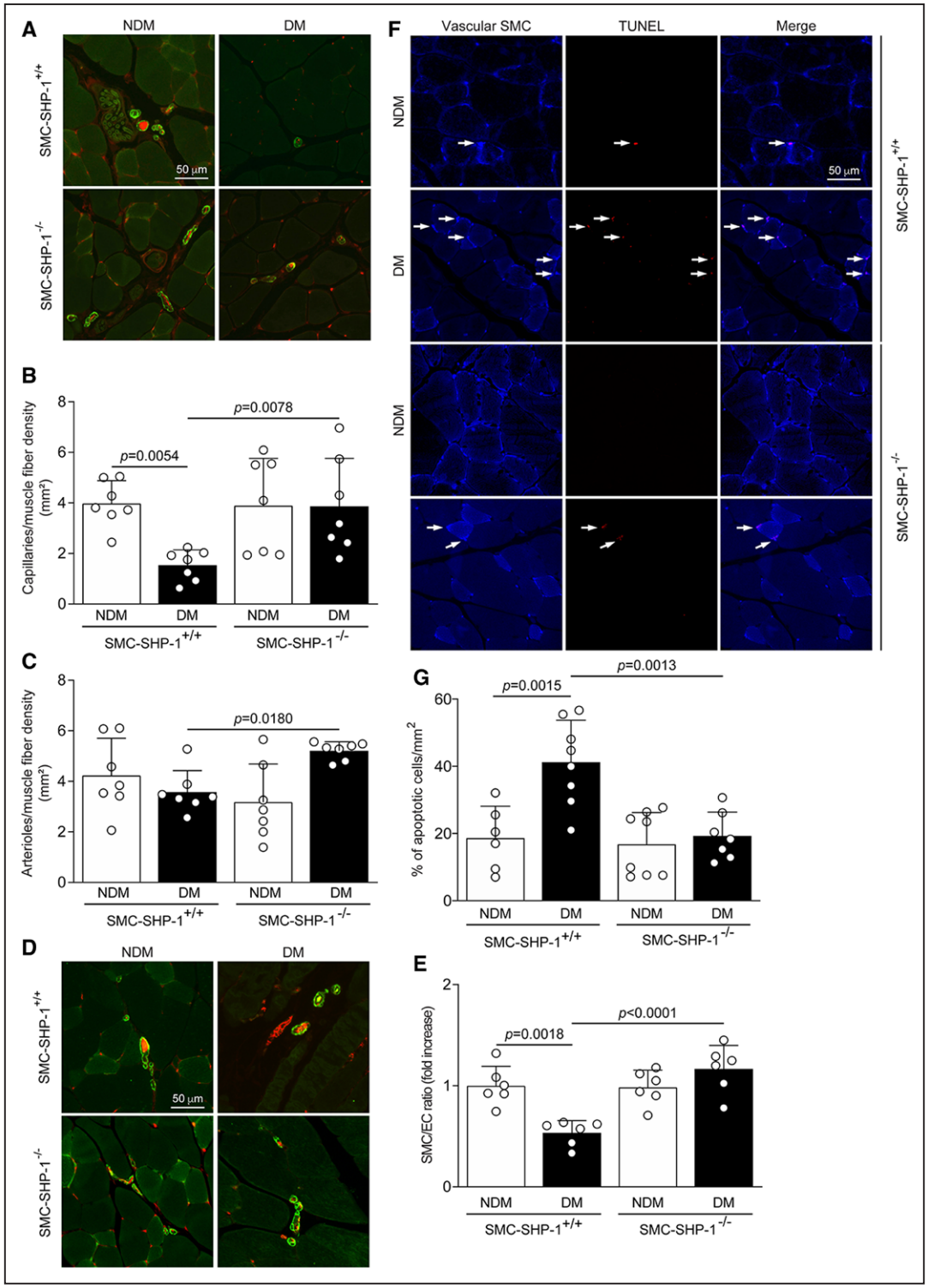

Figure 5. Deletion of SHP-1 (Scr homology 2-containing phosphatase-1) in smooth muscle cell (SMC) enhanced small vessel formation and SMC/endothelial cell (EC) ratio and prevented vascular SMC apoptosis in diabetes.
Immunofluorescence images of (**A** and **D**) endothelial cells (CD31; yellow), α-smooth muscle actin (blue). Quantification of the number of (**B**) capillaries smaller than 10 µm and (**C**) arterioles (10–30 µm) was normalized by muscle fiber density as well as (**E**) the endothelial cells/smooth muscle cell ratio in the ischemic adductor muscle of nondiabetic mice (NDM; white bars) and diabetic mice (DM; black bars) smooth muscle cell (SMC)-SHP-1$^{+/+}$ and SMC-SHP-1$^{-/-}$ mice. **F**, Immunofluorescence of vascular cells (α-smooth muscle actin; blue), apoptotic positive cells (red) and (**G**) quantification of the number of apoptotic positive vascular SMC per mm^2 in the ischemic adductor muscle of NDM (white bars) and DM (black bars) SMC-SHP-1$^{+/+}$ and SMC-SHP-1$^{-/-}$ mice. Results are shown as mean±SD of 7 mice per group (**A**–**C**), 6 mice per group (**D** and **E**), and 6–8 mice per group (**F** and **G**). One-way ANOVA with Tukey post hoc test.

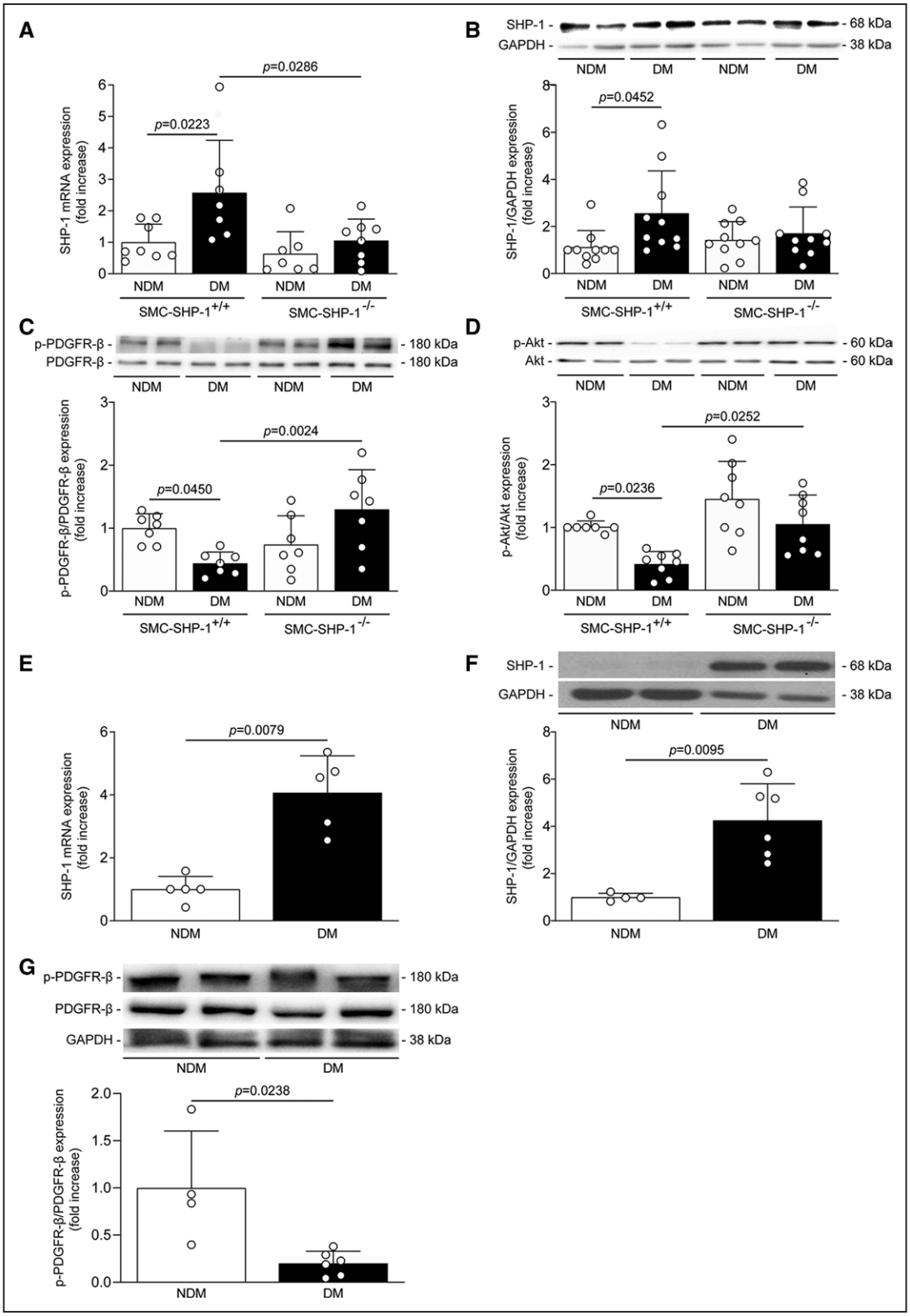

Figure 6. Elevated SHP-1 (Scr homology 2-containing phosphatase-1) expression in ischemic muscle of diabetic mice and patients reduced PDGFR (platelet-derived growth factor receptor)-β activation.
Expression of (**A**) SHP-1 mRNA, (**B**) SHP-1 protein, (**C**) PDGFR-β, and (**D**) Akt phosphorylation in ischemic muscle of nondiabetic (NDM; white bars) and diabetic (DM; black bars) smooth muscle cell (SMC)-SHP-1$^{+/+}$ and SMC-SHP-1$^{-/-}$ mice. mRNA (**E**) and protein expression of (**F**) SHP-1 and (**G**) phospho-PDGFR-β in the gastrocnemius muscle of patients that suffered from an amputation. **A** and **E**, GAPDH gene was used for mRNA normalization. **B**, **C**, **D**, and **F**, The densitometry quantitation was measured. Results are shown as mean±SD of 10 mice per group (**B**), 7–8 mice per group (**A**, **C**, and **D**) as well as 5 (**E**) and 4–6 (**F** and **G**) patients per group. **A**–**D**, One-way ANOVA with Tukey post hoc test. **E**–**G**, Mann-Whitney test.

were blunted in control GFP-overexpressed vascular SMC (Figure 3D through 3F).

Ablation of SHP-1 in SMCs Restored Blood Flow and Prevented Limb Necrosis Following Ischemia and Diabetes

As a definitive proof that SHP-1 plays a role in SMC function during ischemia and diabetes, we have generated an SMC-specific SHP-1 deficient mouse by crossing the *Ptpn6*$^{flox/flox}$ mice with the Tagln-rtTa and the Cre recombinase under the control of the reverse tetracycline responsive promoter element. The expression of SHP-1 was completely abolished in aortic vascular SMC of mice following doxycycline treatment (Figure 4A). To certify that the deletion was specific to SMC, we have isolated other tissues, and no differences in SHP-1 expression were observed in lung endothelial cells, heart, and peritoneal macrophages between control and SMC-specific SHP-1 deficient mice (Figure 4A). Despite the absence of the SHP-1 gene in SMC, body weight loss, and elevated fasting blood glucose levels caused by diabetes were similar between the 2 groups (Table II in the Data Supplement) and had no effect on mouse behavior and basic physiological parameters, such as food intake, energy expenditure, or sleeping time (Table III in the Data Supplement). The validation of the inducible SMC-specific SHP-1 null mouse model allowed us to pursue with the hindlimb ischemia experiments. Therefore, ligation of the femoral artery was performed following doxycycline diet, and blood flow reperfusion of the lower limb was followed for 4 weeks using laser Doppler imaging (Figure 4B). Diabetic SMC-SHP-1$^{+/+}$ mice displayed 45% blood flow reperfusion after 4 weeks as compared to 73% in nondiabetic mice (P=0.0199). The deletion of SHP-1 in SMCs of diabetic animals improved blood flow reperfusion to 63% (P=0.0346; Figure 4C). This was associated with reduced limb necrosis and amputation as compared to untreated diabetic mice (Figure 4D). Furthermore, the structure of the ischemic muscle (measured by the muscle fiber interspace) in diabetic SMC-specific SHP-1 null mice was preserved as compared to diabetic SMC-SHP-1$^{+/+}$ mice (Figure 4E and 4F).

Decreased Vascular Density and SMC/EC Ratio, as Well as Increased Vascular SMC Apoptosis, Were Prevented With SHP-1 Deletion Specifically in SMC of Diabetic Ischemic Muscle

We measured the vascular density of the ischemic muscle of all groups of mice. As expected, diabetes prevented collateral vessel formation and reduced the number of neocapillaries (<10 µm) in the ischemic muscle of SMC-SHP-1$^{+/+}$ mice by 60% (P=0.0054) as compared to nondiabetic littermate controls (Figure 5A and 5B). Excision of the SHP-1 gene in SMC led to a significant increase in the number of capillaries, from 1.5 to 3.9 capillaries/mm^2, suggesting improved revascularization of the ischemic diabetic lower limb (P=0.0078; Figure 5A and 5B). In addition, we observed a significant increase in arteriole (10–30 µm diameter) density in the ischemic muscle of diabetic SMC-SHP-1$^{-/-}$ mice as compared to diabetic controls (P=0.0180; Figure 5C). This observation was further supported by the presence of SMC surrounding endothelial cells as a marker of vessel maturation was reduced by half in the muscle of diabetic mice (P=0.0018). Interestingly, the deletion of SHP-1 in SMC significantly (P<0.0001) enhanced the ratio of CD31-positive vessels that were also surrounded by SMCs to a similar level as the nondiabetic control mice, reflecting increase maturation and stabilization of small vessels (Figure 5D and 5E). Another consequence of impaired angiogenesis in diabetic mice could be attributed to vascular cell apoptosis during ischemia. Since we observed elevated necrosis and amputation in diabetic mice that retained SHP-1 gene, measurements of vascular apoptosis were performed in the ischemic muscles of each group. Ischemic muscle of diabetic SMC-SHP-1$^{+/+}$ mice exhibited a 2-fold increase (P=0.0015) of positive apoptotic vascular cells (Figure 5F and 5G) in parallel with a 50% decrease expression of anti-apoptotic protein Bcl-xL (P=0.0009; Figure II in the Data Supplement) as compared to nondiabetic SMC-SHP-1$^{+/+}$ mice. Interestingly, removing the SHP-1 gene in SMC was able to completely prevent (P=0.0013) the number of apoptotic vascular cells (Figure 5F and 5G) and preserve Bcl-xL expression (P=0.0356, Figure II in the Data Supplement) to a similar extent as the nondiabetic SMC-SHP-1$^{+/+}$ mice.

Deletion of SHP-1 Specifically in SMC Restored In Vivo PDGFR-β and Akt Phosphorylation in Diabetes

We have reported that SHP-1 expression was increased in the ischemic muscle of diabetic mice.[7] Our current study confirmed our previous observation that both SHP-1 mRNA and protein expression were elevated by diabetes following femoral artery ligation by 2.6-fold (P=0.0223) and 2.3-fold (P=0.0452), respectively (Figure 6A and 6B). The ablation of the SHP-1 gene only in SMC was able to significantly reduce diabetes-induced SHP-1 mRNA levels and decrease its protein expression by 59% in the ischemic muscle of SMC-SHP-1$^{-/-}$ mice as compared to diabetic SMC-SHP-1$^{+/+}$ mice (Figure 6A and 6B). We have then measured the activation of PDGFR-β and downstream effectors of the PDGF signaling pathway. The phosphorylation of PDGFR-β and Akt were blunted in ischemic muscle of diabetic SMC-SHP-1$^{+/+}$ mice as compared to nondiabetic

SMC-SHP-1$^{+/+}$ mice (Figure 6C and 6D). Interestingly, diabetes-induced inhibition of PDGFR-β phosphorylation was fully reestablished in the ischemic muscle of diabetic SMC-SHP-1$^{-/-}$ mice (Figure 6C and 6D). Beside PDGF signaling, several proangiogenic genes are downregulated by diabetes in the ischemic muscle, including PDGF, PDGFR-β, VEGF-A, Flk-1 (fetal liver kinase 1)/KDR (kinase insert domain receptor), and eNOS (endothelial nitric oxide synthase), while others (HIF-1α [hypoxia-inducible factor-1α], SDF-1 [stromal cell-derived factor 1], and FGF2 [fibroblast growth factor 2]) were unaffected (Figure III in the Data Supplement). Knocking out the SHP-1 gene specifically in SMC was able to restore essential SMC-specific genes related to a proper angiogenic response, which included PDGFR-β (P=0.0020) and VEGF-A (P=0.0259) mRNA expression, but without affecting diabetes-induced reduction of eNOS and Flk-1/KDR mRNA expression (Figure III in the Data Supplement). Interestingly, both the enhanced expression of SHP-1 and the reduced PDGFR-β activation in diabetic rodents were corroborated in human. Indeed, SHP-1 mRNA and protein expression were significantly elevated in the ischemic muscle of diabetic patients as compared to nondiabetic patients that underwent lower extremity amputation (Figure 6E and 6F). Concurrently, the phosphorylation of PDGFR-β was significantly reduced in the hypoxic muscle of diabetic patients as compared to nondiabetic individuals suggesting a deregulation of PDGF signaling (Figure 6G).

DISCUSSION

Over the last several years, the development of new strategies that favor the creation of new blood vessels as an alternative noninterventional approach to treat PAD has gained strong interest. However, revascularization therapies involving the intramuscular injection of genes coding for growth factors or cells (mainly progenitor cells) have not shown significant clinical benefits in diabetes.[24] Therefore, further investigations are needed to find new therapeutic targets to enhance vessel formation. In our current study, we have investigated the role of SHP-1 as a negative regulator induced by diabetes that prevents SMC natural function in the context of ischemia. By removing the SHP-1 gene specifically in SMC, blood flow reperfusion, vascular cell apoptosis, and SMC response to PGDF were restored in ischemic muscle of diabetic mice. In addition, our in vitro experiments demonstrated that high glucose level exposure preserved both SHP-1 activity and binding ability under hypoxia and inhibition of SHP-1 restored PDGF-induced proliferation, migration, and signaling pathway activation in SMC exposed high glucose concentrations.

Newly formed blood vessels require that SMC surround the endothelium to become functional arteries. This step is essential to maintain vessel capacity to control vascular leakage and diameter under elevated pressure. In addition, vascular mural cells participate to vessel stabilization during muscle regeneration.[25] Therefore, favoring SMC proliferation and migration by several factors, PDGF being the most predominant and potent factor, is a key process of vessel maturation. PDGF-B or PDGFR-β deficient mice die during late gestation from cardiovascular complications.[26,27] Until they reach E16-19, these mice appear healthy and normal. Following that period, edema formation, dilation of large blood vessels and the heart, and rupture of capillaries start to appear suddenly.[26] We and others have reported that variation in PDGF signaling, rather than expression, was linked to morphological abnormalities in the retina and critical limb ischemia in animal models of diabetes.[7,16] Our current study clearly demonstrated, that despite the hypoxia environment, the activation of SHP-1 by diabetes in SMC leads to inhibition of PDGF signaling action along with reduced SMC proliferation and migration capacity.

Other factors have been shown to be important for SMC proliferation and migration such as the HIF-1α and FGF2.[28] Although our data did not demonstrate any variation in HIF-1α and FGF2 mRNA expression, previous studies indicated that the loss of hypoxic signals in vascular SMC impaired the ability of mice to recover from ischemia following femoral artery ligation. Borton et al[29] have reported that the deletion of the HIF-β subunit in SMC altered SMC proliferation and migration as well as compromised limb reperfusion. Others have shown that FGF2 is an important element in the arteriogenic response of SMC.[30] However, growth factor monotherapy of FGF2 has shown disappointing outcomes in the context of diabetes due to FGF2 glycation.[31]

Due to the importance of SMC in the development of restenosis and atherosclerosis, it has been proposed that hyperglycemia, through the activation of protein kinase C and oxidative stress, enhanced SMC proliferation as a contributing factor to accelerated formation of atherosclerosis in diabetes.[32–34] However, others have shown that PDGF-BB expression is decreased in diabetic ischemic muscles[15] and that exposure of methylglyoxal, a glucose metabolite, reduced VEGF and PDGF expression in SMC.[35,36] Our data are in agreement with these latest studies since combined hypoxia and high glucose level exposure reduced PDGF signaling action on SMC proliferation and migration. A potential explanation for the discrepancy of our data with previous authors is that in our study, SMCs were exposed to hypoxia, a condition that replicates to some extent the hindlimb ischemic environment. Other mechanisms have been proposed to explain the unresponsiveness of SMC to PDGF in diabetes. Previous studies indicated that various microRNA are deregulated by hyperglycemia and could be a potential explanation for PDGF inhibition.[37] While it has been shown that microRNA can regulate SHP-1 expression,[38] it remains to be investigated if diabetes influenced SHP-1 activity in SMC through the modulation of microRNA expression.

Although some preclinical studies of growth factor administration have shown some beneficial effects, it becomes increasingly evident that single growth factor administration is insufficient as compared to approaches using multiple factors.[12,39] Interestingly, our data indicated that restoring SMC function in the diabetic ischemic muscle was sufficient to reestablish blood flow reperfusion. One possible reason for this observation is that SMCs are able to secrete several important growth factors that are critical for the angiogenic process such as VEGF. Indeed, our results indicated that inhibition of SHP-1 in SMC increased VEGF mRNA expression in the context of diabetes, suggesting that localized secretion of VEGF that could have supported endothelial cell functionalities for efficient limb revascularization. However, this hypothesis need further investigation if this increase gene expression translates into elevated VEGF secretion as well as to characterize the role of SHP-1 in SMC and endothelial cell interaction during the angiogenic process.

SHP-1 is known to play a critical role in the regulation of protein tyrosine kinase receptor activation. Notably, our group reported that high glucose level treatment enhanced SHP-1 expression in pericytes, affecting PDGF signaling.[16] Another group reported that SMC exposed to oxidized low-density lipoprotein rather than high glucose exposure modulated SHP-1 expression.[40] However, these previous studies were not performed under hypoxia, a condition known to deactivate the catalytic activity of PTP.[41] Indeed, chronic hypoxia enhanced PDGFR-β phosphorylation, SMC proliferation, and migration upon PDGF stimulation through decreased expression and activity of PTP.[22] Our data also confirmed that SHP-1 activity is decreased in hypoxia condition. Therefore, our study provides for the first time evidence that despite being exposed to an environment favoring SHP-1 inactivation, high glucose levels sustained SHP-1 activity to inhibit PDGF actions. While inhibition of SHP-1 as a therapeutic target has been previously shown in a nondiabetic condition[42] and diet-induced obesity model,[43] systemic inhibition of SHP-1 may not be suitable. Therefore, local treatment to modulate SHP-1 expression and activity will depend on the objective of increasing (in the case of critical limb ischemia) or decreasing (in the case of restenosis) SMC proliferation and migration in diabetes.

In conclusion, our current study provided additional knowledge on how diabetes deregulates PDGF action in the context of hypoxia/ischemia in the diabetic muscle. In addition, our study highlighted the importance of reestablishing SMC function during the angiogenic process in a context of critical limb ischemia to ensure stabilization and maturation of the newly formed vessels.

ARTICLE INFORMATION

Received February 24, 2021; accepted July 14, 2021.

Affiliations

Research Center of the Centre Hospitalier Universitaire de Sherbrooke (C.M., T.B., J.L., E.B., S.R., V.B., M.P., A.G., F.L., P.G.), Department of Surgery (M.-A.D.), and Division of Endocrinology, Department of Medicine (P.G.), Université de Sherbrooke, Québec, Canada.

Acknowledgments

C. Mercier, T. Brazeau, J. Lamoureux, E. Boisvert, S. Robillard, V. Breton, and F. Lizotte performed experiments. A. Guay performed animal care and researched data. C. Mercier, T. Brazeau, F. Lizotte, and P. Geraldes analyzed the data and wrote the article. All authors have approved the submitted article and support its conclusion. The authors gratefully acknowledge Anne Vezina (Department of Cell Anatomy, University of Sherbrooke) for her assistance with the histology technics. P. Geraldes is the guarantor of this work, had full access to all the data, and takes full responsibility for the integrity of the data and the accuracy of data analysis.

Sources of Funding

This work was supported by grants from the Canadian Institute of Health Research (PTJ159627 to P. Geraldes). This work was performed at the Centre Hospitalier Universitaire de Sherbrooke (CHUS) research center, funded by the Fonds de Recherche du Québec – Santé (FRQ-S). P. Geraldes is currently the holder of the Canada Research Chair in Vascular Complications of Diabetes.

Disclosures

None.

Supplemental Materials

Major Resources Table
Data Supplement Figures I–III
Data Supplement Tables I–III

REFERENCES

1. American Diabetes Association. Peripheral arterial disease in people with diabetes. *Diabetes care*. 2003;26:3333–3341. doi: 10.2337/diacare.26.12.3333
2. Al-Delaimy WK, Merchant AT, Rimm EB, Willett WC, Stampfer MJ, Hu FB. Effect of type 2 diabetes and its duration on the risk of peripheral arterial disease among men. *Am J Med*. 2004;116:236–240. doi: 10.1016/j.amjmed.2003.09.038
3. Chou E, Suzuma I, Way KJ, Opland D, Clermont AC, Naruse K, Suzuma K, Bowling NL, Vlahos CJ, Aiello LP, et al. Decreased cardiac expression of vascular endothelial growth factor and its receptors in insulin-resistant and diabetic States: a possible explanation for impaired collateral formation in cardiac tissue. *Circulation*. 2002;105:373–379. doi: 10.1161/hc0302.102143
4. Fadini GP, Albiero M, Bonora BM, Avogaro A. Angiogenic abnormalities in diabetes Mellitus: mechanistic and clinical aspects. *J Clin Endocrinol Metab*. 2019;104:5431–5444. doi: 10.1210/jc.2019-00980
5. Rivard A, Silver M, Chen D, Kearney M, Magner M, Annex B, Peters K, Isner JM. Rescue of diabetes-related impairment of angiogenesis by intramuscular gene therapy with adeno-VEGF. *Am J Pathol*. 1999;154:355–363. doi: 10.1016/S0002-9440(10)65282-0
6. Schratzberger P, Walter DH, Rittig K, Bahlmann FH, Pola R, Curry C, Silver M, Krainin JG, Weinberg DH, Ropper AH, et al. Reversal of experimental diabetic neuropathy by VEGF gene transfer. *J Clin Invest*. 2001;107:1083–1092. doi: 10.1172/JCI12188
7. Lizotte F, Paré M, Denhez B, Leitges M, Guay A, Geraldes P. PKCδ impaired vessel formation and angiogenic factor expression in diabetic ischemic limbs. *Diabetes*. 2013;62:2948–2957. doi: 10.2337/db12-1432
8. Roguin A, Nitecki S, Rubinstein I, Nevo E, Avivi A, Levy NS, Abassi ZA, Sabo E, Lache O, Frank M, et al. Vascular endothelial growth factor (VEGF) fails to improve blood flow and to promote collateralization in a diabetic mouse ischemic hindlimb model. *Cardiovasc Diabetol*. 2003;2:18. doi: 10.1186/1475-2840-2-18
9. Rajagopalan S, Mohler ER 3rd, Lederman RJ, Mendelsohn FO, Saucedo JF, Goldman CK, Blebea J, Macko J, Kessler PD, Rasmussen HS, et al. Regional angiogenesis with vascular endothelial growth factor in peripheral arterial disease: a phase II randomized, double-blind, controlled study of adenoviral delivery of vascular endothelial growth factor 121 in patients with disabling intermittent claudication. *Circulation*. 2003;108:1933–1938. doi: 10.1161/01.CIR.0000093398.16124.29
10. Molin D, Post MJ. Therapeutic angiogenesis in the heart: protect and serve. *Curr Opin Pharmacol*. 2007;7:158–163. doi: 10.1016/j.coph.2006.10.006

11. Betsholtz C. Role of platelet-derived growth factors in mouse development. *Int J Dev Biol*. 1995;39:817–825.
12. Carmeliet P. Angiogenesis in health and disease. *Nat Med*. 2003;9:653–660. doi: 10.1038/nm0603-653
13. Lindahl P, Johansson BR, Levéen P, Betsholtz C. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science*. 1997;277:242–245. doi: 10.1126/science.277.5323.242
14. Enge M, Bjarnegård M, Gerhardt H, Gustafsson E, Kalén M, Asker N, Hammes HP, Shani M, Fässler R, Betsholtz C. Endothelium-specific platelet-derived growth factor-B ablation mimics diabetic retinopathy. *EMBO J*. 2002;21:4307–4316. doi: 10.1093/emboj/cdf418
15. Tanii M, Yonemitsu Y, Fujii T, Shikada Y, Kohno R, Onimaru M, Okano S, Inoue M, Hasegawa M, Onohara T, et al. Diabetic microangiopathy in ischemic limb is a disease of disturbance of the platelet-derived growth factor-BB/protein kinase C axis but not of impaired expression of angiogenic factors. *Circ Res*. 2006;98:55–62. doi: 10.1161/01.RES.0000197842.38758.45
16. Geraldes P, Hiraoka-Yamamoto J, Matsumoto M, Clermont A, Leitges M, Marette A, Aiello LP, Kern TS, King GL. Activation of PKC-delta and SHP-1 by hyperglycemia causes vascular cell apoptosis and diabetic retinopathy. *Nat Med*. 2009;15:1298–1306. doi: 10.1038/nm.2052
17. Mercier C, Rousseau M, Geraldes P. Growth factor deregulation and emerging role of phosphatases in diabetic peripheral artery disease. *Front Cardiovasc Med*. 2020;7:619612. doi: 10.3389/fcvm.2020.619612
18. Lizotte F, Denhez B, Guay A, Gévry N, Côté AM, Geraldes P. Persistent insulin resistance in podocytes caused by epigenetic changes of SHP-1 in diabetes. *Diabetes*. 2016;65:3705–3717. doi: 10.2337/db16-0254
19. Paquin-Veillette J, Lizotte F, Robillard S, Béland R, Breton MA, Guay A, Despatis MA, Geraldes P. Deletion of AT2 receptor prevents SHP-1-induced VEGF inhibition and improves blood flow reperfusion in diabetic ischemic hindlimb. *Arterioscler Thromb Vasc Biol*. 2017;37:2291–2300. doi: 10.1161/ATVBAHA.117.309977
20. Drapeau N, Lizotte F, Denhez B, Guay A, Kennedy CR, Geraldes P. Expression of SHP-1 induced by hyperglycemia prevents insulin actions in podocytes. *Am J Physiol Endocrinol Metab*. 2013;304:E1188–E1198. doi: 10.1152/ajpendo.00560.2012
21. Denhez B, Rousseau M, Dancosst DA, Lizotte F, Guay A, Auger-Messier M, Côté AM, Geraldes P. Diabetes-induced DUSP4 reduction promotes podocyte dysfunction and progression of diabetic nephropathy. *Diabetes*. 2019;68:1026–1039. doi: 10.2337/db18-0837
22. ten Freyhaus H, Dagnell M, Leuchs M, Vantler M, Berghausen EM, Caglayan E, Weissmann N, Dahal BK, Schermuly RT, Ostman A, et al. Hypoxia enhances platelet-derived growth factor signaling in the pulmonary vasculature by down-regulation of protein tyrosine phosphatases. *Am J Respir Crit Care Med*. 2011;183:1092–1102. doi: 10.1164/rccm.200911-1663OC
23. Markova B, Herrlich P, Rönnstrand L, Böhmer FD. Identification of protein tyrosine phosphatases associating with the PDGF receptor. *Biochemistry*. 2003;42:2691–2699. doi: 10.1021/bi0265574
24. Grochot-Przeczek A, Dulak J, Jozkowicz A. Therapeutic angiogenesis for revascularization in peripheral artery disease. *Gene*. 2013;525:220–228. doi: 10.1016/j.gene.2013.03.097
25. Fadini GP, Spinetti G, Santopaolo M, Madeddu P. Impaired regeneration contributes to poor outcomes in diabetic peripheral artery disease. *Arterioscler Thromb Vasc Biol*. 2020;40:34–44. doi: 10.1161/ATVBAHA.119.312863
26. Levéen P, Pekny M, Gebre-Medhin S, Swolin B, Larsson E, Betsholtz C. Mice deficient for PDGF B show renal, cardiovascular, and hematological abnormalities. *Genes Dev*. 1994;8:1875–1887. doi: 10.1101/gad.8.16.1875
27. Soriano P. Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. *Genes Dev*. 1994;8:1888–1896. doi: 10.1101/gad.8.16.1888
28. Zhou M, Sutliff RL, Paul RJ, Lorenz JN, Hoying JB, Haudenschild CC, Yin M, Coffin JD, Kong L, Kranias EG, et al. Fibroblast growth factor 2 control of vascular tone. *Nat Med*. 1998;4:201–207. doi: 10.1038/nm0298-201
29. Borton AH, Benson BL, Neilson LE, Saunders A, Alaiti MA, Huang AY, Jain MK, Proweller A, Ramirez-Bergeron DL. Aryl hydrocarbon receptor nuclear translocator in vascular smooth muscle cells is required for optimal peripheral perfusion recovery. *J Am Heart Assoc*. 2018;7:e009205. doi: 10.1161/JAHA.118.009205
30. Lederman RJ, Mendelsohn FO, Anderson RD, Saucedo JF, Tenaglia AN, Hermiller JB, Hillegass WB, Rocha-Singh K, Moon TE, Whitehouse MJ, et al; TRAFFIC Investigators. Therapeutic angiogenesis with recombinant fibroblast growth factor-2 for intermittent claudication (the TRAFFIC study): a randomised trial. *Lancet*. 2002;359:2053–2058. doi: 10.1016/s0140-6736(02)08937-7
31. Facchiano F, Lentini A, Fogliano V, Mancarella S, Rossi C, Facchiano A, Capogrossi MC. Sugar-induced modification of fibroblast growth factor 2 reduces its angiogenic activity in vivo. *Am J Pathol*. 2002;161:531–541. doi: 10.1016/S0002-9440(10)64209-5
32. Campbell M, Allen WE, Silversides JA, Trimble ER. Glucose-induced phosphatidylinositol 3-kinase and mitogen-activated protein kinase-dependent upregulation of the platelet-derived growth factor-beta receptor potentiates vascular smooth muscle cell chemotaxis. *Diabetes*. 2003;52:519–526. doi: 10.2337/diabetes.52.2.519
33. Mori S, Takemoto M, Yokote K, Asaumi S, Saito Y. Hyperglycemia-induced alteration of vascular smooth muscle phenotype. *J Diabetes Complications*. 2002;16:65–68. doi: 10.1016/s1056-8727(01)00189-1
34. Nakamura J, Kasuya Y, Hamada Y, Nakashima E, Naruse K, Yasuda Y, Kato K, Hotta N. Glucose-induced hyperproliferation of cultured rat aortic smooth muscle cells through polyol pathway hyperactivity. *Diabetologia*. 2001;44:480–487. doi: 10.1007/s001250051646
35. Cantero AV, Portero-Otín M, Ayala V, Auge N, Sanson M, Elbaz M, Thiers JC, Pamplona R, Salvayre R, Nègre-Salvayre A. Methylglyoxal induces advanced glycation end product (AGEs) formation and dysfunction of PDGF receptor-beta: implications for diabetic atherosclerosis. *FASEB J*. 2007;21:3096–3106. doi: 10.1096/fj.06-7536com
36. Nakano T, Kumiko T, Mizumasa T, Kuroki Y, Tsuruya K, Kitazono T. The glucose degradation product methylglyoxal induces immature angiogenesis in patients undergoing peritoneal dialysis. *Biochem Biophys Res Commun*. 2020;525:767–772. doi: 10.1016/j.bbrc.2020.02.048
37. Yang J, Zeng P, Yang J, Liu X, Ding J, Wang H, Chen L. MicroRNA-24 regulates vascular remodeling via inhibiting PDGF-BB pathway in diabetic rat model. *Gene*. 2018;659:67–76. doi: 10.1016/j.gene.2018.03.056
38. Batista L, Bourachot B, Mateescu B, Reyal F, Mechta-Grigoriou F. Regulation of miR-200c/141 expression by intergenic DNA-looping and transcriptional read-through. *Nat Commun*. 2016;7:8959. doi: 10.1038/ncomms9959
39. Cao R, Bråkenhielm E, Pawliuk R, Wariaro D, Post MJ, Wahlberg E, Leboulch P, Cao Y. Angiogenic synergism, vascular stability and improvement of hind-limb ischemia by a combination of PDGF-BB and FGF-2. *Nat Med*. 2003;9:604–613. doi: 10.1038/nm848
40. Qi W, Li Q, Liew CW, Rask-Madsen C, Lockhart SM, Rasmussen LM, Xia Y, Wang X, Khamaisi M, Croce K, et al. SHP-1 activation inhibits vascular smooth muscle cell proliferation and intimal hyperplasia in a rodent model of insulin resistance and diabetes. *Diabetologia*. 2017;60:585–596. doi: 10.1007/s00125-016-4159-1
41. Weibrecht I, Böhmer SA, Dagnell M, Kappert K, Ostman A, Böhmer FD. Oxidation sensitivity of the catalytic cysteine of the protein-tyrosine phosphatases SHP-1 and SHP-2. *Free Radic Biol Med*. 2007;43:100–110. doi: 10.1016/j.freeradbiomed.2007.03.021
42. Sugano M, Tsuchida K, Maeda T, Makino N. SiRNA targeting SHP-1 accelerates angiogenesis in a rat model of hindlimb ischemia. *Atherosclerosis*. 2007;191:33–39. doi: 10.1016/j.atherosclerosis.2006.04.021
43. Krüger J, Wellnhofer E, Meyborg H, Stawowy P, Östman A, Kintscher U, Kappert K. Inhibition of Src homology 2 domain-containing phosphatase 1 increases insulin sensitivity in high-fat diet-induced insulin-resistant mice. *FEBS Open Bio*. 2016;6:179–189. doi: 10.1002/2211-5463.12000