

MicroRNA-221 inhibits the transition of endothelial progenitor cells to mesenchymal cells via the PTEN/FoxO3a signaling pathway

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Conflict of interest

None declared

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Abstract

Background. Coronary heart disease is one of the most common cardiovascular diseases worldwide and is often associated with vascular endothelial injury. Endothelial–mesenchymal transition (EndMT) is an important process in vascular endothelial injury.

Objectives. This study investigated the function of miR-221 in the EndMT process of endothelial progenitor cells (EPCs).

Materials and methods. Transforming growth factor beta (TGF-β1) was used to induce EndMT in EPCs, and SM22α expression was detected using immunocytochemistry. Western blot was used to detect alpha smooth muscle actin (αSMA) expression, and miR-221 function was evaluated using inhibitors or mimics of the miR-221 sequences that were transfected into EPCs. Reverse transcription polymerase chain reaction (RT-PCR) was used to detect the expression of miR-221 and western blot was used to detect the expression of αSMA, myocardin, phosphatase and tensin homolog (PTEN), p-FoxO3a, and FoxO3a in EPCs. Finally, the expression of the miR-221 target genes was determined using RT-PCR.

Results. The expression of SM22α and αSMA increased in EPCs treated with TGF-β1, while the expression of miR-221 was decreased in EPCs on the 5th day, when compared with the control. The expression of SM22α increased after inhibiting miR-221 in EPCs treated with TGF-β1 and this was reversed by the overexpression of miR-221. The expression of αSMA and myocardin was significantly increased after inhibiting miR-221 in EPCs treated with TGF-β1 and decreased in EPCs overexpressing miR-221. Conversely, PTEN was increased in TGF-β1-treated EPCs and decreased following the overexpression of miR-221. The decrease in phosphorylated-FoxO3a expression in EPCs was accompanied by an increase in αSMA expression, which was reversed in the presence of miR-221 mimics. This effect was nearly abolished following the addition of PTEN cDNA.

Conclusions. The overexpression of miR-221 inhibits EndMT in EPCs, possibly by interacting with PTEN to regulate FoxO3a signaling, to facilitate the repair of the endothelium by EPCs.

Key words: EPC, miR-221, EndMT, PTEN/FoxO3a

Background

Coronary heart disease is one of the most common cardiovascular diseases worldwide and is caused by vascular endothelial injury. It has been demonstrated that once the vascular endothelium is damaged, the endothelial progenitor cells (EPCs) migrate from the bone marrow (BM) to the ischemic tissue and differentiate into mature vascular endothelial cells in an attempt to repair the injured site.¹ However, accumulating evidence suggests that EPCs contribute to intimal hyperplasia during this process. For example, BM-derived EPCs have been shown to augment intimal hyperplasia by migrating into the intima of balloon-injured carotid arteries,² and our previous study showed that BM-derived EPCs can transdifferentiate into smooth muscle lineage cells that are positive for alpha smooth muscle actin (α SMA), producing an endothelial-to-mesenchymal transition (EndMT).³ These results suggest that the EndMT capacity of EPCs may contribute to intimal hyperplasia. This is further supported by evidence that EndMT increases the thickness of the intimal layer in pulmonary arteries.⁴ However, the mechanisms underlying the EndMT process in EPCs have not yet been fully elucidated.

MicroRNAs (miRs) are short (only 21–23 nucleotides), highly conserved, non-coding RNA sequences that bind to the 3'-untranslated region of a variety of mRNA targets and function to regulate gene expression at the post-transcriptional level by inhibiting the translation or promoting mRNA degradation.^{5,6} Previous microarray-based expression profiles have shown that several miRs are expressed in EPCs, including miR-126, miR-21, miR-27a, and miR-221.^{7,8} Furthermore, some studies have shown that both miR-21 and miR-127 are involved in mature endothelial cell EndMT that can be induced by TGF- β 1 and TGF- β 2.^{8,9} In addition, miR-221, an endothelial-specific miR, has been shown to exert anti-angiogenic, anti-inflammatory and pro-apoptotic effects by targeting both ZEB2 and Ets-1.^{10,11} Finally, miR-221 maintains the angiogenic capacity of EPCs by inhibiting p27 expression.¹² However, few studies have described the role of miR-221 in the EndMT process of EPCs.

Previous investigations have confirmed that phosphatase and tensin homolog (PTEN) is a direct target of miR-221.¹³ In vascular endothelial cells, downregulation of miR-221 decreased cell viability, migration and invasion, while all of these effects were reversed in response to the overexpression of PTEN.¹³ Furthermore, recent studies have shown that a decrease in PTEN activates the phosphorylation of FoxO3a.^{14,15} In our previous study, we showed that phosphorylated FoxO3a plays an inhibitory role in EndMT by inactivating the Smad3/4 pathway in the nucleus of EPCs.¹⁶ Given this, we hypothesized that the activation of the miR-221/PTEN/FoxO3a pathway may inhibit EndMT in EPCs.

Objectives

This study was designed to determine the function of miR-221 in the EndMT process of EPCs and to explore the potential signaling pathways underlying these functions.

Materials and methods

Ethical approval

All animal experiments were approved by the Animal Research Ethics Committee of the No. 9 People's Hospital affiliated with the Shanghai Jiao Tong University School of Medicine, Shanghai, China. All animals were sacrificed using bilateral thoracotomy during anesthesia.

Cell isolation and culture

Bone marrow-derived EPCs were isolated from male Sprague Dawley rats, as described previously.² Ficoll-Isopaque Plus (Histopaque-1077; Sigma-Aldrich, St. Louis, USA) density gradient centrifugation was used to isolate mononuclear cells from rat bone marrow. The cells were cultured in endothelial cell growth medium (EGM2; Lonza, Basel, Switzerland) consisting of endothelial cell basal medium-2 (EBM-2), supplemented with 20% fetal bovine serum (FBS). Cells were then cultured on a fibronectin-coated dish at 37°C in a 5% CO₂ incubator. The medium was removed after 4–7 days, and cells were gently washed with phosphate-buffered solution (PBS). The attached cells were then used for further experiments. The CD31 fluorescence staining was then performed as previously described⁷ to identify vascular endothelial cells.

Inducing EndMT in EPCs

Endothelial progenitor cell EndMT was induced as previously described.¹⁷ Briefly, the cells were seeded on culture plates in Ham's F-12 media containing 5% FBS, and then treated with TGF- β 1 (5 ng/mL) for 7 days.

Immunocytochemistry

The EPCs were cultured on cell slides and then rinsed with PBS, before being fixed with 4% paraformaldehyde phosphate buffer for 15 min at room temperature. Slides were then blocked for 2 h in PBS containing 5% normal goat serum and 2% bovine serum albumin (BSA), and incubated with monoclonal antibodies (diluted 1:500) against SM22 α (Cat. No. SAB1400272; Sigma-Aldrich) overnight at 4°C. Cells were washed 5 times in PBS and incubated with AlexaFluor® 594-conjugated goat anti-mouse/rabbit antibody diluted 1:500 (Santa Cruz Biotechnology, Dallas, USA) for 1 h at room temperature. The mean fluorescence intensity of SM22 α was

evaluated using ImageJ software (RRID:SCR_003070; National Institutes of Health (NIH), Bethesda, USA).

Real-time PCR

Total RNA was isolated from EPCs using TRIzol reagent (Cat. No. 10296028, Lot No. 79348502; Life Technologies, Carlsbad, USA), as described in the manufacturer's instructions. Extracted RNA was then reverse transcribed to cDNA using a Thermo Cycler (Applied Biosystems, Waltham, USA) and real-time polymerase chain reaction (RT-PCR) was performed using a real-time PCR machine (LightCycler 480 II; Roche, Basel, Switzerland). The mRNA was quantified using a TaKaRa reverse transcription assay (Cat. No. R0037A; TaKaRa, Tokyo, Japan) and SYBR II (Cat. No. RR820A; TaKaRa). Gene-specific primers were synthesized by Sangon Biotech (Shanghai, China) and mRNA levels were quantified using the $2^{-\Delta\Delta Ct}$ method (Table 1).

Transfection

The miR-221 mimics and inhibitors were synthesized using the Qiagen (Hilden, Germany) reagents and the miR-221 mimic sequence was set as 5'-AGCUACAU-UGUCUGCUGGGUUUC-3'. The gene ID of PTEN in National Center for Biotechnology Information (NCBI) was NM_000314 and both, the PTEN cDNA and siRNAs, were synthesized by Sangon Biotech. Cells were seeded in six-well plates and transfected with the miR-221 mimic, miR-221 inhibitor or their negative controls using Lipofectamine 3000 transfection reagent (Cat. No. 11668019; Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Transfections were performed on days 4 and 7. All subsequent experiments were performed 24 h after the final transfection.

Western blot

Total protein from the EPCs was extracted using radioimmunoprecipitation assay (RIPA) buffer and then separated using 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) before being transferred to polyvinylidene fluoride (PVDF) membranes using electrophoresis, and probed with primary antibodies against α SMA (Cat.

No. CBL171; Merck Millipore, Billerica, USA), myocardin (Cat. No. 14760S; Cell Signaling Technology, Danvers, USA), PTEN (Cat. No. sc-7974; Santa Cruz Biotechnology, Dallas, USA), p-FoxO3a (Cat. No. sc-101683; Santa Cruz Biotechnology), FoxO3a (Cat. No. 2497s, Cell Signaling Technology), and β -actin (Cat. No. 3700; Cell Signaling Technology). Appropriate horseradish peroxidase (HRP)-conjugated secondary antibodies were then used to facilitate visualization, and protein bands were detected using an electrochemiluminescence (ECL) system (Fusion FX7; Vilber, Lemont, USA).

Statistical analyses

All data were initially tested using the one-sample Kolmogorov–Smirnov normality test and F-test. This highlighted that not all data were normally distributed and that some data variance was uneven. All experiments were repeated 5 times and therefore we used the more reliable nonparametric tests. The data were expressed as maximum, minimum, median, percentiles 25, and percentiles 75, and analyzed with the Kruskal–Wallis H test followed by a Mann–Whitney U test for post hoc comparison, using the SPSS v. 13.0 software (SPSS Inc., Chicago, USA). The values of $p \leq 0.05$ were considered statistically significant.

Results

TGF- β 1 induces EndMT in EPCs

Endothelial progenitor cells were identified as described in our previous research.⁷ The cells cultured from mononuclear cells showed a cobblestone-like appearance, while EPCs treated with TGF- β 1 showed a spindle-shaped appearance (Fig. 1A,B). Immunofluorescent staining showed that EPCs treated with TGF- β 1 had a high SM22 α expression (Fig. 1C,D) and the expression of α SMA also increased in response to TGF- β 1 (Fig. 1E).

miR-221 expression was decreased following EPC EndMT

The miR-221 expression continuously increased in EPCs cultured in medium without TGF- β 1. In contrast, when

Table 1. The primer sequences used for this investigation

Gene	Forward 5' → 3'	Reverse 5' → 3'
miR-221	CGCGAGCTACATTGTCTGCTG	AGTGCAGGGTCCGAGGTATT
PTEN	TTGAAGACCATAACCCACACACAGC	CATTACACCAGTCCGTCCTTTCCC
PIK3R1	ACTGAAGCAGATGTTGAACAAC	CATCGATCATTCCAAGTCCAC
FoxO3a	TGGACGCCTGGACCGACTTC	GCTCCGTGCTCGCAAGATG
ESR1	CTGCTGCTGGAGATGCTGGATG	CTGGCTCTGGCTAGGCTCCTC
MMP-1	TGTTTCGCCTTCTACAGAGGAGACC	CTGGTGGGAATGTGTGAGCAAGTC
GAPDH	GACATGCCCGCTGGAGAAAC	AGCCCAGGATGCCCTTAGT

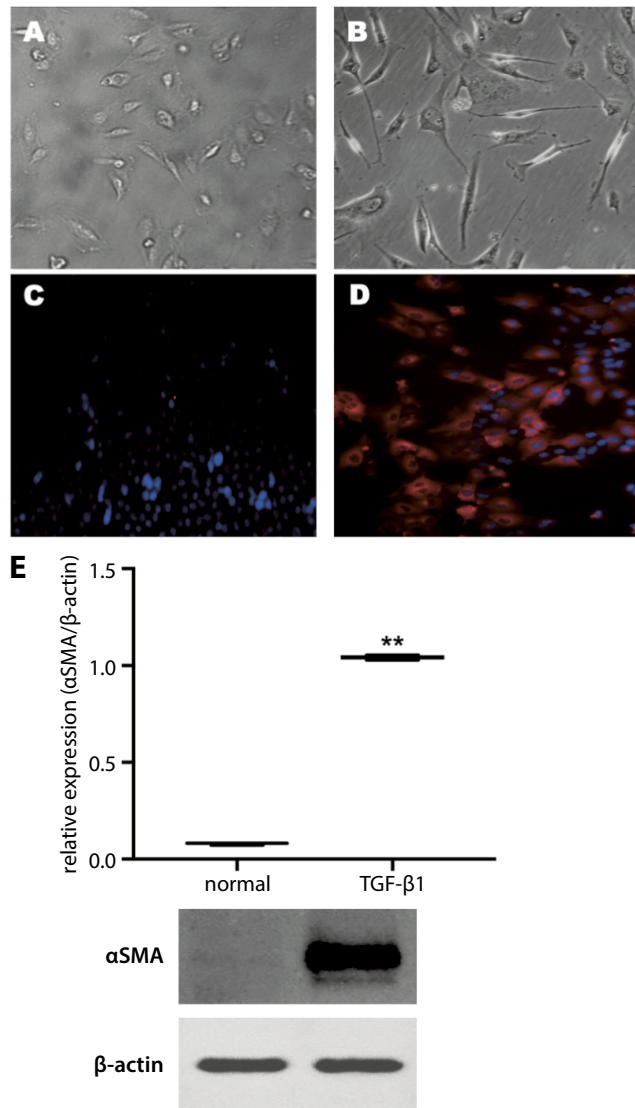


Fig. 1. TGF- β 1 induces endothelial-mesenchymal transition (EndMT) in endothelial progenitor cells (EPCs). A and B. Morphology of EPCs under a light microscope; A. EPCs cultured in endothelial cell basal medium-2 (EBM-2) containing 20% fetal bovine serum (FBS); B. The EPCs treated with TGF- β 1; C and D. Immunofluorescence staining of SM22 α in EPCs (red: SM22 α ; blue: DAPI); C. The EPCs cultured in EBM-2 containing 20% FBS; D. The EPCs treated with TGF- β 1; E. The expression of α SMA in EPCs with or without TGF- β 1 was detected with western blot (data expressed as maximum, minimum, median, percentiles 25 and percentiles 75, and analyzed with Kruskal-Wallis H test followed by Mann-Whitney U test for post hoc comparison using the SPSS v. 13.0 software). Normal – EPCs cultured in EBM-2 containing 20% FBS; TGF β 1 – EPCs treated with TGF- β 1; ** $p < 0.01$

EPCs were treated with TGF- β 1, the miR-221 expression remained comparable over the first 3 days and then began to gradually decrease compared to controls (Fig. 2A).

Overexpression of miR-221 inhibits TGF- β 1-induced EndMT of EPCs

To demonstrate the role of miR-221 in EPC EndMT, EPCs were transfected with either a miR-221 inhibitor

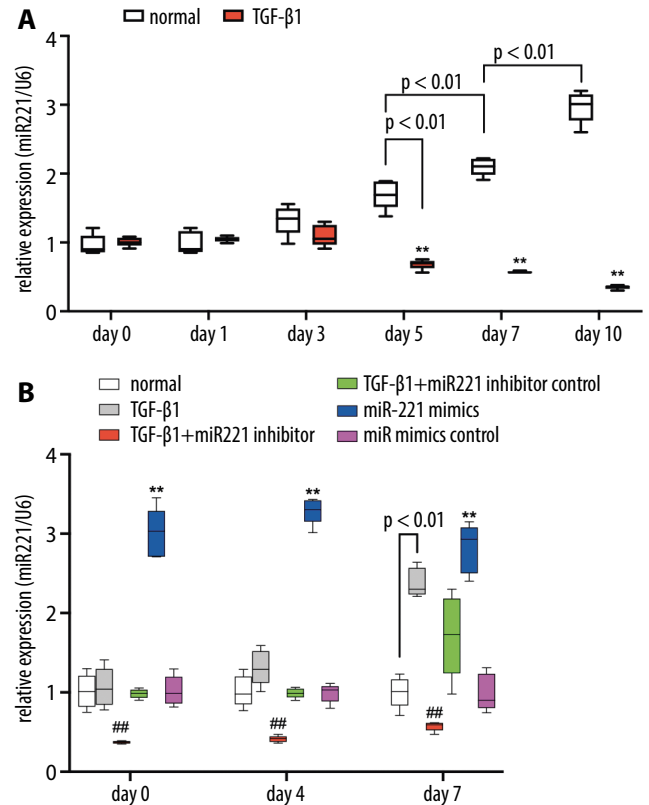


Fig. 2. The expression of miR-221 in endothelial progenitor cells (EPCs) during endothelial-mesenchymal transition (EndMT). A. The expression of miR-221 in EPCs with or without TGF- β 1 over 10 days and detected with reverse transcription polymerase chain reaction (RT-PCR). Normal – EPCs cultured in endothelial cell basal medium-2 (EBM-2) containing 20% fetal bovine serum (FBS); TGF β 1 – EPCs treated with TGF- β 1; **normal compared to TGF- β 1, $p < 0.01$; B. The expression of miR-221 every 3 days following the treatment with miR-221 mimics and inhibitors as detected with RT-PCR. The data were expressed as maximum, minimum, median, percentiles 25 and percentiles 75, and analyzed with Kruskal-Wallis H test, followed by Mann-Whitney U test for post hoc comparison. Normal – EPCs cultured in EBM-2 containing 20% FBS; TGF β 1 – EPCs treated with TGF- β 1; TGF β 1 + miR-221 inhibitor – EPCs transfected with miR-221 inhibitor then treated with TGF- β 1; TGF β 1 + miR-221 inhibitor control – EPCs transfected with the negative control of miR-221 inhibitor then treated with TGF- β 1; miR-221 mimics – EPCs cultured in EBM-2 containing 20% FBS and transfected with miR-221 mimics; miR-221 mimics control – EPCs cultured in EBM-2 containing 20% FBS and transfected with the negative control of miR-221 mimics; ** normal compared to miR-221 mimics, $p < 0.01$; ## TGF- β 1 compared to TGF- β 1 + miR-221 inhibitor, $p < 0.01$

or miR-221 mimic (Fig. 2B), and then treated with TGF- β 1. Endothelial progenitor cells transfected with the miR-221 inhibitor exhibited a spindle-shaped appearance, while EPCs transfected with miR-221 mimics presented with a cobblestone-like appearance (Fig. 3A–C). Immunofluorescent staining revealed that the SM22 α expression significantly increased or decreased in response to the miR-221 inhibitor or miR-221 mimic, respectively (Fig. 3D–G). The expression of both α SMA and myocardin was significantly increased in the presence of the miR-221 inhibitor and attenuated in the presence of miR-221 mimics in EPCs treated with TGF- β 1 (Fig. 4A,B).

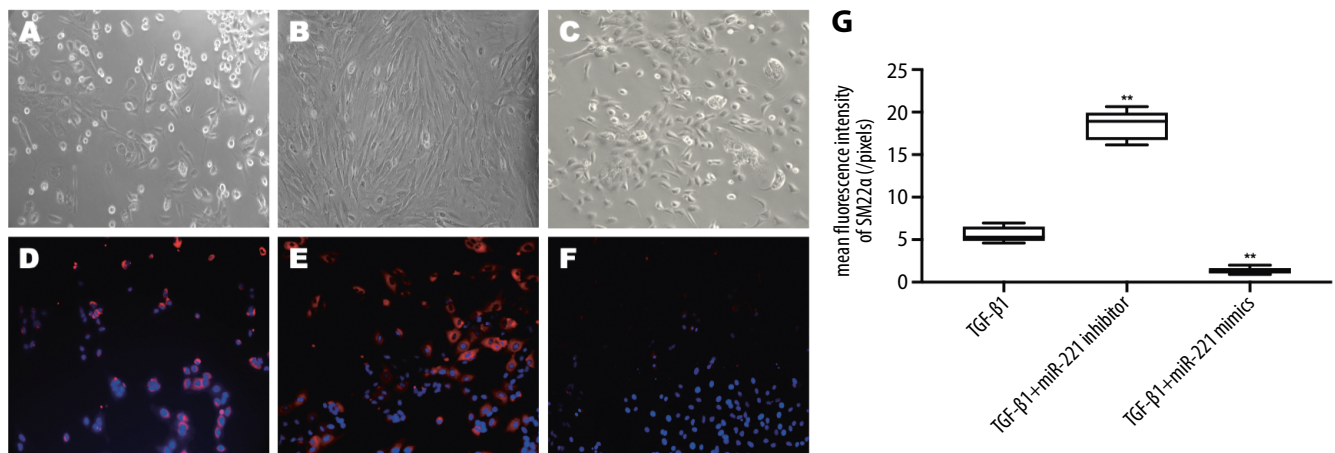


Fig. 3. Morphological and SM22α expression changes in endothelial progenitor cells (EPCs) following miR-221 overexpression or silencing during endothelial-mesenchymal transition (EndMT). A–C. Morphological changes in EPCs evaluated using light microscopy; A. The EPCs treated with TGF-β1; B. The EPCs transfected with miR-221 inhibitors and treated with TGF-β1; C. The EPCs transfected with miR-221 mimics and treated with TGF-β1; D–F. Immunofluorescence staining of SM22α in EPCs. The expression of SM22α in EPCs during EndMT following miR-221 overexpression or silencing; D. The EPCs treated with TGF-β1; E. Silencing of miR-221 in EPCs treated with TGF-β1; F. Overexpression of miR-221 in EPCs treated with TGF-β1; G. Mean fluorescence intensity of SM22α in EPCs following miR-221 overexpression or silencing during EndMT. Data expressed as maximum, minimum, median, percentiles 25 and percentiles 75, and analyzed with Kruskal–Wallis H test followed by Mann–Whitney U test for post hoc comparison. TGFβ1 – EPCs treated with TGF-β1; TGFβ1 + miR-221 inhibitor – EPCs transfected with miR-221 inhibitor and treated with TGF-β1; TGFβ1 + miR-221 mimics – EPCs transfected with miR-221 mimics and treated with TGF-β1; ** $p < 0.01$

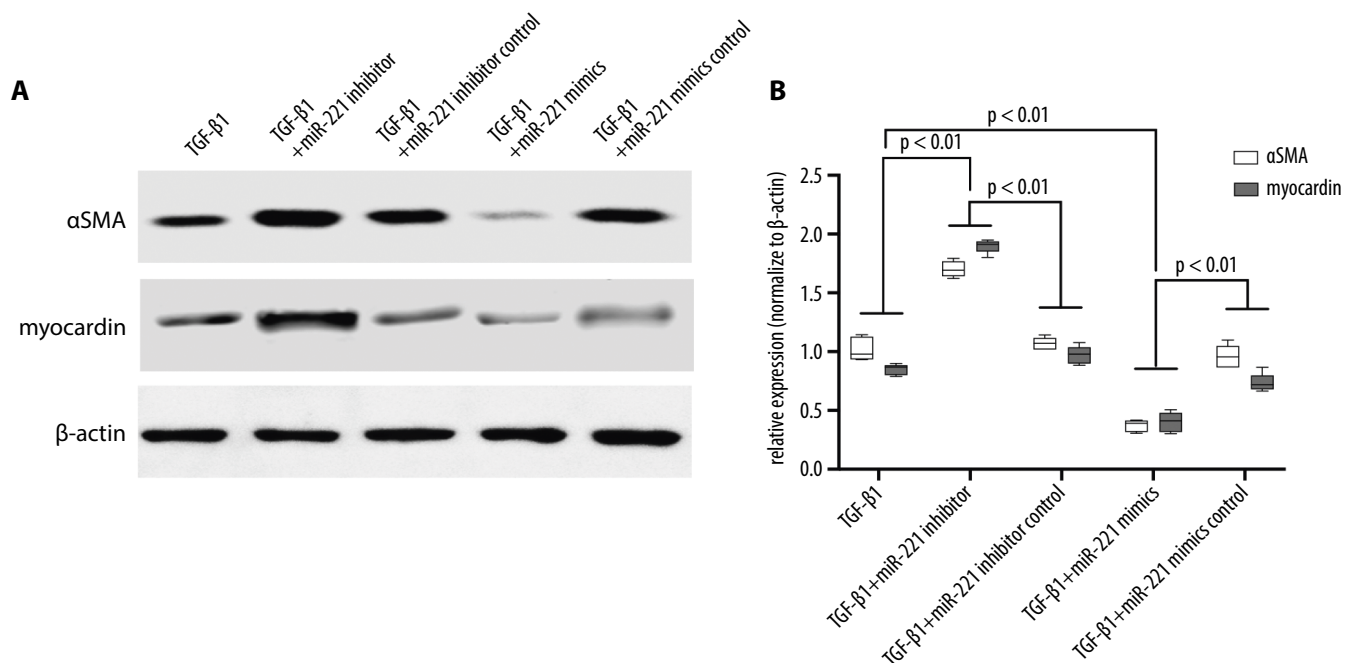


Fig. 4. Expression of αSMA and myocardin in endothelial progenitor cells (EPCs) undergoing endothelial-mesenchymal transition (EndMT). A. Western blot describing αSMA and myocardin expression during EndMT in EPCs; B. The relative expression of αSMA and myocardin under these conditions. Data expressed as maximum, minimum, median, percentiles 25 and percentiles 75, and analyzed with Kruskal–Wallis H test followed by Mann–Whitney U test for post hoc comparison. TGFβ1 – EPCs treated with TGF-β1; TGFβ1 + miR221 inhibitor – EPCs transfected with miR-221 inhibitor then treated with TGF-β1; TGFβ1 + miR221 inhibitor control – EPCs transfected with the negative control of miR-221 inhibitor and treated with TGF-β1; TGFβ1 + miR221 mimics – EPCs transfected with miR-221 mimics and treated with TGF-β1; TGFβ1 + miR221 mimics control – EPCs transfected with the negative control of miR-221 mimics and treated with TGF-β1

miR-221 regulates the PTEN/FoxO3 signaling pathways in TGF-β1-induced EndMT

The expression of several potential targets of miR-221 in EPCs undergoing TGF-β1-dependent EndMT was

assessed with RT-PCR. The expression of PTEN was shown to be significantly higher in EPCs treated with TGF-β1, while the expression of PIK3R1, FoxO3a, ESR1, and MMP-1 was comparable between the 2 groups (Fig. 5A). The expression of αSMA was increased in TGF-β1-treated EPCs and was attenuated in response to PTEN siRNA. However,

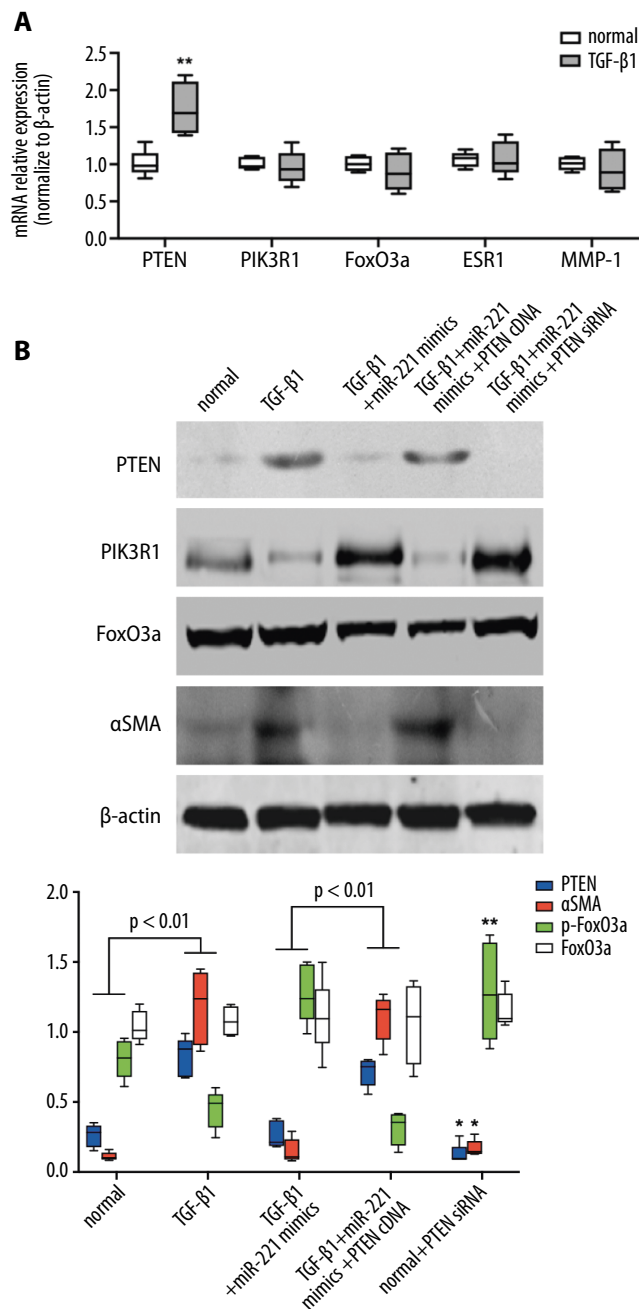


Fig. 5. Expression of miR-221 related genes in endothelial progenitor cells (EPCs) during endothelial-mesenchymal transition (EndMT). **A.** The expression of phosphatase and tensin homolog (PTEN), PIK3R1, FoxO3a, ESR1, and MMP-1 during EndMT was detected with reverse transcription polymerase chain reaction (RT-PCR). Normal – EPCs cultured in endothelial cell basal medium-2 (EBM-2) containing 20% fetal bovine serum (FBS); TGFβ1 – EPCs treated with TGF-β1; TGFβ1 + miR221 mimics – miR-221 overexpressing EPCs treated with TGF-β1; TGFβ1 + miR221 mimics + PTEN cDNA – miR-221 and PTEN overexpressing EPCs treated with TGF-β1; normal + PTEN siRNA – PTEN silenced EPCs cultured in EBM-2 containing 20% FBS; *normal compared to normal + PTEN siRNA 5, p < 0.05; **normal compared to normal + PTEN siRNA, p < 0.01

the increase in αSMA expression was significantly attenuated by the addition of miR-221 mimics in EPCs treated with TGF-β1, which was reversed in response to PTEN cDNA (Fig. 5B). At the same time, the total expression of FoxO3a remained unchanged. However, phosphorylated FoxO3a expression was notably reduced in response to TGF-β1, which was reversed following the addition of the miR-221 mimics. This reversal was nearly completely abolished following the addition of the PTEN cDNA. In addition, the phosphorylation of FoxO3a was significantly augmented in EPCs treated with PTEN siRNA compared to normal cells (Fig. 5B).

Discussion

It has been widely reported that EPCs are beneficial in the treatment of coronary heart disease, as these cells can differentiate into mature vascular endothelial cells. However, accumulating evidence suggests that EPCs can also differentiate into mesenchymal cells, which may aggravate intimal hyperplasia and contribute to atherogenesis.^{18,19} Several studies have shown that TGF-β1 induces the EndMT process in EPCs in vitro. However, only half of the EPCs transplanted into the impaired endothelium were transformed into smooth muscle cells (SMCs) following TGF-β1 treatment, while the others acted to enhance intimal hyperplasia in vivo.² These results suggest that the EndMT process in EPCs can be regulated. In this study, we found that a deficiency in miR-221 may facilitate TGF-β1-dependent EndMT in EPCs. Furthermore, miR-221 inhibits the EndMT process by interacting with PTEN, which regulates PTEN/FoxO3 signaling.

The SM22α and αSMA are mesenchymal markers used to detect EndMT in EPCs, as described previously.⁷ In this study, the expression of SM22α and αSMA significantly increased in EPCs after the treatment with TGF-β1, suggesting that EPCs underwent EndMT.⁷ It has also been reported that EPCs derived from rat bone marrow are able to undergo EndMT after treatment with TGF-β1,² while human circulating EPCs only differentiated into mesenchymal cells following direct contact with smooth muscle cells,¹⁹ indicating a complex regulatory system in different tissues.

Some studies have reported that miRs are closely associated with cellular transdifferentiation. The miRNA-221 exerts anti-angiogenic, anti-inflammatory and pro-apoptotic effects in EPCs through their interactions with ZEB2 and Ets-1.^{10,11} In ectopic endometrial tissues and ectopic endometrial stromal cells, the inhibition of miR-221 expression leads to decreased cellular proliferation.²⁰ In our study, we found that the expression of miR-221 was markedly decreased for up to 5 days following TGF-β1 treatment, and was accompanied by an increase in αSMA expression. This suggests that miR-221 is involved in the EndMT process of EPCs.

To determine the role of miR-221 in EndMT, miR-221 inhibitor or mimics were repeatedly transfected into EPCs following TGF- β 1 treatment on days 1, 4 and 7, to induce either upregulation or downregulation of miR-221. As a result, the expression of SM22 α , α SMA and myocardin in EPCs undergoing EndMT was either increased or decreased following transfection with miR-221 inhibitors and miR-221 mimics, respectively. The previous study suggests that myocardin plays a critical role in the development of cardiac myocytes and vascular SMCs. Loss of myocardin results in a synthetic, dedifferentiated phenotype that leads to atherosclerosis.²¹ These results suggest that miR-221 inhibits the EndMT process in EPCs by suppressing the expression of α SMA and myocardin.

Previous studies have also reported that the PI3K/Akt/FoxO3 signaling pathway plays an important role in the self-renewal of hematopoietic stem cells.²² In our previous study, we also found that miR-126 targets PI3KR2 to inhibit EPC EndMT through the PI3K/Akt/FoxO3 pathway.⁷ In this study, we evaluated several potential target genes for miR-221, including *PTEN*, *PI3KR1*, *FoxO3a*, *ESR1*, and *MMP-1*, in EPCs undergoing EndMT. We found that the expression of *PTEN* was increased in EPCs treated with TGF- β 1, while its expression was inhibited by overexpression of miR-221. The expression levels of *PI3KR2*, *FoxO3a*, *ESR1*, and *MMP-1* were comparable between the 2 groups. These results suggest that *PTEN* might be a target of miR-221 and regulate the EndMT process in EPCs. This was supported by the finding that miR-221 overexpression amplified PI3K signaling to promote precursor B-cell retention by targeting *PTEN*.²³

In addition, we found that FoxO3a expression was slightly decreased in EPCs treated with TGF- β 1. A downstream effector of the *PTEN*/Akt pathway, FoxO3a, is localized within the nucleus, where it drives transcription. However, when phosphorylated, the protein translocates to the cytoplasm.²⁴ One example of this kind of regulation can be seen in the differential regulation of onco- and anti-oncogene expression in cells following the nuclear translocation of phosphorylated AKT.^{14,25} Interestingly, we found that the expression of phosphorylated FoxO3a (p-FoxO3a) was significantly increased when *PTEN* was suppressed, while overexpression of *PTEN* led to the inhibition of p-FoxO3a expression. This further suggests that *PTEN* decreases the phosphorylation of FoxO3a, thereby suppressing its nuclear translocation in EPCs undergoing TGF- β 1-dependent EndMT. Furthermore, we found that miR-221 overexpression led to a decrease in *PTEN* expression and an increase in α SMA expression, both of which were reversed by *PTEN* overexpression. Finally, our previous study demonstrated that the phosphorylation of FoxO3a negatively regulates EPC EndMT.⁷ Taken together, these findings suggest that miR-221 may lead to *PTEN* downregulation and the activation of the FoxO3a signaling pathway, thereby inhibiting the EndMT process in EPCs.

Limitations


In the present study, we report that the overexpression of miR-221 inhibits EndMT in EPCs, but as all experiments in this study were conducted in vitro, there should be some focus on validating these findings in vivo. We believe that miR-221 interacts with *PTEN* to regulate FoxO3a/Smad4 transcription, but we did not evaluate Smad4 expression in this study, as these observations were recorded in our previous paper.⁷ Our findings suggest that miR-221 may be a potential target during the treatment of in-stent restenosis in coronary artery disease, and facilitate the inhibition of intimal hyperplasia through EndMT signaling.


Conclusions


In summary, we found that the overexpression of miR-221 inhibited EndMT in EPCs. This phenomenon is likely the result of the interaction between miR-221 and its target gene, *PTEN*, effecting a change in the regulation of FoxO3a phosphorylation.


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