Phosphodiesterase Type 5 Inhibition Reverts Prostate Fibroblast-to-Myofibroblast Trans-Differentiation

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Phosphodiesterase type 5 (PDE5) inhibitors have been demonstrated to improve lower urinary tract symptoms secondary to benign prostatic hyperplasia (BPH). Because BPH is primarily driven by fibroblast-to-myofibroblast trans-differentiation, this study aimed to evaluate the potential of the PDE5 inhibitor vardenafil to inhibit and reverse trans-differentiation of primary human prostatic stromal cells (PrSC). Vardenafil, sodium nitroprusside, lentiviral-delivered short hairpin RNA-mediated PDE5 knockdown, sodium orthovanadate, and inhibitors of MAPK kinase, protein kinase G, Ras homolog family member (Rho) A, RhoA/Rho kinase, phosphatidylinositol 3 kinase and protein kinase B (AKT) were applied to PrSC treated with basic fibroblast growth factor (fibroblasts) or TGFβ1 (myofibroblasts) in vitro, in chicken chorioallantoic membrane xenografts in vivo, and to prostatic organoids ex vivo. Fibroblast-to-myofibroblast trans-differentiation was monitored by smooth muscle cell actin and IGF binding protein 3 mRNA and protein levels. Vardenafil significantly attenuated TGFβ1-induced PrSC trans-differentiation in vitro and in chorioallantoic membrane xenografts. Enhancement of nitric oxide/cyclic guanosine monophosphate signaling by vardenafil, sodium nitroprusside, or PDE5 knockdown reduced smooth muscle cell actin and IGF binding protein 3 mRNA and protein levels and restored fibroblast-like morphology in trans-differentiated myofibroblast. This reversal of trans-differentiation was not affected by MAPK kinase, protein kinase G, RhoA, or RhoA/Rho kinase inhibition, but vardenafil attenuated phospho-AKT levels in myofibroblasts. Consistently, phosphatidylinositol 3 kinase or AKT inhibition induced reversal of trans-differentiation, whereas the tyrosine phosphatase inhibitor sodium orthovanadate abrogated the effect of vardenafil. Treatment of prostatic organoids with vardenafil ex vivo reduced expression of myofibroblast markers, indicating reverse remodeling of stroma towards a desired higher fibroblast/myofibroblast ratio. Thus, enhancement of the nitric oxide/cyclic guanosine monophosphate signaling pathway by vardenafil attenuates and reverts fibroblast-to-myofibroblast trans-differentiation, hypothesizing that BPH patients might benefit from long-term therapy with PDE5 inhibitors. (Endocrinology 153: 5546–5555, 2012)

Benign prostatic hyperplasia (BPH) is characterized by progressive enlargement and reorganization of the stromal compartment of the gland, in particular increased extracellular matrix deposition and trans-differentiation of fibroblasts into myofibroblasts, the mitogenic secretome of which promotes proliferation, angiogenesis, and tumorigenesis (1–4). TGFβ1 is considered to be a key inducer of pathogenic stromal reorganization, and we and others have demonstrated that TGFβ1 induces fibroblast-to-myofibroblast trans-differentiation (5–7).

BPH is commonly associated with bothersome lower urinary tract symptoms (LUTS). Inhibition of the phospho-

Abbreviations: AKT, Protein kinase B; bFGF, basic fibroblast growth factor; BPH, benign prostatic hyperplasia; CAM, chorioallantoic membrane; cGMP, cyclic guanosine monophosphate; CNN1, calponin-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMBS, hydroxymethylbilane synthase; IGFBP3, IGF binding protein 3; LUTS, lower urinary tract symptoms; MEK, MAPK kinase; Na3VO4, sodium orthovanadate; NO, nitric oxide; NOX4, reduced nicotinamide adenine dinucleotide phosphate oxidase 4; PDE5, phosphodiesterase type 5; PEK, phosphatidylinositol 3 kinase; PKG, protein kinase G; PrSC, prostatic stromal cell; qPCR, quantitative PCR; Rho, ras homolog family member; ROCK, RhoA/Rho kinase; ROS, reactive oxygen species; SCR, scrambled; shRNA, short hairpin RNA; SMA, smooth muscle cell actin; Smad, mothers against decapentaplegic homolog; SNP, sodium nitroprusside; SOD2, sodium dismutase 2.
phodiesterase type 5 (PDE5), an enzyme expressed in the stromal compartment of the prostate (7) that metabolizes the second messenger cyclic guanosine monophosphate (cGMP), has been demonstrated to improve LUTS (for review see Refs. 8, 9). The precise mechanisms underlying these beneficial effects are unclear. However, it appears that PDE5 inhibition impacts several pathways involved in BPH/LUTS, including increased smooth muscle relaxation and reduced stromal cell proliferation (9, 10) via enhancing nitric oxide (NO)/cGMP signaling. We and others previously demonstrated antiproliferative effects of the PDE5 inhibitors tadalafil (7, 11) and vardenafil (11, 12) on prostatic stromal cells (PrSC). In addition to these effects, we recently reported that tadalafil attenuated fibroblast-to-myofibroblast trans-differentiation, a hallmark of stromal remodeling (7).

In vivo fibroblast-to-myofibroblast trans-differentiation occurs via a two-step process that is initiated by changes in mechanical tension of the extracellular matrix that are transmitted to the fibroblast cytoskeleton via Ras homolog family member (Rho) A/Rho kinase (ROCK) signaling (13). Consequently, fibroblasts adopt an activated phenotype and deposit new extracellular matrix components (14). Soluble factors and cytokines, in particular the extra domain-A splice variant of cellular fibronectin and TGFβ, especially TGFβ1, play a key role in the differentiation to the α-smooth muscle cell actin (SMA)-expressing myofibroblast phenotype (14). The effects of TGFβ1 are mediated via mothers against decapentaplegic homolog (Smad) 2/3 activation and Smad-independent regulation of MAPK and phosphatidylinositol 3 kinase (PI3K) and protein kinase B (AKT) pathways (15–17). The RhoA/ROCK pathway seems to be the predominant pathway that regulates myofibroblast contraction (14) and has been shown to regulate the expression of α-SMA in smooth muscle cells (18). Furthermore, RhoA has been shown to modulate TGFβ-induced smooth muscle cell differentiation via cross talk with Smad (19). The PDE5 inhibitors vardenafil and sildenafil have been shown to inhibit RhoA/ROCK in a NO/cGMP-dependent protein kinase G (PKG)-dependent manner in the bladder stroma and in vascular smooth muscle cells, respectively (20, 21). We previously demonstrated that PrSC fibroblast-to-myofibroblast trans-differentiation downstream of TGFβ1 is driven by a prooxidant shift in redox homeostasis due to elevated production of reduced nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4)-derived reactive oxygen species (ROS) and concomitant decreases in anti-oxidant enzymes like sodium dismutase 2 (SOD2) (17).

In the present study, we evaluated the potential of the specific PDE5 inhibitor vardenafil that has been shown to significantly improve LUTS secondary to BPH (22), to inhibit and moreover to revert stromal remodeling as characterized by fibroblast-to-myofibroblast trans-differentiation, and investigated the pathway underlying trans-differentiation reversal using inhibitors of MAPK kinase (MEK), PKG, RhoA, ROCK, PI3K, AKT, and the tyrosine phosphatase inhibitor sodium orthovanadate (Na3VO4).

Materials and Methods

Reagents

Reagents were from Sigma-Aldrich (St. Louis, MO) unless otherwise specified. Human recombinant TGFβ1 was from R&D Systems (Minneapolis, MN). Kinase inhibitors and concentrations employed were as follows: MEK inhibitor PD98059 (50 μM; Calbiochem, San Diego, CA); PKG inhibitor KT5823 (1 μM; Calbiochem); RhoA inhibitor C3 exoenzyme (1 μg/ml; Calbiochem); ROCK inhibitor Y27632 (5 μM; PI3K inhibitor LY-294002 (20 μM; Calbiochem); and AKT inhibitor triciribine (20 μM). Antibodies were obtained as follows: AKT, phospho-AKT, phospho-p44/42 MAPK (ERK1/2), PDE5 (Cell Signaling Technology, Beverly, MA); α-tubulin, vimentin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA); IGF binding protein 3 (IGFBP3) (R&D Systems); α-SMA (Sigma, St. Louis, MO); and Smad-independent secondary antibodies (antimouse from Promega Corp., Madison, WI; antirabbit from Dianova, Hamburg, Germany; and antigoat from Dako). Tissue culture grade vardenafil was kindly provided by Peter Sandner (Bayer HealthCare, Wuppertal, Germany).

Culture of primary cells and prostatic organoids, trans-differentiation, and lentiviral-mediated PDE5 knockdown

Human primary PrSC were established as described previously (23) and cultured in stromal cell growth medium (Quanta 333; PAA Laboratories, Pasching, Austria). Fibroblast-to-myofibroblast trans-differentiation was induced by 1 ng/ml TGFβ1, whereas control cells were incubated with 1 ng/ml basic fibroblast growth factor (bFGF) in RPMI 1640 (PAA Laboratories) supplemented with 1% charcoal-treated bovine calf serum (HyClone Laboratories, Logan, UT) and antibiotics for 72 h as described (7). For trans-differentiation reversal studies, cells were subsequently stimulated for additional 72 h with bFGF, TGFβ1, or TGFβ1 with vardenafil, sodium nitroprusside (SNP), and/or kinase inhibitors and Na3VO4.

For PDE5 knockdown, 72-h trans-differentiated PrSC were transduced with lentiviral particles at multiplicity of infection 8 and analyzed 6 d after transfection (media were replaced by fresh medium containing bFGF or TGFβ1 after 72 h). Production of lentiviral particles was carried out according to the manufacturer’s protocol (Addgene, Cambridge, MA) as described previously (24). The target sequence (5-gacagcttgtgatctttctgcaatt) was located within the coding region of PDE5, scrambled short hairpin RNA (shRNA) vector (Addgene plasmid 1864) was used as control.

Human prostatic organoids were established from radical prostatectomy from tissue-wedges from the ventral prostate
Preparation of onplants and ex ovo chick chorioallantoic membrane (CAM) assay

Onplants were prepared as described previously (25). Briefly, native, nonpepsinized type I rat-tail collagen (BD Biosciences, San Jose, CA) was neutralized with 0.1 M NaOH solution and mixed with 10 × DMEM (Life Technologies, Inc., Carlsbad, CA) on ice. Then, 3 × 10^3 lentiviral transduced or normal PrSC were added to 30 μl of this solution and dropped on a 3 × 4 mm NITEX nylon mesh (Sefar, Inc., Depew, NY). After incubation at 37°C for 45 min, onplants were submersed in RPMI 1640 medium and cultured at 37°C in a humidified atmosphere containing 5% CO₂ for 3 d. Subsequently, onplants were applied to the chicken CAM.

The CAM assay was performed as described elsewhere (25) with slight modifications. Fertilized white leghorn chicken eggs (SPF eggs; Charles River, Germantown, MD) were incubated at 37°C with 80% humidity for 3 d. Subsequently, eggs were opened (ex ovo cultures) and transferred to plastic weighing boats. Ex ovo cultures were covered with a square Petri dish and incubated at 37°C and 80% humidity for 5 d. Then, PrSC collagen-onplants containing vardenafil (1.7 ng/μl) or distilled water as control were applied to CAM (four equal onplants/CAM) and incubated for 3 d. For histological analysis, onplants were excised from the CAM, fixed in 4% paraformaldehyde solution, and processed for paraffin sectioning.

IHC, immunofluorescence, Western blot analysis, and quantitative real-time PCR

IHC, immunofluorescence, and Western blot analysis were performed as described previously (6, 7).

mRNA extraction, cDNA synthesis and quantitative PCR (qPCR) were performed as described elsewhere (7, 17). Primer sequences are given in Table 1. For PrSC experiments, cDNA concentrations were normalized by the internal standard hydroxymethylbilane synthase (HMBS); for prostatic organoids, cDNA was normalized to HMBS and eukaryotic translation elongation factor 1α1 (EEF1A1).

Statistical analysis

Results are expressed as mean values ± SEM. Statistical differences between treatments were calculated by paired Student’s t test and considered significant when P < 0.05 (*, P < 0.05; **, P < 0.01).

Results

Vardenafil inhibits prostatic fibroblast-to-myofibroblast trans-differentiation

To investigate the potential of vardenafil to inhibit fibroblast-to-myofibroblast trans-differentiation in vitro, PrSC were differentiated with TGFβ1. Effective trans-differentiation is characterized by typical changes in cell morphology from the thin and elongated phenotype of fibroblasts to the flattened phenotype of myofibroblasts. Treatment with vardenafil maintained PrSC in a fibroblast-like phenotype in the presence of TGFβ1, indicating inhibition of fibroblast-to-myofibroblast trans-differentiation (Fig. 1A). At molecular level, trans-differentiation can be monitored by the induction of marker genes like SMA and IGFBP3. Although stimulation with TGFβ1 significantly enhanced mRNA levels of both markers, treatment with vardenafil significantly attenuated the potential of TGFβ1 to induce trans-differentiation (Fig. 1B), which was verified at the protein level by Western blot analysis (Fig. 1C).

In the past three decades, the CAM assay developed to an accepted and reliable in vivo model to replace animal experiments for testing different substances and chemotherapeutics (26–29). To study trans-differentiation in an in vivo environment, a PrSC CAM xenograft model was established. Due to the growth factor-rich environment in the developing embryo, PrSC in the onplant trans-differ-

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**Table 1. Primer sequences**

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differentiated into myofibroblasts in vivo, as determined by SMA expression. However, SMA staining was reduced in onplants treated with vardenafil, indicating that PDE5 inhibition attenuated fibroblast-to-myofibroblast trans-differentiation in vivo (Fig. 2A). To confirm that this was indeed mediated via inhibition of PDE5, specific lentiviral-delivered shRNA was employed. Consistently, PDE5 shRNA attenuated SMA expression in PrSC compared with control scrambled (SCR) shRNA (Fig. 2B). The presence of human stromal cells in the onplants was verified by IHC for human vimentin (Fig. 2).

**PDE5 inhibition by vardenafil reverses fibroblast-to-myofibroblast trans-differentiation**

We previously reported that trans-differentiated PrSC overcome TGFβ1-induced growth arrest by mitogenic stimulation but maintained expression of SMA and calponin-1 (CNN1), indicating a stable conversion to a myofibroblast phenotype (6). To determine whether PDE5 inhibition restored the fibroblast phenotype to predifferentiated cells, PrSC were trans-differentiated for 72 h and subsequently treated with different concentrations of vardenafil in the presence of TGFβ1 for additional 72 h, whereas control cells were maintained with either bFGF (fibroblast) of TGFβ1 (myofibroblast) (Fig. 3A). Vardenafil attenuates fibroblast-to-myofibroblast trans-differentiation in vitro. PrSC were incubated with bFGF, TGFβ1, or TGFβ1 + vardenafil as indicated for 72 h before phase contrast microscopy (A), qPCR (B), or Western blotting (C) for the trans-differentiation markers SMA and IGFBP3. Note the thin, elongated, and light-refractive phenotype of bFGF-treated PrSC (fibroblasts) in comparison with the flattened and less light-refractive morphology of TGFβ1-differentiated PrSC (myofibroblasts). A and C, Images are representative of at least three independent experiments using different donors. B, Values represent mean ± s.e.m. Significance is indicated (*, P < 0.05; **, P < 0.01; n = 5). C, GAPDH served as loading control.
enafil-treated cells adopted a fibroblast-like morphology and stained less intense for SMA in immunofluorescence, indicating that PDE5 inhibition induced a reversal of trans-differentiation (Fig. 3B). Consistently, vardenafil reduced the mRNA levels of the myofibroblast markers SMA and IGFBP3 in a dose-dependent manner that was significant at 50 µM of the inhibitor (Fig 3C). This was verified at the protein level by Western blot analysis (Fig. 3D).

Enhancement of NO/cGMP signaling reverses fibroblast-to-myofibroblast trans-differentiation
To confirm that the observed partial reversal of trans-differentiation upon vardenafil treatment was mediated via elevated cGMP levels, the soluble NO donor SNP was used. As with vardenafil, SNP dose dependently reduced SMA and IGFBP3 levels of in vitro trans-differentiated myofibroblasts (Fig. 4, A and B).

Although vardenafil is highly specific for PDE5 (30), this does not exclude potential interactions with other molecules. To verify that the reversal of fibroblast-to-myofibroblast trans-differentiation via vardenafil was by direct inhibition of PDE5, we analyzed the effect of specific lentiviral-delivered shRNA. PDE5 shRNA significantly reduced PDE5 mRNA and protein levels compared with cells treated with SCR shRNA (Fig. 4C). Additionally, PDE5 knockdown significantly reduced mRNA levels of the myofibroblast markers in trans-differentiated PrSC in the presence of TGFβ1 (Fig. 4D), which was verified at the protein level (Fig. 4E), indicating that the effect of vardenafil was derived from a specific inhibition of PDE5.

PDE5 inhibition reduces myofibroblast marker levels in ex vivo-treated prostatic organoids and restores SOD2 levels ex vivo and in vitro
Next, we addressed the question whether reversal of trans-differentiation by vardenafil is limited to freshly in vitro-differentiated myofibroblast or can be applied to in vivo-generated myofibroblasts in a three-dimensional tissue. Thus, prostatic organoids from prostatectomy tissue were cultured in serum-free medium and treated with vardenafil for 7 d ex vivo. Subsequently, mRNA levels of myofibroblast-related genes were compared with control-treated organoids. A panel of markers significantly regulated during TGFβ1-induced trans-differentiation of PrSC in vitro was employed (Fig. 5A). SMA, CNN1, and PLN that were up-regulated during trans-differen-
tion were found significantly reduced, whereas SOD2 that was down-regulated during trans-differentiation was found significantly induced in vardenafil-treated organoids (Fig. 5B). The TGFβ1-induced genes IGF1 and NOX4 were not significantly regulated upon vardenafil treatment of organoids, although there was a trend for IGF1 to be reduced (P = 0.15). These data indicate re-modeling of the stroma toward a higher fibroblast/myofibroblast ratio by PDE5 inhibition in prostatic organoids and thus a reversed trans-differentiation of in vitro-generated myofibroblasts to a fibroblast-like phenotype.

PrSc myofibroblast trans-differentiation is driven by a prooxidant shift in redox homeostasis due to induction of ROS-producing NOX4 and concomitant decrease in ROS-scavenging enzymes (17). The findings obtained in prostatic organoids indicate that reversal of trans-differentiation upon PDE5 inhibition does not affect NOX4 levels but restores expression of antioxidant enzymes like SOD2 to counteract the prooxidant shift. To further substantiate this finding, NOX4 and SOD2 mRNA levels were analyzed during vardenafil-induced reversal of trans-differentiation in in vitro-predifferentiated cells treated according to Fig. 3A. Indeed, NOX4 levels were unaffected by PDE5 inhibition (Fig. 5C), whereas SOD2 levels were restored to almost control (bFGF) levels upon treatment with 50 µM vardenafil (Fig. 5D). To verify that regulation of SOD2 expression was specific for PDE5 inhibition, the effect of lentiviral shRNA on NOX4 and SOD2 expression was investigated in PrSc. Consistently, PDE5 knockdown by PDE5 shRNA significantly induced SOD2 expression but did not affect NOX4 levels in PrSc compared with SCR shRNA-treated cells (Fig. 5E).

**FIG. 4.** Enhancement of NO/cGMP signaling reverses fibroblast-to-myofibroblast trans-differentiation in vitro. PrSc were trans-differentiated with TGFβ1 for 72 h and subsequently stimulated with SNP or subjected to lentiviral transduction in the presence of TGFβ1 before qPCR or Western blotting for the trans-differentiation markers SMA and IGFBP3. Control cells were maintained in medium containing bFGF. SNP dose dependently attenuated SMA and IGFBP3 mRNA levels after 72 h (A) and reduced myofibroblast marker protein levels to a similar extent than treatment with 50 µM vardenafil (V) (B). Lentiviral-delivered PDE5 shRNA significantly reduced PDE5 mRNA and protein levels compared with SCR control shRNA as determined at d 6 after transduction (C) and reduces SMA and IGFBP3 mRNA (D) and protein levels in trans-differentiated myofibroblasts in the presence of TGFβ1 (E). A, C, and D. Values represent mean ± SEM. Significance is indicated (*, P < 0.05; **, P < 0.01; n = 5). B, C, and E, GAPDH served as loading control, images are representative of at least three independent experiments using different donors.

**Reversal of myofibroblast trans-differentiation is mediated via inactivation of PI3K/AKT signaling**

The signaling pathway underlying vardenafil-induced reversal of trans-differentiation was investigated using specific kinase inhibitors. We previously reported that PDE5 inhibition reduced proliferation of PrSc via PKG and attenuated trans-differentiation via the MEK pathway (7). However, neither the PKG inhibitor KT5823 nor the MEK inhibitor PD98059 abrogated the potential of vardenafil to reverse trans-differentiation (Fig. 6A). PDE5 inhibition has been demonstrated to inhibit RhoA/ROCK downstream of NO/cGMP/PKG (20, 21). To investigate whether vardenafil-induced reversal of trans-differentiation was mediated via inhibition of RhoA signaling, the effect of RhoA inhibition by C3 exoenzyme and ROCK inhibition by Y27632 on myofibroblast markers was tested. Likewise the PKG inhibitor, neither C3 exoenzyme nor Y27632 significantly affected IGFBP3 and SMA mRNA levels in myofibroblasts and vardenafil-treated...
myofibroblasts (Fig. 6C). In agreement with the finding that vardenafil-induced reversal of myofibroblast trans-differentiation was unaffected by MEK inhibition, short-term treatment of trans-differentiated PrSC with vardenafil did not enhance phospho-ERK1/2 levels but attenuated phospho-AKT levels (Fig. 6B), indicating that inactivation of PI3K/AKT signaling might underlie reversal of trans-differentiation. Consistently, the PI3K inhibitor LY-294002 significantly reduced SMA and IGFBP3 mRNA and protein levels (Fig. 6, A and D). Because LY-294002 besides PI3K also inhibits other kinases, such as glycogen synthase kinase-3 (31), we additionally used the highly selective AKT inhibitor triciribine. Likewise PI3K inhibition, triciribine significantly reduced SMA and IGFBP3 levels synergistically with vardenafil, indicating that attenuation of PI3K/AKT signaling is causative for PDE5 inhibition-induced reversal of myofibroblast trans-differentiation (Fig. 6C). Consistently, the broad-spectrum phosphatase inhibitor Na3VO4 that acts as a phosphate analog and inhibits tyrosine phosphatases and other enzymes, including alkaline phosphatases and ATPases, abrogated the potential of vardenafil to reverse trans-differentiation (Fig. 6, A and D). Taken together, these findings indicate that PDE5 inhibition-induced reversal of fibroblast-to-myofibroblast trans-differentiation is mediated via inactivation of PI3K/AKT signaling potentially via phosphatases that inactivate AKT.

**Discussion**

Stromal remodeling via fibroblast-to-myofibroblast trans-differentiation promotes development and progression of BPH. Based on the reported beneficial effects of PDE5 inhibitors on LUTS secondary to BPH (8, 9), we investigated the potential of the PDE5 inhibitor vardenafil to inhibit and revert fibroblast-to-myofibroblast trans-differentiation of PrSC and demonstrate here that vardenafil not only attenuated but also reversed trans-differentiation.

Besides inhibition of trans-differentiation in vitro, vardenafil and shRNA-mediated PDE5 knockdown inhibited trans-differentiation in an in vivo CAM xenograft
model. These results further substantiate our previous findings using PDE5 inhibition by tadalafil and small interfering RNA-mediated PDE5 knockdown in vitro (7), indicating that enhancement of the NO/cGMP signaling pathway attenuates PrSC trans-differentiation. These data are in line with previous studies implicating the NO/cGMP pathway in fibroblast-to-myofibroblast conversion in other tissues. In fibroblast cultures from human Peyronie’s disease plaques sildenafil and the cGMP analog 8-bromo-cGMP reduced SMA levels (32) and sildenafil in combination with the soluble guanylyl cyclase activator BAY58–2667 inhibited human lung fibroblast-to-myofibroblast conversion (33). 8-Bromo-cGMP inhibited TGFβ1-induced trans-differentiation of cardiac fibroblasts isolated from wild-type mice (34). Similarly, the soluble guanylyl cyclase stimulator BAY41–2272 elevated intracellular cGMP levels and inhibited myofibroblast conversion in cultured cardiac fibroblasts and reduced the number of myofibroblasts in cardiac fibrosis in rats with hypertension induced by suprarenal aortic constriction (35).

We demonstrate that enhancement of the NO/cGMP pathway by SNP, vardenafil, or PDE5 knockdown does not only inhibit fibroblast-to-myofibroblast conversion but moreover restores the fibroblast phenotype in trans-differentiated PrSC. To our knowledge, this is the first study reporting a reversal of stromal remodeling as characterized by fibroblast-to-myofibroblast trans-differentiation in the prostate. A reversal of corneal myofibroblasts by FGF/heparin has been reported previously (36), and vardenafil treatment reduced myofibroblast numbers and total size of preformed TGFβ1-induced Peyronie’s disease plaques in a rat model (37).

In a three-dimensional coculture models of porcine skin fibrosis, SOD significantly lowered the levels of TGFβ1 and SMA, indicating reversal of myofibroblasts into normal fibroblasts (38). Moreover, the ROS scavenger N-ace-
tylcysteine significantly decreases SMA and type I collagen levels in fibroblasts isolated from patients with idiopathic pulmonary fibrosis (39). We previously demonstrated that PrSC trans-differentiation is associated with a sustained prooxidant shift driven by induction of the ROS-producing enzyme NOX4 and a concomitant down-regulation of several ROS-scavenging enzymes, including SOD2 (17). Consistently, trans-differentiation was inhibited by SOD supplementation (17). Interestingly, in the present study, vardenafil treatment of prostatic organoids and prefibrotized myofibroblasts or PDE5 knockdown in PrSC did not affect NOX4 levels but restored SOD2 expression (Fig. 5). Thus, PDE5 inhibition appears to enhance the antioxidative potential of PrSC thereby counteracting the TGFβ1-induced sustained prooxidant shift.

Unlike inhibition of trans-differentiation that is mediated via the MEK pathway (7), reversal was neither affected by MEK inhibition nor by PKG, RhoA, or ROCK inhibition. In contrast, vardenafil attenuated phospho-AKT levels in trans-differentiated myofibroblasts, and PI3K or AKT inhibition induced reversal of the phenotype. Regulation of AKT signaling by cGMP has been described in guanylyl cyclase C (that generates cGMP) knockout mice, where AKT signaling was elevated in intestinal cells and could be repressed by oral cGMP supplementation (40). Moreover, phosphatase inhibition abrogated vardenafil-induced reversal of trans-differentiation, indicating that reversal due to enhanced NO/cGMP signaling is mediated via activation of phosphatases that inactivate protein kinases like AKT.

Enhancement of the NO/cGMP pathway partially but not completely reversed trans-differentiation in our model system. However, one has to keep in mind that this partial reversal occurred in the continued presence of the TGFβ1-mediated prooxidant shift that partly reverses the in vivo situation. Vardenafil treatment in the absence of TGFβ1 completely restores trans-differentiation markers to the levels of bFGF-treated fibroblasts (data not shown).

In conclusion, enhancement of the NO/cGMP signaling pathway by PDE5 inhibitors like vardenafil attenuates and reverts fibroblast-to-myofibroblast trans-differentiation, a central process underlying stromal remodeling in BPH. Reversibility of trans-differentiation suggests that prostatic fibroblasts and myofibroblasts are not terminally differentiated cell types but rather alternative and convertible phenotypes. Our findings indicate that BPH patients might benefit from long-term therapy with PDE5 inhibitors that attenuate and revert stromal remodeling. PDE5 inhibition seems to affect BPH/LUTS via several mechanisms. Although increased smooth muscle cell relaxation mediates a readily relieve of LUTS, reduced stromal cell proliferation and attenuation of myofibroblast formation might stop the progression of BPH, and reversal of myofibroblast trans-differentiation provides the potential for regression of disease upon long-term therapy.

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