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Phosphodiesterase type 5 inhibitor tadalafil reduces prostatic fibrosis via MiR-3126-3p/FGF9 axis in benign prostatic hyperplasia

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Abstract

Myofibroblast buildup and prostatic fibrosis play a crucial role in the development of benign prostatic hyperplasia (BPH). Treatments specifically targeting myofibroblasts could be a promising approach for treating BPH. Tadalafil, a phosphodiesterase type 5 (PDE5) inhibitor, holds the potential to intervene in this biological process. This study employs prostatic stromal fibroblasts to induce myofibroblast differentiation through TGFβ1 stimulation. As a result, tadalafil significantly inhibited prostatic stromal fibroblast proliferation and fibrosis process, compared to the control group. Furthermore, our transcriptome sequencing results revealed that tadalafil inhibited FGF9 secretion and simultaneously improved miR-3126-3p expression via TGFβ1 suppression. Overall, TGFβ1 can trigger pro-fibrotic signaling through miR-3126-3p in the prostatic stroma, and the use of tadalafil can inhibit this process.

Keywords Benign prostatic hyperplasia, Myofibroblast differentiation, Phosphodiesterase 5 inhibitor, miR-3126-3p, FGF9

Introduction

Benign prostatic hyperplasia (BPH) impacts approximately 60% of males aged 60 years and above globally [1, 2]. It causes bladder outlet obstruction (BOO), resulting in lower urinary tract symptoms (LUTS) that can greatly affect the quality of life in men [3–5]. BPH is characterized by the benign proliferation of epithelial and stromal cells of the prostate gland, resulting in increased prostate gland volume [6, 7]. Furthermore, studies have indicated that BPH is closely associated with changes in the stromal microenvironment, with fibroblast-to-myofibroblast trans-differentiation (FMT) playing a pivotal role [8–10]. The FMT process results in the accumulation of myofibroblasts, which are the primary producers of extracellular matrix (ECM) components and are central to the fibrosis observed in BPH tissue [11].

The standard treatment of BPH has mostly involved the use of α-adrenergic receptor blockers such as tamsulosin,

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terazosin, and alfuzosin, as well as 5-α-reductase inhibitors such as finasteride and dutasteride [12]. Nonetheless, a subset of patients may experience limited symptomatic relief or, in specific cases, observe disease progression despite pharmacotherapy [13].

Currently, phosphodiesterase-type-5 (PDE-5) inhibitors are clinically approved for the treatment of erectile dysfunction (ED) [14], LUTS [15], and pulmonary artery hypertension (PAH) [16]. Regarding the treatment of LUTS, it has been discovered that PDE5 inhibitors enhance the NO-cGMP pathway, resulting in smooth muscle relaxation, inhibition of proliferation of prostate stromal cells, and downregulation of the inflammatory response of the prostate [17, 18]. Tadalafil has not been widely used in China for the pharmacological treatment of BPH. Previous studies revealed a significant increase in stromal proliferation within BPH tissues [19]. In addition, tadalafil has demonstrated efficacy in suppressing stromal fibrosis, yet the precise mechanisms remain unclear [20, 21]. Thus, our objective is to thoroughly investigate the impact of tadalafil on fibrosis in prostate stromal cells and elucidate the underlying mechanisms of its effects.

MicroRNAs (miRNAs) are a specific type of small RNAs derived from stem-loop regions within longer RNA transcripts. These miRNAs play a crucial role in controlling gene expression by binding themselves to target messenger RNAs (mRNAs) at the post-transcriptional level [22, 23]. Numerous studies have demonstrated the pivotal roles of miRNAs in the diagnosis and prognosis of prostate cancer and BPH [22, 24]. Aberrant expression of miRNAs has been associated with various diseases. Among various types of miRNAs, miR-3126-3p has been identified as an important contributor to multiple pathophysiological processes, including bone remodeling [25], antiviral activity [26], and tumorigenesis [27]. However, the specific impact of miR-3126-3p in BPH remains unclear. The fibroblast growth factor (FGF) family, which consists of 18 receptor-binding members and 4 FGF receptors, plays a crucial role in regulating various biological processes through paracrine, autocrine, or endocrine mechanisms [28]. Recent studies highlighted the significant role of FGF9 in prostate stromal remodeling, which is associated with poor prostate cancer prognosis [29, 30].

In this study, we investigated the inhibitory effects of tadalafil on prostatic stromal fibroblast proliferation and myofibroblast trans-differentiation. During this process, we observed a concurrent increase in miR-3126-3p expression and downregulation of FGF9 protein level. Furthermore, we have established that tadalafil interferes with the inhibitory effect of TGFβ1 on miR-3126-3p, resulting in a reduction in the expression and secretion of FGF9. This ultimately impedes the process by which fibroblasts undergo differentiation into myofibroblasts.

Methods and materials

Reagents

Highly pure tadalafil (HY-90,009 A), hydrocortisone (HY-N0583), insulin (Cat# HY-P73243), human recombinant TGFβ1 (HY-P70543), and human basic fibroblast growth factor (HY-P7331A) were purchased from MedChemExpress (NJ, USA). The antibiotic/antimycotic solution (C100C8) was purchased from NCM Biotech (Suzhou, China).

Cell culture

Prostate-derived fibroblasts (PrFc) were established as described previously [31]. WPMY-1 (immortalized prostate stromal fibroblasts) acquired from Cell Bank of the Chinese Academy of Sciences(Shanghai, China) was cultured in DMEM (HyClone, South Logan, UT, USA) containing 5% fetal bovine serum (FBS) (VivaCell, Shanghai, China) and 1% penicillin-streptomycin solution (Gibco). All cells were cultured in the incubator with 5% CO_2 at 37 °C.

Inducing trans-differentiation

PrFc and WPMY-1 were cultured in phenol red-free DMEM supplemented with 1% charcoal-stripped fetal bovine serum (VivaCell, Shanghai, China) and 1% penicillin-streptomycin in the presence of 1 ng/mL human recombinant TGFβ1 or 1 ng/mL human basic fibroblast growth factor as a control when the cell density reached approximately 30%. Subsequently, the cells were cultured for 72 h, followed by the addition of tadalafil for subsequent experiments.

Cell transfection

The miR-3126-3p mimic, miR-3126-3p inhibitor, miR-NC mimic, and miR-NC inhibitor were synthesized by RiboBio (Guangzhou, China). The sequences of mimic and inhibitor are as follows: miR-3126-3p mimic, sense: 5′-CAUCUGGCAUCCGUCACACAGA-3′, antisense: 5′-UCUGUGUGACGGAUGCCAGAUG-3′. miR-3126-3p inhibitor, sense: 5′-UCUGUGUGACGGAUGCCAGAUG-3′. MiR-NC mimic sense: 5′-UUUGUACUACACAAAAGUACUG-3′, antisense: 5′-CAGUACUUUUGUGUAGUACAAA-3′.MiR-NC inhibitor, sense: 5′-CAGUACUUUUGUGUAGUACAAA-3′. PrFc and WPMY-1 were incubated into six-well plates with 30–40% density. After cell trans-differentiation, the miR-3126-3p mimic, miR-3126-3p inhibitor, miR-NC mimic, and miR-NC inhibitor were transfected into PrFc or WPMY-1 by lipofectamine 3000 reagents (Invitrogen, USA) according to the protocols.

Cell viability assay

The Cell Counting Kit-8 (CCK8, DOJINDO, Japan) and EdU staining were performed to detect cell proliferation.

For the CCK8 assay, PrFc was incubated in 96-well plates and continued to cultivate for 1-, 3-, 5-, and 7 days with tadalafil at 0 nM, 5 nM, 15 nM, and 25 nM. After the CCK8 reagent was added to the medium, optical density (OD) at 450 nm was measured on a microplate reader. In addition, the BeyoClick™ EdU Cell Proliferation Kit (Beyotime, China) was utilized to assess the PrFc proliferation. All operations followed the protocols.

Western blot analysis

Total protein extraction was conducted, and protein expression was assessed through Western blot analysis. The primary antibodies were utilized as follows: anti-COL1A1 (Proteintech, 67288-1-Ig), anti-αSMA (Sigma-Aldrich, A5228), anti-IGFBP3 (Proteintech, 10189-2-AP), anti-Smad2/3 (Cell Signaling Technology, 8685 S), anti-Phospho-Smad2/Smad3 (Cell Signaling Technology, 8828 S), anti-GAPDH (Cell Signaling Technology, 5174 S), and anti-FGF9 (Absin, abs139052).

RNA extraction and qRT-PCR

All extracted RNA was isolated from cultured cells using Trizol reagent (Takara, Japan) according to the procedure. Prime Script RT reagent Kit (Takara, Japan) was utilized for reverse transcription. The qRT-PCR was performed with the SYBR Prime Script RT PCR kit (Takara, Japan). The GAPDH was defined as the internal reference; meanwhile, the 2-ΔΔCt values were utilized to determine the relative expression of mRNA. The primer sequences are provided in the Supplementary Table 1.

IF and IHC

IF and IHC were performed as previously described [32]. Masson's trichrome staining was performed using the Trichrome Stain (Masson) Kit (Sigma-Aldrich, USA), following the manufacturer's protocol. The primary antibodies used in IF and IHC, including Vimentin (Cell Signaling Technology, D21H3), αSMA, IGFBP3, and FGF9, were obtained from the same source as those used in the WB mentioned above. Cells and tissues were observed and photographed under a fluorescence microscope (Leica Microsystems, Germany).

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed to analyze FGF9 levels released from PrFc and WPMY-1. The cell supernatant was collected to detect the FGF9 content using the FGF9-specific ELISA kit (Multisciences Biotech, China) according to the manufacturer's instructions.

Dual-luciferase reporter assay

Luciferase reporter constructs were generated from OBiO Technology to encode the NC 3'UTR, wild-type FGF9 3'UTR region, or mutant FGF9 3'UTR region. The pRL-TK vector, encoding Renilla luciferase from OBiO Technology, was used as a control for transfection and normalization of luciferase assays. HEK293T cells were co-transfected with the luciferase reporter plasmids, miR3126-3p/miR-NC mimic, and the internal control Renilla luciferase plasmid. Luciferase activities were measured 48 h after transfection using the Dual-luciferase assay kit (Promega, USA) following the manufacturer's protocol.

Patient-derived explant model

The PDE model is a model that can reflect the tissue microenvironment and the responsiveness of epithelial and stromal cells to drugs [33]. Fresh BPH tissue was obtained by transurethral resection of the prostate from the Urology Center, Shanghai General Hospital. Our study has been ethically reviewed and approved by our institutional ethics committee, with informed consent obtained from both patients and their families (Ethics number: 2022SQ190). Briefly, fresh tissue was placed in sterile DMEM/F-12 medium on ice and transported to the lab within 30 min. After the eschar of fresh tissue was removed, the BPH tissue was divided into 3–5 mm cubes. Next, these cubes were cultured on the sterile gelatin sponge in 6 cm dishes containing 5 ml of medium (DMEM/F12 buffered with 10% FBS, 0.01 mg/ml hydrocortisone, 0.01 mg/ml insulin, and antibiotic/antimycotic solution). After fresh tissue was cultured for 24 h, tadalafil (0, 25 μM) was added to the medium, and the tissue was further cultured for 1 Day.

Statistical analysis

The data are presented as the mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism 9 software. Statistical significance was assessed using either ANOVA or t-tests, as appropriate. A p-value of <0.05 was considered statistically significant.

Results

PDE5i tadalafil reduces viability and trans-differentiation of the prostatic fibroblasts

We analyzed the single-cell sequencing dataset from GSE172357, which included normal prostate and BPH tissue samples that comprise both glandular and stromal nodules [34]. As shown in Figure S1, various immune cells and myofibroblasts were significantly increased in BPH tissues. Subsequently, PrFc was isolated and primarily cultured from BPH tissue specimens. It has been reported that PDE5 inhibitors can reduce the proliferation and trans-differentiation of prostatic fibroblasts through the cGMP/PKG and cGMAP/MEK pathways [35]. Nevertheless, PKG and MEK hold significant biological roles across diverse tissue types. FMT is a prominent feature of prostatic stromal fibroblasts in BPH. Our

objective is to gain a better understanding of the mechanisms through which PDE5 inhibitors influence this process [36].

Initially, we added tadalafil to a fresh culture medium with varying concentrations and proceeded to culture the cells for 1 week. We found that tadalafil significantly suppressed PrFc proliferation (Fig. 1A-B). To ensure that the observed inhibitory effects on proliferation were not caused by toxicity or off-target effects resulting from high drug concentrations, we additionally determined the IC50 value of tadalafil in PrFc (Figure S2). Since the TGF-β1/Smads pathway is involved in the FMT process [37], we examined the RNA and protein expression of FMT markers, including Collagen I, αSMA, IGFBP3, SAMD2/3, and p-SMAD2/3, in PrFc and WPMY-1 (Fig. 1C-F). Our results showed that tadalafil reduced the expression of Collagen I, αSMA, IGFBP3, and p-SMAD2/3 in PrFc and WPMY-1 cells when compared to the control group. Moreover, immunofluorescence staining showed that tadalafil decreased αSMA immunofluorescence in both PrFc and WPMY-1 cells (Fig. 1G-H). This suggests that tadalafil can hinder FMT.

MiR-3126-3p reversed FMT progress in prostatic fibroblasts

To elucidate the molecular mechanism by which tadalafil inhibits the FMT of prostate stromal fibroblasts, we

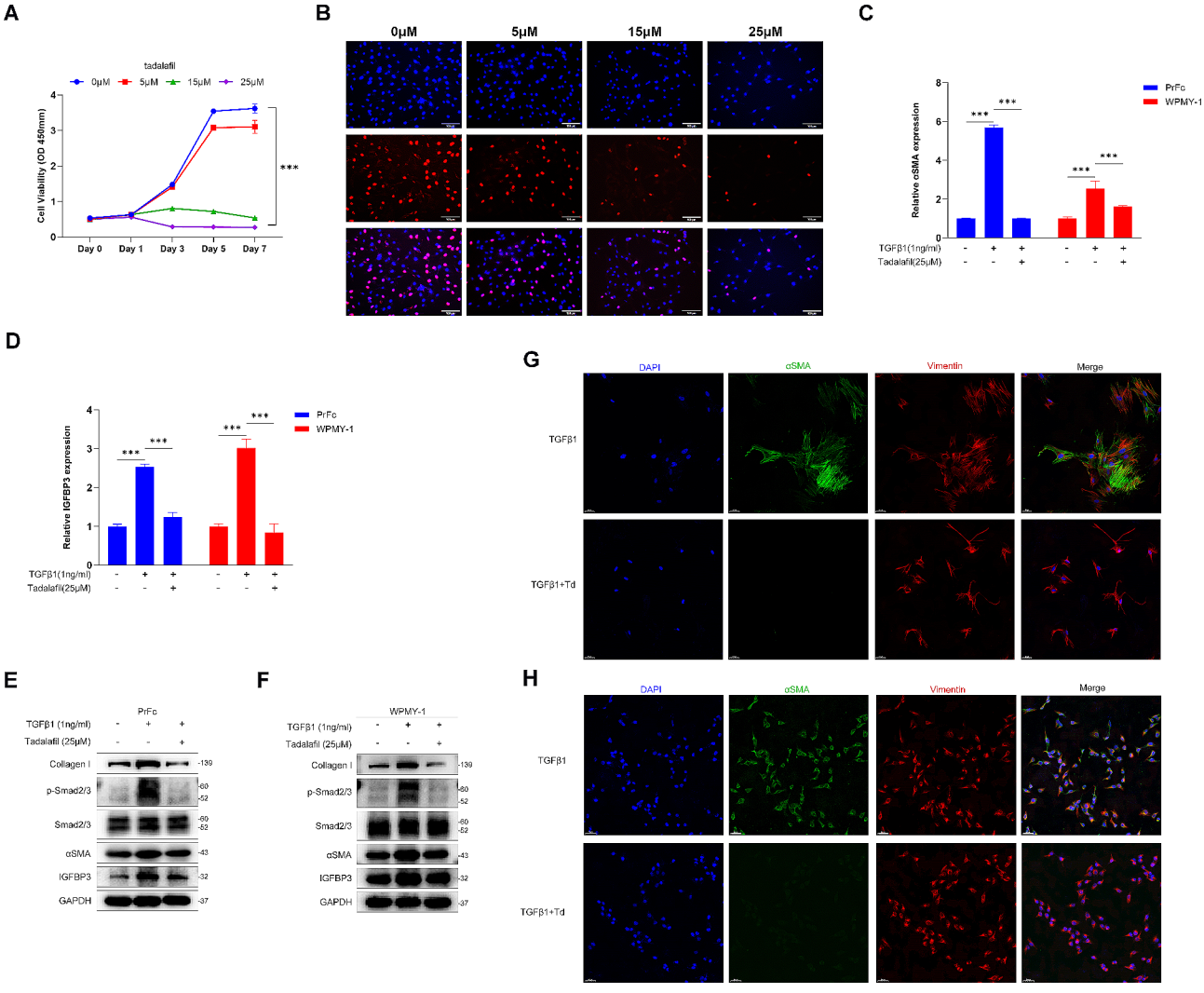

Fig. 1 PDE5 Inhibitor Tadalafil Suppresses Proliferation and FMT in Prostate Stromal Cells. (**A**) WPMY-1 cells were subjected to various concentrations of tadalafil treatment for 1 week, and cellular proliferation vitality was assessed on 0, 1, 3, 5, and 7 days. (**B**) WPMY-1 cells were exposed to various concentrations of tadalafil for 48 h, and alterations in cell proliferation were evaluated by EdU incorporation assay. (**C**-**D**) After a 72-hour treatment of PrFc or WPMY-1 cells with 1 ng/mL TGFβ1, a continued 48-hour exposure to 25 μM tadalafil was administered, and the mRNA expression levels of αSMA and IGFBP3 were quantified using qRT-PCR. (**E**-**F**) Following a 72-hour activation of PrFc and WPMY-1 cells with 1 ng/mL TGFβ1, subsequent treatment with 25 μM tadalafil for 48 h was carried out, and the expression levels of proteins related to the TGFβ pathway were examined. (**G**-**H**) Immunofluorescence staining was employed to analyze levels of Vimentin and αSMA expression in PrFc and WPMY-1 cells following a 48-hour treatment with 25μM tadalafil or TGFβ1. (*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$)

conducted bioinformatics analysis on the differential miRNA expression before and after TGFβ1 treatment, using data from the GSE205378 dataset [38]. As shown in Fig. 2A, 7 miRNAs were significantly upregulated while 6 miRNAs were significantly downregulated in TGFβ1-treated PrFc. Subsequently, we performed qRT-PCR analysis to assess the expression levels of various mature miRNAs in TGFβ1-treated PrFc with or without tadalafil treatment. After receiving tadalafil treatment, there was a distinct and exclusive upregulation in miR-3126-3p expression (Fig. 2B). Therefore, we selected miR-3126-3p for further study. In addition, to investigate the impact of miR-3126-3p on FMT in PrFc, we transfected miR-3126-3p mimic into the cells and examined the expression of FMT-related markers at both the RNA and protein levels. As shown in Fig. 2C-F, miR-3126-3p mimic significantly suppressed Collagen I, αSMA, IGFBP3, and p-SMAD2/3 in PrFc and WPMY-1 cells. Nevertheless, miR-3126-3p had no suppressive effect on cell proliferation in both PrFc and WPMY-1 cells (Figure S3 A-B). To further confirm whether tadalafil could inhibit FMT in PrFc and WPMY-1 through miR-3126-3p, we transfected miR-3126-3p inhibitor into PrFc and WPMY-1. Subsequently, we used a western blot to assess the protein expression levels of Collagen I, αSMA, IGFBP3, SMAD2/3, and p-SMAD2/3. As shown in Fig. 2G-H,

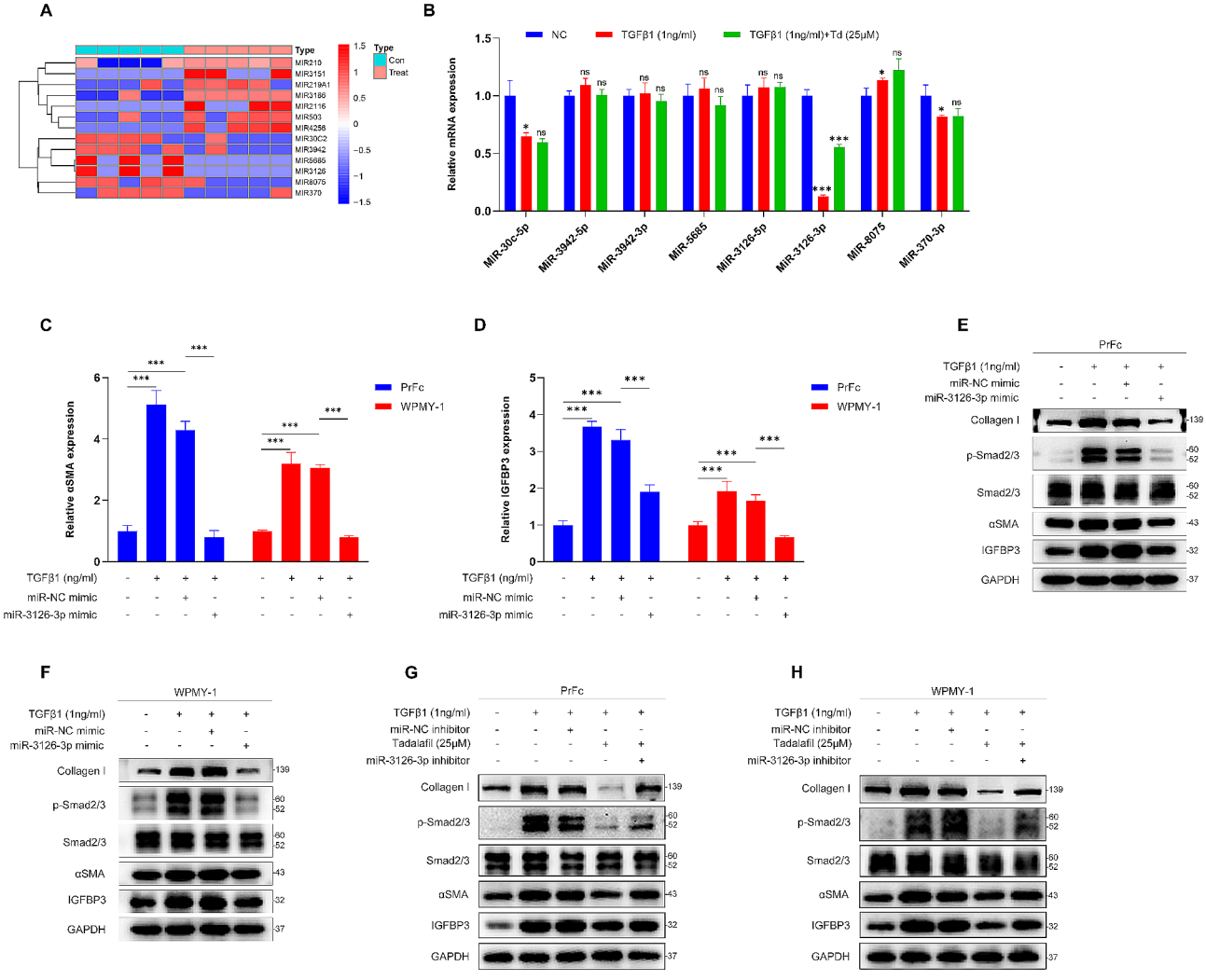

Fig. 2 MiR-3126-3p is suppressed by TGFβ1, which can be restored by Tadalafil. (**A**) Heatmap illustrates the differential expression of miRNAs in PrFc between the control group and the TGFβ1-treated group. (**B**) Following a 72-hour activation of PrFc and WPMY-1 cells with 1 ng/mL TGFβ1, a subsequent 48-hour treatment with 25 μM tadalafil was conducted. The histogram shows the result of qRT-PCR analysis of the expression levels of 8 mature miRNAs. (**C**-**D**) Activated PrFc and WPMY-1 cells were transfected with miR-NC mimic or miR-3126-3p mimic, and after 48 h of continued incubation, αSMA and IGFBP3 mRNA levels were analyzed using qRT-PCR. (**E**-**F**) After a 72-hour activation of PrFc and WPMY-1 cells with 1 ng/mL TGFβ1, separate transfections with miR-NC mimic or miR-3126-3p mimic were performed, and the levels of proteins associated with the TGFβ pathway were assessed. (**G**-**H**) After a 72-hour treatment of PrFc and WPMY-1 cells with 1ng/mL TGFβ1, a subsequent addition of 25 μM tadalafil or transfection with miR-NC inhibitor/miR-3126-3p inhibitor for 48 h was conducted, and the levels of proteins associated with the TGF pathway were measured. (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$)

the co-administration of tadalafil and a miR-3126-3p inhibitor to PrFc and WPMY-1 cells resulted in a notable reduction in the inhibitory effect of tadalafil on FMT.

FGF9 is a potential target of mir-3126-3p in prostate stromal cells

To elucidate the potential target genes of miR-3126-3p in prostate stromal fibroblasts, we used the "multiMiR" package to predict its target genes. FGF9 has been reported to promote epithelial cell proliferation through autocrine and paracrine mechanisms [39]. Using miRTarBase, we predicted the binding site of miR-3126-3p in the 3'UTR region of FGF9. Subsequently, we designed a luciferase reporter vector containing the wild-type or mutant FGF9 3'UTR region. To verify whether miR-3126-3p could directly target the FGF9 3'UTR region, we performed a dual-luciferase reporter gene assay. The results demonstrated that the luciferase activity of the FGF9 wild-type 3'UTR was significantly decreased when co-transfected with the miR-3126-3p mimic, as compared to the transfection of the miR-NC mimic (Fig. 3A). In contrast, there was no interaction between miR-3126-3p and the vector containing the FGF9 mutant 3'UTR.

Overexpression of miR-3126-3p in both PrFc and WPMY-1 cells resulted in a significant decrease in the mRNA levels of FGF9 (Fig. 3B). In addition, Western blot analysis showed that transfection of miR-3126-3p inhibited the protein expression of FGF9 in PrFc and WPMY-1

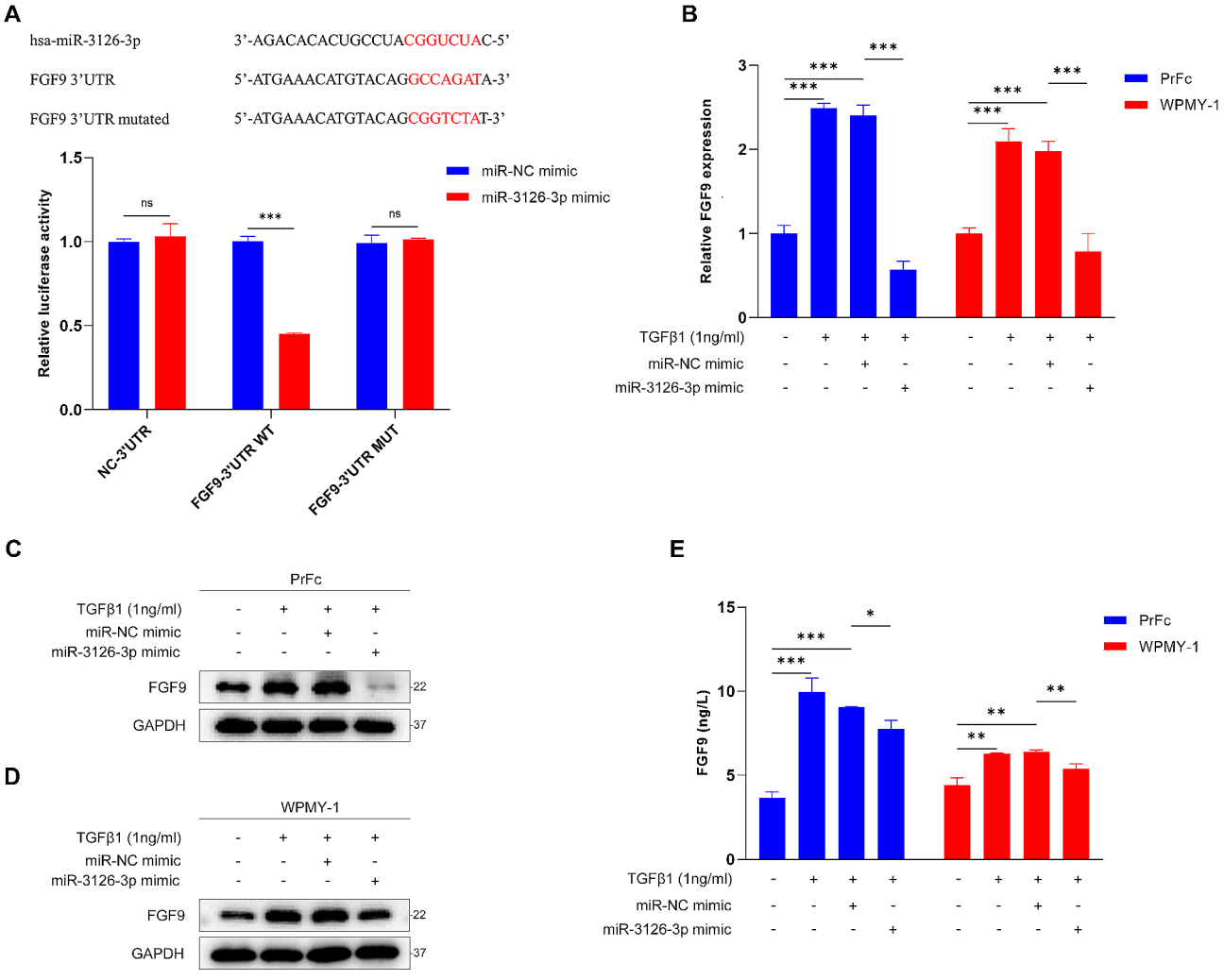

Fig. 3 Targeting and suppression of *FGF9* expression and secretion by miR-3126-3p. (**A**) The control group utilized miR-NC mimic, while the experimental group employed miR-3126-3p mimic. Both groups were co-transfected with pRL-TK and the binding sites of *FGF9* 3'UTR region (WT or MUT) plasmids. Cellular lysis was performed after 48 h of transfection, and Firefly luciferase and Renilla luciferase activities were quantified at wavelengths of 560 nm and 465 nm, respectively ($n=3$). (**B**) qRT-PCR analysis was conducted to evaluate *FGF9* mRNA expression levels in activated PrFc and WPMY-1 cells after a 48-hour transfection with miR-NC mimic or miR-3126-3p mimic ($n=3$). (**C**-**D**) Following a 72-hour activation of PrFc and WPMY-1 cells with TGFβ1 (1ng/mL), miR-NC mimic and miR-3126-3p mimic were individually transfected for 48 h to assess FGF9 protein levels ($n=3$). (**E**) After a 72-hour activation of PrFc and WPMY-1 cells with 1ng/mL TGFβ1, further transfection with miR-NC mimic or miR-3126-3p mimic was performed for 48 h. Supernatants were collected, and FGF9 levels were quantified using ELISA ($n=3$). (*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$)

cells (Fig. 3C-D). Furthermore, we measured FGF9 levels in the culture supernatants by ELISA and found that miR-3126-3p also suppressed FGF9 secretion (Fig. 3E). Overall, FGF9 is a potential target of miR-3126-3p in prostate stromal cells.

MiR-3126-3p inhibits the FMT process via the suppression of FGF9

To thoroughly examine the mechanism by which miR-3126-3p reduces FGF9 expression in stromal fibroblasts, we restored FGF9 expression in stromal cells that were co-transfected with miR-3126-3p mimic. As illustrated in Fig. 4A and B, when compared to transfection with miR-3126-3p mimic alone, the introduction of FGF9 resulted in a marked augmentation of the expression levels of fibrotic-associated proteins in PrFc and WPMY-1 cells, such as Collagen I, p-SMAD2/3, αSMA, and IGFBP3. Collectively, these findings demonstrate that the inhibitory impact of miR-3126-3p on FMT is achieved through the suppression of FGF9.

PDE5i tadalafil inhibits FMT via suppression of FGF9 secretion

To further clarify the mechanism by which tadalafil inhibits the FMT process, we investigated to determine if its effect is achieved by promoting the expression of miR-3126-3p, which in turn targets the expression of FGF9. We initially examined the mRNA and protein expression levels of FGF9 in prostate stromal fibroblasts treated with tadalafil. As shown in Fig. 4C-E, tadalafil significantly inhibited FGF9 expression compared to the control group. In addition, ELISA results further confirmed the inhibitory effect of tadalafil on FGF9 secretion (Fig. 4F). Subsequently, we evaluated whether tadalafil exerts its inhibitory effect on FGF expression via miR-3126-3p. Prostate stromal fibroblasts were transfected with miR-3126-3p inhibitor while receiving concurrent treatment with tadalafil. As depicted in Fig. 5A-C, the inhibitory effect of tadalafil on FGF9 was reversed by co-transfection with a miR-3126-3p inhibitor, as compared to treatment with tadalafil alone. In addition, we added tadalafil and recombinant human FGF9 to TGFβ1-activated prostate stromal fibroblasts. Through qRT-PCR and WB analysis, we found that compared to tadalafil alone, co-administration of rhFGF9 significantly increased the mRNA and protein expression levels of FMT-related markers (Collagen I, αSMA, IGFBP3, SMAD2/3, p-SMAD2/3) (Fig. 5D-G). This indicates that tadalafil inhibits the FMT process in prostate stromal cells by suppressing FGF9 expression.

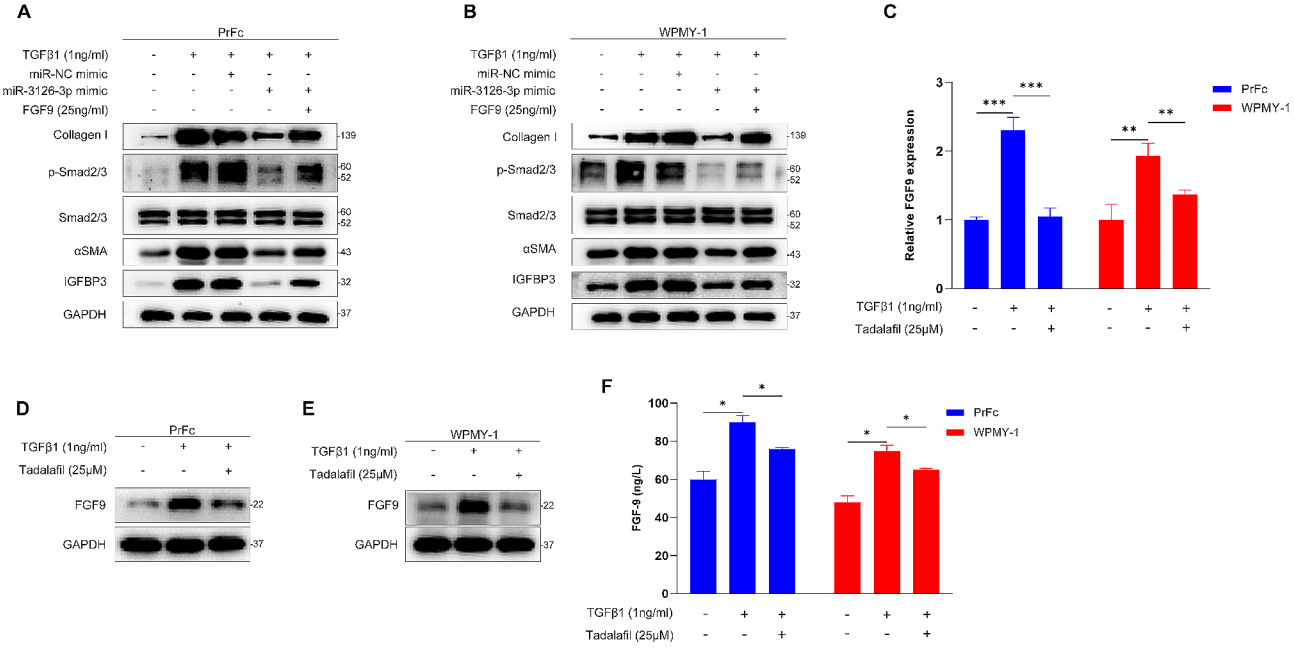

Fig. 4 MiR-3126-3p suppresses prostatic FMT by targeting *FGF9*. (**A**-**B**) Following a 72-hour activation of PrFc or WPMY-1 cells with 1ng/mL TGFβ1, subsequent treatment with miR-NC mimic/miR-3126-3p mimic or FGF9 (25ng/mL) was conducted for 48 h. The expression levels of proteins associated with the TGF pathway were then evaluated. (**C**) FGF9 mRNA expression levels in TGFβ1 (1ng/mL)-activated PrFc and WPMY-1 cells following tadalafil treatment were assessed using qRT-PCR. (**D**-**E**) PrFc and WPMY-1 cells were stimulated with TGFβ1 (1ng/mL) for 72 h and subsequently treated with tadalafil (25μM) for 48 h to evaluate FGF9 protein expression levels ($n = 3$). (**F**) ELISA was employed to quantify FGF9 levels in the supernatants of PrFc and WPMY-1 cells treated with TGFβ1 (1ng/mL) and tadalafil (25μM). (*$p \leq 0.05$, > **$p \leq 0.01$, ***$p \leq 0.001$)

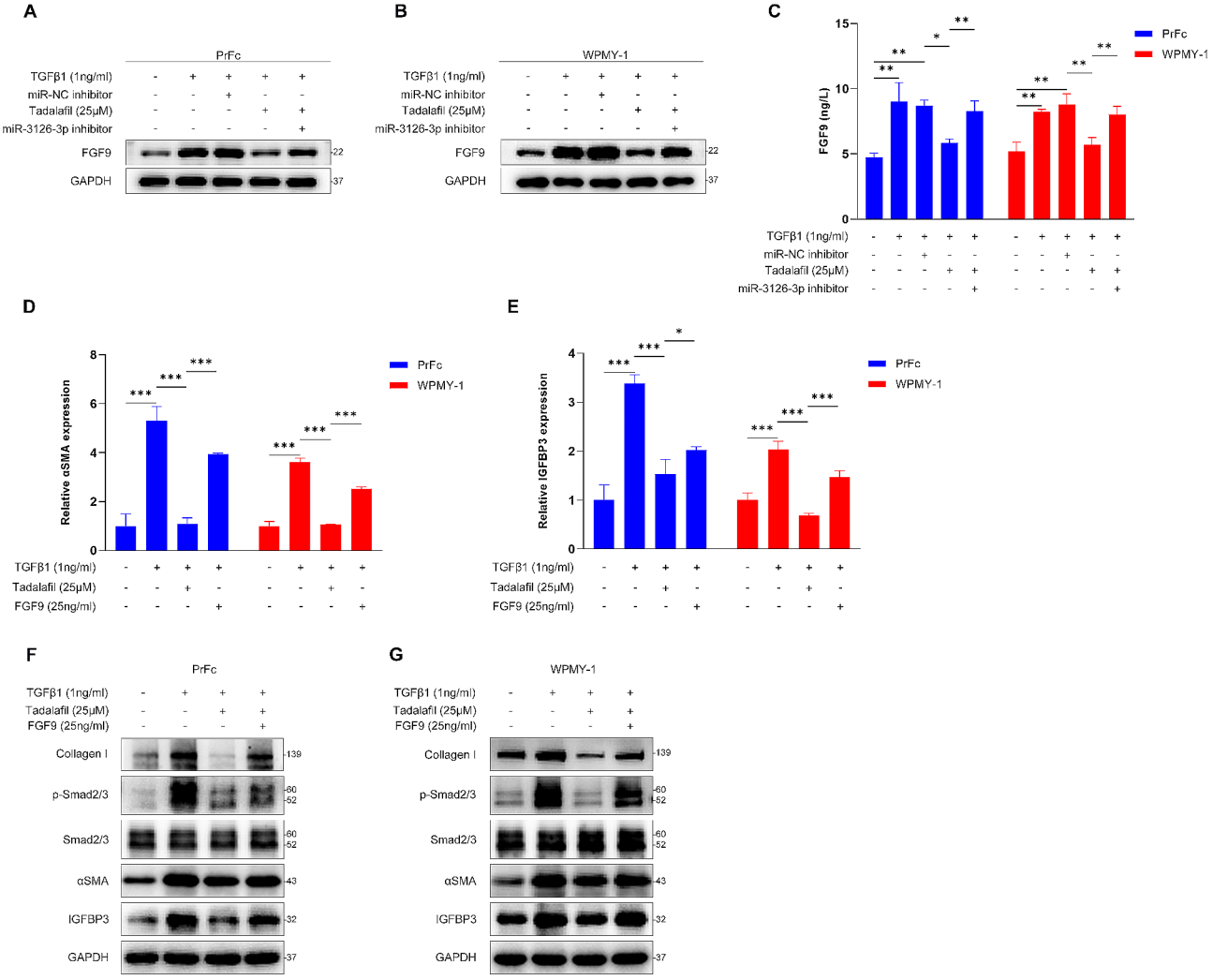

Fig. 5 Tadalafil restrains FMT of prostatic stromal fibroblasts through suppression of FGF9. (**A**-**B**) After 72-hour activation of PrFc and WPMY-1 cells with 1ng/mL TGFβ1, subsequent treatment with tadalafil (25μM) or transfection with miR-NC inhibitor/miR-3126-3p inhibitor was performed for 48 h. The protein levels of FGF9 were then assessed. (**C**) Activated PrFc and WPMY-1 cells were treated with tadalafil (25μM) or transfected with miR-NC inhibitor/miR-3126-3p inhibitor, and FGF9 levels in the supernatants were measured using ELISA. (**D**-**E**) qRT-PCR analyses were conducted to ascertain the αSMA and IGFBP3 mRNA expression levels in TGFβ1 (1ng/mL)-activated PrFc and WPMY-1 cells upon addition of tadalafil (25μM) and FGF9 (25ng/mL). (**F**-**G**) Following 72-hour TGFβ1 (1ng/mL) activation, PrFc and WPMY-1 cells were treated with tadalafil (25μM) and FGF9 (25ng/mL) for 48 h to determine the expression levels of FMT-related proteins ($n = 3$). (*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$)

PDE5i tadalafil reduces FMT by decreasing FGF9 in human BPH tissues

To verify the impact of tadalafil on the inhibitory effect of FMT in prostate stromal fibroblasts, we conducted an in vitro culture of human BPH tissues and treated them with 25 μM tadalafil (Fig. 6A). Masson's trichrome staining demonstrated that tadalafil significantly inhibited tissue fibrosis compared to the control group (Fig. 6B). Meanwhile, we performed qRT-PCR to assess the expression levels of miR-3126-3p in the tissues. As shown in Fig. 6C, the expression of miR-3126-3p was significantly upregulated in the tadalafil treated tissues. Additionally, we assessed the protein levels in the tissues using Western blotting and IHC. Consistent with previous in vitro experiments in PrFc and WPMY-1 cells, tadalafil significantly reduced the expression of FMT-related proteins Collagen I, αSMA, and IGFBP3 in the tissues (Fig. 6D-F). Furthermore, tadalafil was also found to suppress the protein levels of FGF9 in human BPH tissues (Fig. 6G).

Discussion

BPH is an age-related disease primarily characterized by the non-malignant proliferation of epithelial and stromal cells in the transitional zone of the prostate [40]. Recent research has shown that FMT plays a major role in the development of BPH. Prostatic fibrosis leads to the deposition of collagen tissue, which reduces urethral compliance and the expression of elastic proteins, thereby

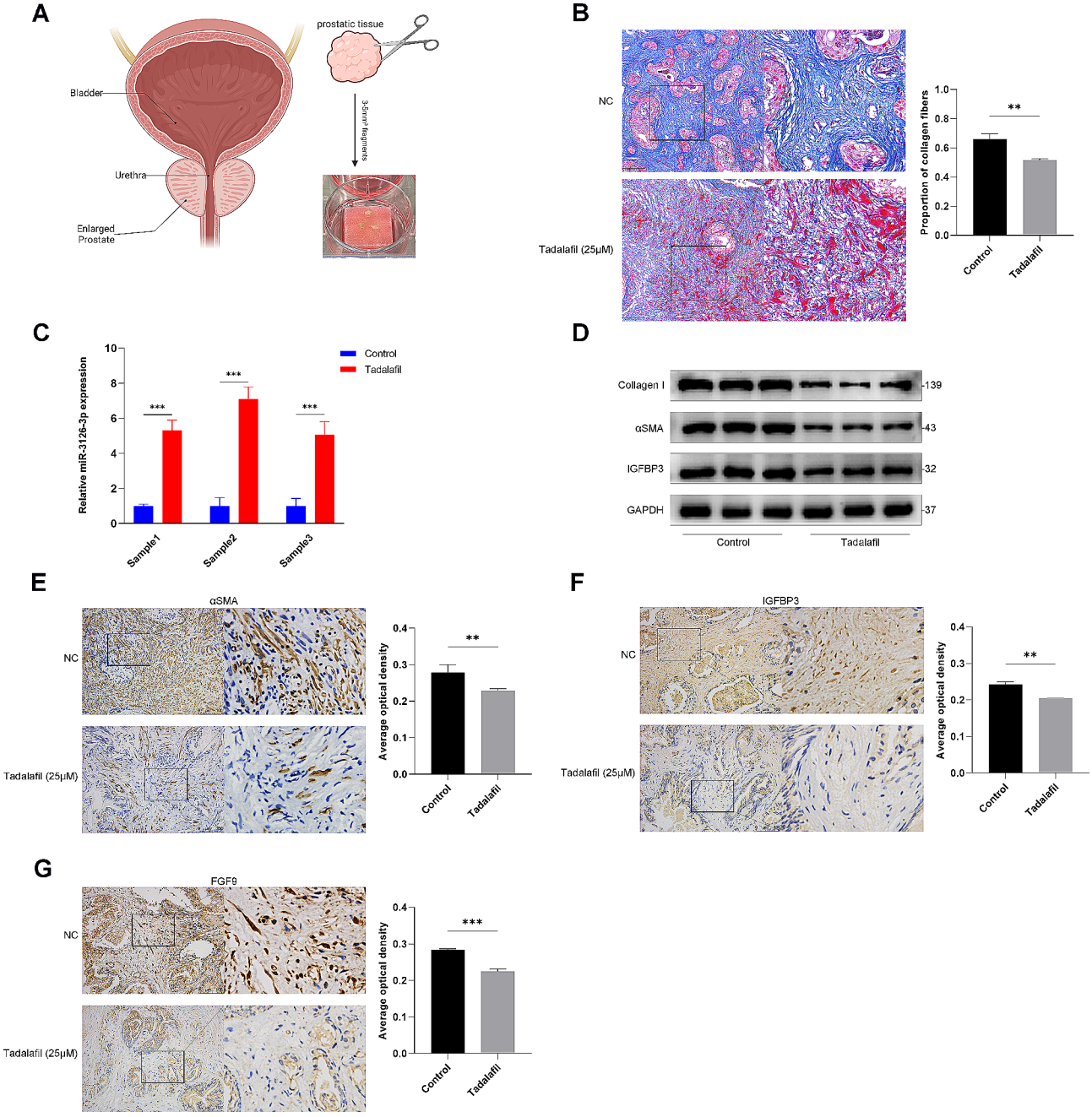

Fig. 6 Influence of PDE5 Inhibitor Tadalafil on Prostatic Tissue Fibrosis within the PDE Model. (**A**) Illustration outlining the construction process of the PDE model. (**B**) Masson's trichrome staining to evaluate the effect of tadalafil treatment (25μM) on prostate tissue fibrosis. (**C**) qRT-PCR comparison of miR-3126-3p expression levels between the tadalafil treatment tissues (25μM) and the control tissues. (**D**) Western blot analysis to detect the protein expression levels of Collagen I, αSMA, and IGFBP3 in the control group and the tadalafil treatment (25μM) group across the three PDE models. (**E**) Immunohistochemical assessment of αSMA staining density in PDE model after tadalafil treatment (25μM). (**F**) Evaluation of IGFBP3 expression by immunohistochemical analysis in the PDE model following tadalafil treatment (25μM) ($n = 3$). (**G**) Immunohistochemical investigation of FGF9 expression in the PDE model following tadalafil treatment (25μM) ($n = 3$). Values are presented as mean ± SD. (*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$)

increasing urethral pressure [41, 42]. Additionally, there are several clinical cases in which small-volume prostates exhibit severe fibrosis. Several studies have defined this condition as progressive BPH [9]. Alpha-reductase inhibitors (ARIs) are currently the first-line treatment for BPH. They significantly reduce prostate size and alleviate BPH-related LUTS by inhibiting dihydrotestosterone (DHT) synthesis through targeting 5-alpha reductase [13]. Nevertheless, their efficacy may be limited in BPH patients who have a predominant condition of stromal

hyperplasia and fibrosis [8]. Unfortunately, studies that investigated the inhibitory effect and potential mechanisms of drugs on fibrosis in prostatic stromal fibroblasts are limited.

PDE5 inhibitors are crucial in the treatment of ED, pulmonary arterial hypertension, and cardiovascular diseases. They achieve this by the inhibition of the NO/cGMP pathway [14–16]. The European Association of Urology currently recommends 5 mg of tadalafil per day as another effective treatment option for moderate to severe BPH [43]. Previous studies have shown that PDE5 is highly expressed in BPH stromal tissue, and low concentrations of PDE5 inhibitors can inhibit prostate stromal cell proliferation, while high concentrations can inhibit epithelial cell proliferation [35, 44]. This indicates that the effectiveness of PDE5 inhibitors in treating BPH may be associated with the relaxing effect of increased intracellular cGMP in stromal cells. Additionally, Morelli et al. found that PDE5 inhibitors can control prostatic inflammation and inhibit related fibrotic processes by improving tissue oxygenation in the prostate [45]. Our study shows that PDE5 inhibitor tadalafil can significantly inhibit the proliferation and FMT processes of prostate stromal fibroblasts.

MicroRNAs, a type of non-coding RNA, have emerged as a new class of mRNA translation regulators that influence protein stability. Recent advances in the field of microRNAs and their role in BPH have provided an interesting avenue for understanding the molecular mechanisms underlying the inhibition of prostate stromal cell fibrosis by PDE5 inhibitors [46]. For example, Zhou et al. identified that miR-222-3p/XBP1-mediated autophagy may play a crucial regulatory role in BPH [47]. Through in-depth analysis of PrFc sequencing data treated with bFGF/TGFβ1 and qRT-PCR, we identified that miR-3126-3p may play an important role in the inhibition effect of tadalafil on prostate stromal cell fibrosis. Furthermore, no studies have reported the mechanistic role of miR-3126-3p in prostate tissue. Our findings showed that miR-3126-3p could suppress fibrosis by targeting FGF9 expression.

Notably, this study reveals that tadalafil can reduce fibrosis in prostatic stromal cells by inhibiting FGF9, a secreted protein belonging to the FGF family. Studies have shown that FGF9 plays a critical role in the progression of various tumors, including lung adenocarcinoma, gastric cancer, and hepatocellular carcinoma [48–50]. Furthermore, FGF9 is also vital in neurodevelopment and neuroprotection via FGF-FGFR ligand-receptor interactions [51]. One study suggests that FGF9 can promote the formation of reactive stroma in the prostate cancer microenvironment [52]. In addition, Chen et al. demonstrate that in the presence of androgen deprivation therapy (ADT), CAFs can secrete FGF9 through paracrine signaling, which promotes the proliferation of prostate cancer cells [30]. Our study found that the addition of FGF9 in the culture medium significantly attenuated the inhibitory effect of tadalafil on prostate stromal cell fibrosis. This suggests that FGF9 plays an indispensable role in the anti-fibrotic effects of PDE5 inhibitors in BPH. In our study, we treated TGFβ1-activated stromal fibroblasts with both tadalafil and rhFGF9. This successfully rescued the FMT phenotype, indicating that tadalafil can inhibit the FMT process by suppressing FGF9. Nevertheless, we did not observe a complete rescue. Thus, we hypothesize that FGF9 is only partially responsible for tadalafil's inhibition of FMT. As a result, we cannot rule out the possibility that tadalafil will affect other targets involved in the FMT process. Figure 7 depicts the molecular mechanism by which tadalafil upregulates miR-3126-3p to suppress FGF9.

Interestingly, our findings establish miR-3126-3p as a promising therapeutic target for inhibiting prostate fibrosis in BPH patients treated by the PDE5i tadalafil. Our study confirmed that PDE5 inhibitors can inhibit the FMT process in prostate stromal fibroblasts via the miR-3126-3p/FGF9 signaling axis. Furthermore, we discovered that PDE5 inhibitors inhibit prostate stromal fibroblast proliferation as well as fibrosis, implying that tadalafil's antiproliferative effects may be mediated by other pathways. Nonetheless, since fibrosis is a critical factor in BPH progression, we believe that identifying this novel mechanism by which PDE5 inhibitors suppress prostate stromal fibrosis will have significant clinical implications.

Our study has several limitations to be addressed. The rescue experiments employed miRNA inhibitors, which could result in off-target effects. Using genetic techniques to modulate specific genes could help improve the credibility of our findings. Moreover, it is imperative to conduct additional research to investigate the function of FGF9 derived from activated stromal fibroblasts in the microenvironment of BPH tissue. To address this issue, transgenic mice should be employed to verify the role of FGF9 in the pathogenesis of BPH. Furthermore, it is worth investigating whether increasing the expression of miR-3126-3p can enhance the therapeutic effects of tadalafil.

Conclusion

Our study verifies that tadalafil inhibits the regulatory effect of TGFβ1 on miR-3126-3p, resulting in the upregulation of miR-3126-3p. Simultaneously, miR-3126-3p acts by specifically targeting and suppressing the expression of FGF9, thereby inhibiting the FMT process in stromal fibroblasts. Ultimately, this study provides a basis for future investigations into the role of FGF9 in BPH and

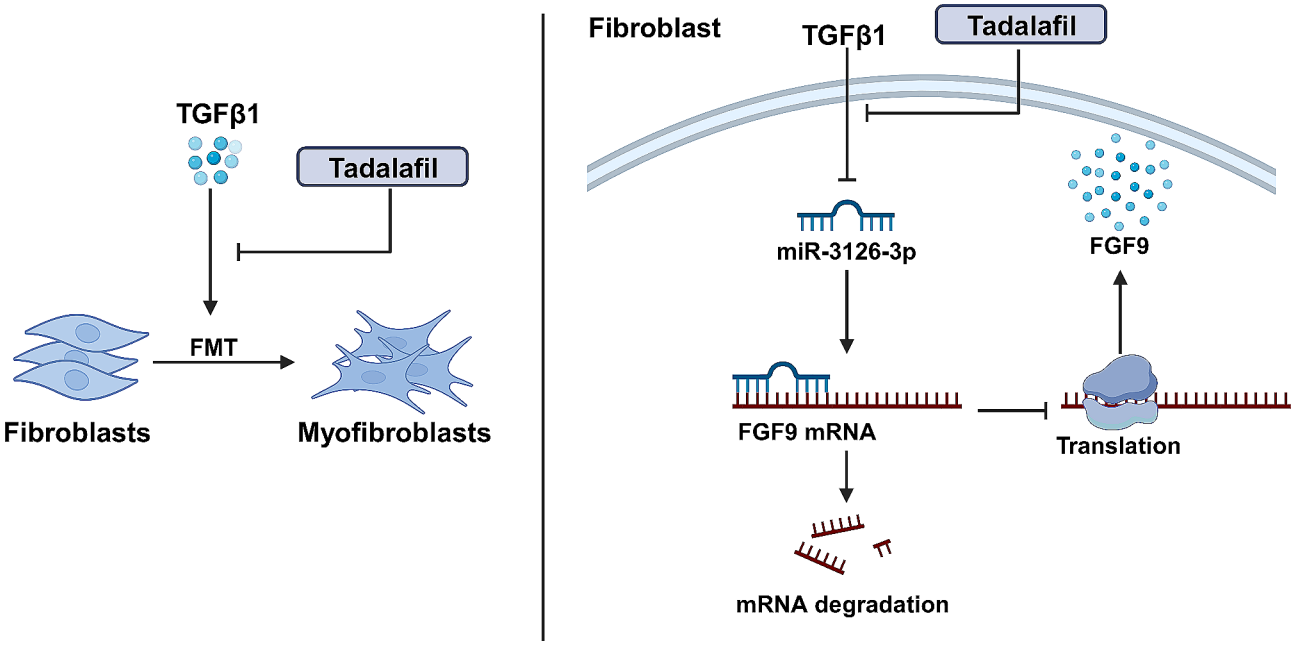

Fig. 7 Mechanistic depiction of PDE5 inhibitor-mediated inhibition of prostatic FMT process. Tadalafil can counteract the inhibitory impact of TGFβ1 on miR-3126-3p, ultimately boosting the expression of miR-3126-3p. Following this, miR-3126-3p selectively binds to the 3'UTR region of FGF9 mRNA, thereby curtailing both the translation and secretion of FGF9. This intricate series of events culminates in the suppression of FMT differentiation within the prostate

reveals a potential approach for the treatment of BPH by identifying a new target for PDE5 inhibitor therapy.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13062-024-00504-y.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Author contributions

TL and YZ designed and completed original manuscript writing; ZZ, LG, YZ and WW implemented data analysis and literature collection; YZ and XZ reviewed the manuscript; YR, CJ and DC supervised and funded this work. All authors read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Competing interests

The authors declare no competing interests.

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