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# MiR-647 promotes proliferation and migration of ox-LDL-treated vascular smooth muscle cells through regulating PTEN/PI3K/AKT pathway

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**Abstract. – OBJECTIVE: Aberrant microRNAs (miRNAs) play vital roles in various human diseases, including atherosclerosis (AS). MiR-647 expression was highly elevated in AS samples. Therefore, this study aimed at exploring the role and mechanism of miR-647 on AS progression.**

**PATIENTS AND METHODS: Human aorta vascular smooth muscle cells (HA-VSMCs) were treated with oxidized modified low-density lipoprotein (ox-LDL) to establish the AS model *in vitro.* The qRT-PCR assay was used to detect the expression of miR-647 and PTEN mRNA. The levels of PTEN protein, PI3K, AKT, p-PI3K, and p-AKT were measured using Western blot. Cell proliferation and migration were determined by Cell Counting Kit-8 (CCK-8) assay and transwell assay, respectively. The target of miR-647 was verified using the dual-luciferase reporter assay.**

**RESULTS: Our data supported that miR-647 was upregulated and PTEN was downregulated in the serum of AS patients and ox-LDL-treated HA-VSMCs. The proliferation and migration of ox-LDL-treated HA-VSMCs were promoted by miR-647 overexpression or PTEN knockdown, while they were suppressed following miR-647 depletion or high PTEN expression. Moreover, PTEN was a direct target of miR-647. PTEN antagonized miR-647-mediated regulatory effects on cell proliferation and migration. Additionally, the PI3K/AKT signaling pathway was involved in miR-647/PTEN-mediated regulation in ox-LDL-treated HA-VSMCs.**

**CONCLUSIONS: MiR-647 promoted the proliferation and migration of ox-LDL-treated HA-VSMCs at least partly by targeting the PTEN/PI3K/AKT pathway. Targeting miR-647 may be a promising method for AS treatment.**

*Key Words:*

*Atherosclerosis (AS), MiR-647, Phosphatase and tensin homolog (PTEN), Proliferation, Migration.*

## Abbreviations

AS=atherosclerosis, PTEN=phosphatase and tensin homolog, VSMCs=vascular smooth muscle cells, ox-LDL=Oxidized modified low-density lipoprotein, ATCC=American Type Culture Collection, FBS=fetal bovine serum, qRT-PCR=RNA extraction, reverse transcription, quantitative real-time PCR, CCK-8=Cell Counting Kit-8.

## Introduction

Cardiovascular disease is the leading cause of death and disability worldwide[1]. Atherosclerosis (AS), the hallmark of cardiovascular disease, involves the formation of an atherosclerotic plaque in the artery wall, leading to some complications such as myocardial infarction and stroke[2]. The etiology of AS is complicated, including endothelial cells injury and apoptosis, hyperproliferation of vascular smooth muscle cells (VSMCs), macrophages recruitment, and inflammatory cytokines production[3]. Oxidized modified low-density lipoprotein (ox-LDL) has been demonstrated to enhance the proliferation and migration of VSMCs and participates in AS progression[4]. We explored the regulatory mechanisms involved in AS by employing ox-LDL-treated VSMCs as AS model.

MicroRNAs (miRNAs) are endogenous, small non-coding RNAs with about 19-23 nucleotides in length that direct post-transcriptional suppression of gene expression in diverse eukaryotic lineages[5]. Mature miRNAs are located on a ribonucleoprotein complex, called RNA-induced silencing complex (RISC) where miRNAs bind to the 3'-untranslated region (3'-UTR) of their target mRNAs, resulting in target mRNA degradation or/and translational inhibition[5,6]. Increasing evidence[7,8] suggested that aberrant miRNAs play vital roles in various hu-



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man diseases, including AS. MiR-647 expression was reported to be upregulated in AS samples with stenosis and ox-LDL-treated VSMCs[9]. Moreover, the level of circulating miR-647 was negatively correlated with that of circulating long noncoding RNA atherosclerotic plaque pathogenesis associated transcript (APPAT), and the latter depletion was associated with severe stenosis in AS[9]. However, the role and mechanism of miR-647 on AS progression are still indefinable.

The expression of phosphatase and tensin homolog (PTEN) was demonstrated to be decreased in intimal VSMCs in atherosclerotic lesions and its deficiency accelerated AS progression in PTEN-null/ApoE$^{-/-}$ double knockout mice[10,11]. In the present work, our data supported that miR-647 was upregulated and PTEN was downregulated in the serum of AS patients and ox-LDL-treated human aorta VSMCs (HA-VSMCs). Moreover, miR-647 promoted the proliferation and migration of ox-LDL-treated HA-VSMCs at least partly by targeting the PTEN/PI3K/AKT pathway.

## Patients and Methods

### Patients

This research was approved by the Ethics Review Committee of the Second Affiliated Hospital of Fujian Medical University. 38 AS patients and 38 healthy volunteers were included in this study, with informed written consent. No conventional treatment was conducted before the samples collection. The healthy volunteers did not suffer from AS disease, cancers, inflammatory diseases, autoimmune diseases or recent infection (less than 1 month). After collection, blood samples were placed at 4°C for 1 h, and serum fractions were separated by centrifugation at 3000 rpm for 5 min. The serum samples were stored at –80°C until RNA extraction.

### Cell Culture and Treatment

HA-VSMCs purchased from American Type Culture Collection (ATCC; Manassas, VA, USA), were cultured with Roswell Park Memorial Institute-1640 (RPMI-1640) medium (Gibco, Rockville, MD, USA) containing 10% heat-inactivated fetal bovine serum (FBS; Gibco, Rockville, MD, USA), 1% penicillin/streptomycin (Mediatech, Herndon, VA, USA) at 37°C in a humidified atmosphere of 95% air and 5% $CO_2$.

To establish the AS model *in vitro*, HA-VSMCs were treated with different concentrations (0, 25, 50, 75, and 100 μg/mL) of ox-LDL (Sigma-Aldrich, St. Louis, MO, USA) or 100 μg/mL of ox-LDL for 24 h.

### Cell Transfection

To explore the role of miR-647, HA-VSMCs were transfected with synthetic miR-647 or negative control mimics (miR-647 mimics or NC; GenePharma, Shanghai, China), modified miR-647 inhibitors (anti-miR-647; GenePharma) or nontargeting control oligonucleotide (anti-NC; GenePharma) using Lipofectamine 2000 reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the instructions of manufacturers.

To observe the effect of PTEN, Lipofectamine 2000 reagent was used to transfect HA-VSMCs with the following nucleic acids: PTEN overexpression plasmid (pcDNA-PTEN; GenePharma) or negative control pcDNA (GenePharma), siRNA targeting PTEN (si-PTEN, GenePharma) or nontargeting control siRNA (siRNA, GenePharma).

### RNA Extraction, Reverse Transcription, Quantitative Real Time PCR (qRT-PCR)

Total RNA was extracted from tissues and HA-VSMCs using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the guidance of manufacturers. Then, the concentration and quality of RNA extracts were detected using Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). For mRNA quantification, cDNA was synthesized by iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) in a 20 μL reaction containing 0.5 μg of total RNA. QRT-PCR was performed using SYBR™ Green Master Mix (Applied Biosystems, Foster City, CA, USA) on a 7900HT thermocycler (Applied Biosystems, Foster City, CA, USA). The relative expression of PTEN mRNA was normalized to GAPDH and calculated by the $2^{-\Delta\Delta Ct}$ method. For miR-647 quantification, reverse transcription PCR was performed using the miScript reverse transcription kit (Qiagen, Hilden, Germany), and qRT-PCR was performed using the miScript SYBR Green PCR kit (Qiagen, Hilden, Germany) with PCR primer specific for miR-647 (Qiagen, Hilden, Germany). MiR-647 expression was normalized against U6. The specific primer sequences were listed as follows: PTEN-Forward, 5'-CGGCAGCATCAAATGTTTCAG-3', PTEN-Reverse 5'-AACTGGCAGGTAGAAGGCAACTC-3'; GAPDH-Forward, 5'-ATGTCG TGGAGTCTACTGGC-3', GAPDH-Reverse, 5'-TGACCTTGCCCACAGCCTTG-3'; miR-647-

Forward, 5'- GTGTTGGCCTGTGGCTG-3', miR-647-Reverse 5'-CTGACCCTCCCTCCTGC-3'; and U6-Forward, 5'-GCGCGTCGTGAAGCGTTC-3', U6-Reverse, 5'-GTGCAGGGTCCGAGGT-3'.

### Cell Proliferation Assay

Cell proliferation was determined using a Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kumamoto, Japan) referring to the protocols of manufacturers. Briefly, HA-VSMCs were seeded into a 96-well plate and were transfected with indicated oligonucleotide or plasmid for 24 h. Then, they were treated with 100 μg/mL of ox-LDL. At the indicated time point, 20 μL of CCK-8 solution was added into each well, followed by the incubation at 37°C for 1 h. The absorbance at 450 nm was detected by a Microplate Reader (VERSAmax, Molecular Devices, Sunnyvale, CA, USA).

### Transwell Assay of Cell Migration

Cell migration was measured using a polycarbonate transwell filter with 8 μm pore (Corning, Corning, NY, USA). 200 μL of serum-free medium containing treated HA-VSMCs was added onto the top chamber of transwell, while 700 μL of growth medium containing 10% fetal bovine serum (FBS) was added into the bottom chamber. After 24 h incubation, the migrated cells were fixed in 4% paraformaldehyde and stained with 0.1% crystal violet (Sigma-Aldrich; St. Louis, MO, USA). Images were captured and the numbers of migrated cells were counted under a microscope (Leica, Wetzlar, Germany).

### Dual-Luciferase Reporter Assay

Bioinformatic analysis of miRNA targets was performed using the online software TargetScan (http://www.targetscan.org/). PTEN 3'-UTR-luciferase reporter plasmid (PTEN-WT) containing the target sequence for miR-647 and seed site-directed mutation plasmid (PTEN-MUT) were designed and synthesized by Applied Biosystems. Lipofectamine 2000 reagent was used to co-transfect subconfluent HA-VSMCs with 100 ng of PTEN-WT or PTEN-MUT and 50 nM of miR-647 mimics or NC. After 48 h transfection, the relative luciferase activity was determined by the dual-luciferase assay system (Promega, Madison, WI, USA), normalized to the firefly luciferase activity.

### Western Blot

HA-VSMCs were lysed in lysis buffer comprising of 50 mM Tris-HCl (pH=7.4), 150 mM NaCl, 1% Triton X-100, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1% sodium dodecyl sulphate (SDS), 0.5% deoxycholate and protease inhibitor cocktail (Roche Diagnostics, Basel, Switzerland). The total protein extracts were resolved on a 10% SDS polyacrylamide gel and blotted onto a polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA). The membranes were blocked in 5% non-fat milk for 1 h, and then were incubated with primary antibodies including anti-PTEN (Abcam, Cambridge, MA, USA; dilution 1:10000), anti-PI3K (Abcam, Cambridge, MA, USA; dilution 1:1000), anti-p-PI3K (Cell Signaling Technology, Danvers, MA, USA; dilution 1:1000), anti-AKT (Cell Signaling Technology, Danvers, MA, USA; dilution 1:1000), anti-p-AKT (Cell Signaling Technology, Danvers, MA, USA; dilution 1:2000) and anti-β-actin (Cell Signaling Technology, Danvers, MA, USA; dilution 1:1000). After incubation with horseradish-peroxidase (HRP)-conjugated secondary antibodies (Abcam, Cambridge, MA, USA; dilution 1:5000), the protein bands were visualized using an enhanced chemiluminescence (ECL) Detection System (GE Healthcare, Chicago, IL, USA) with an Image Reader LAS-3000 software (Fujifilm Holdings KK, Tokyo, Japan).

### Statistical Analysis

All data were analyzed using GraphPad Prism 7.0 software (GraphPad Software Inc., La Jolla, CA, USA) and presented as mean ± standard deviation (SD) from at least three repeated experiments. The comparisons between two groups were analyzed by Student's *t*-test or Mann-Whitney U and among multiple groups were conducted by one-way analysis of variance (ANOVA). $p$-value $< 0.05$ was considered statistically significant.

## Results

### Upregulation of MiR-647 in Serum of AS Patients and ox-LDL-Treated HA-VSMCs

We detected whether miR-647 was aberrantly expressed in AS by qRT-PCR. As shown in Figure 1A, miR-647 expression was significantly elevated in the serum of AS patients compared with healthy control. Subsequently, HA-VSMCs were treated with different concentrations of ox-LDL. These results revealed that ox-LDL treatment in HA-VSMCs resulted in an increase of miR-647 expression in a dose-dependent manner (Figure 1B).

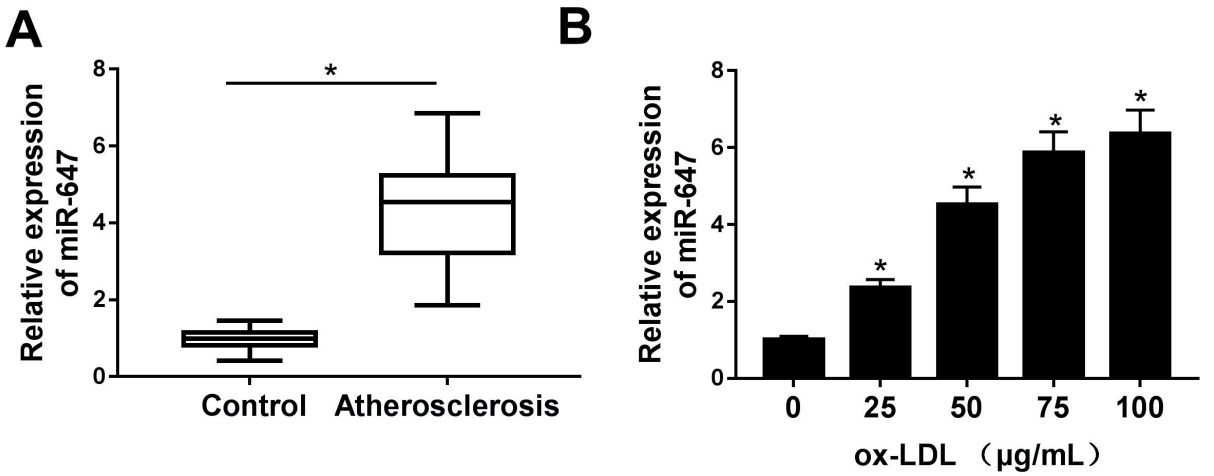


**Figure 1.** The expression of miR-647 was detected in the serum of AS patients and ox-LDL-treated HA-VSMCs. ***A,*** QRT-PCR assay was performed to determine miR-647 expression in serum of AS patients (n=38) and healthy volunteers (n=38). ***B,*** HA-VSMCs were treated with different concentrations (0, 25, 50, 75 and 100 μg/mL) of ox-LDL for 24 h, followed by the measurement of miR-647 expression by qRT-PCR. *$p$<0.05 *vs.* Control or 0 μg/mL of ox-LDL treatment.

### *MiR-647 Overexpression or Knockdown Affected the Proliferation and Migration of ox-LDL-Treated HA-VSMCs*

To investigate the role of miR-647 on AS, gain-of-function and loss-of-function experiments were performed by transfecting with miR-647 mimics or anti-miR-647 into HA-VSMCs prior to ox-LDL treatment. As shown in Figure 2A, in comparison to a corresponding control, the expression of miR-647 was strikingly increased by miR-647 mimics transfection, while it was highly decreased in the presence of anti-miR-647. Subsequent functional experiments revealed that high miR-647 expression resulted in increased proliferation and migration in ox-LDL-treated HA-VSMCs (Figure 2B and 2C). While miR-647 knockdown markedly repressed the proliferation and migration of ox-LDL-treated HA-VSMCs (Figure 2B and 2C).

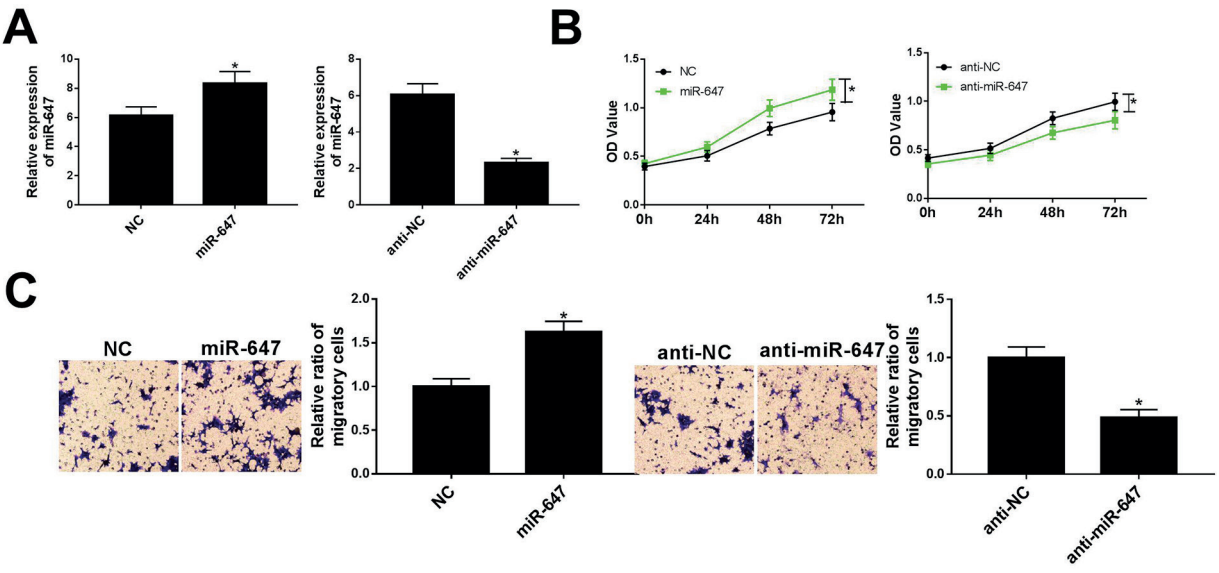


**Figure 2.** The effect of miR-647 on HA-VSMCs proliferation and migration under ox-LDL treatment. HA-VSMCs were transfected with miR-647 mimics, anti-miR-647 or respective control and then were treated with 100 μg/mL of ox-LDL for 24 h, followed by the measurement of miR-647 expression by qRT-PCR *(**A**)*, cell proliferation by CCK-8 assay *(**B**)* and cell migration by transwell assay *(**C**)*. * $p$<0.05 *vs.* NC or anti-NC.

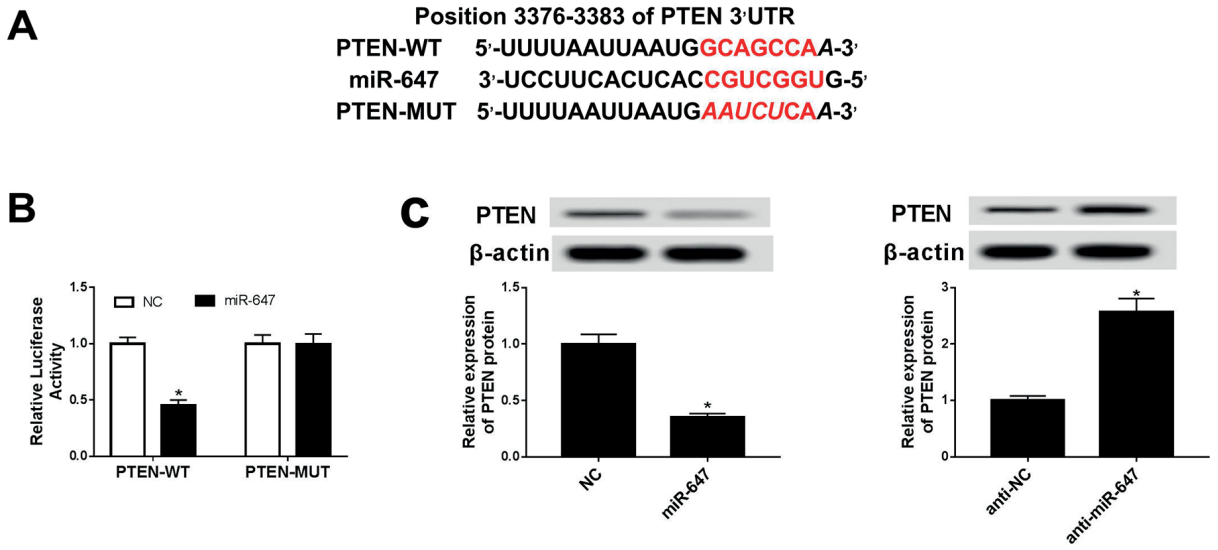


**Figure 3.** PTEN was a direct target of miR-647. ***A,*** Nucleotide resolution of the predicted miR-647 binding sites in 3'-UTR of PTEN mRNA: seed sequence and mutated miR-647 binding sites. ***B,*** Dual-luciferase reporter assay was performed by co-transfecting with PTEN 3'-UTR-luciferase reporter plasmid (PTEN-WT) containing the target sequence for miR-647 or seed site-directed mutation (PTEN-MUT) into HA-VSMCs, and miR-647 mimics or miR-NC mimics. The relative luciferase activity was detected. ***C,*** HA-VSMCs were transfected with miR-647 mimics or anti-miR-647, followed by the determination of PTEN protein expression by Western blot. * $p<0.05$ *vs.* NC or anti-NC.

### *PTEN was a Direct Target of MiR-647*

To further understand the underlying mechanism of miR-647 on AS, we carried out a detailed analysis of its molecular targets. The predicted data by the TargetScan software presented that the 3'-UTR of PTEN mRNA harbored a putative target sequence for miR-647 (Figure 3A). To confirm this, dual-luciferase reporter assay was performed by co-transfecting with PTEN 3'-UTR-luciferase reporter plasmid (PTEN-WT) containing the target sequence for miR-647 or seed site-directed mutation plasmid (PTEN-MUT) into HA-VSMCs and miR-647 mimics. These results revealed that transfection of miR-647 mimics, but not miR-NC mimics, reduced the luciferase activity of PTEN-WT (Figure 3B). Whereas, PTEN-MUT abolished the effect of miR-647 (Figure 3B). Next, we observed whether miR-647 influenced PTEN protein expression in HA-VSMCs. These data demonstrated that the expression of PTEN protein was significantly inhibited by miR-647 overexpression, while it was enhanced by miR-647 knockdown (Figure 3C).

### *Downregulation of PTEN in Serum of AS Patients and ox-LDL-Treated HA-VSMCs*

We determined the expression of PTEN in serum of AS patients and ox-LDL-treated HA-VSMCs. QRT-PCR and Western blot results revealed a significant downregulation of PTEN mRNA and protein levels in serum of AS patients compared with healthy control (Figure 4A and 4B). Likewise, ox-LDL treatment in HA-VSMCs resulted in a decrease of PTEN mRNA expression in a dose-dependent manner (Figure 4C). Notably, PTEN mRNA level was inversely correlated with miR-647 expression in serum of AS patients (Figure 4D).

### *The Effect of PTEN on Proliferation and Migration of ox-LDL-Treated HA-VSMCs*

To explore the role of PTEN on AS, pcDNA-PTEN or si-PTEN were transfected into HA-VSMCs prior to the ox-LDL treatment. Results revealed that, in comparison to their counterpart, PTEN mRNA and protein levels were dramatically increased by pcDNA-PTEN transfection, while they were remarkably decreased in the presence of si-PTEN (Figure 5A and 5B). Further functional experiments showed that cell proliferation and migration were significantly suppressed by PTEN overexpression, while they were highly enhanced by PTEN depletion in ox-LDL-treated HA-VSMCs (Figure 5C and 5D).

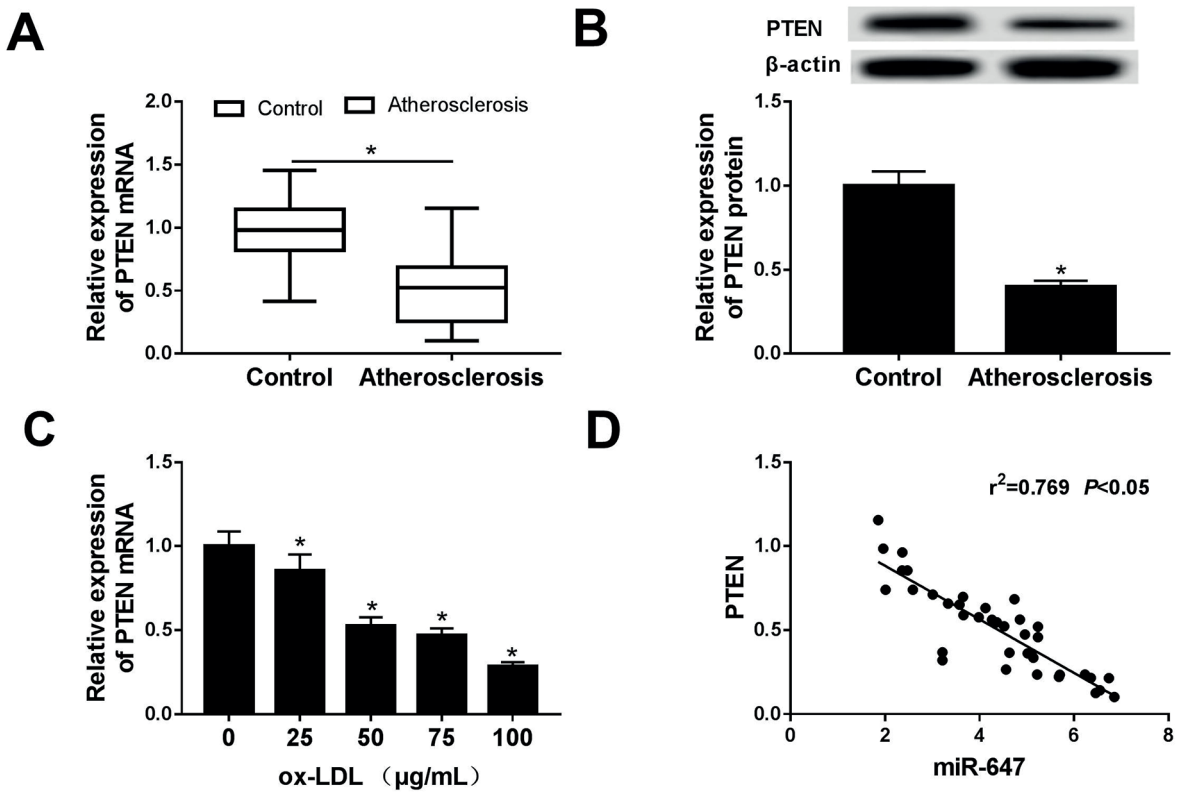


**Figure 4.** PTEN was downregulated in the serum of AS patients and ox-LDL-treated HA-VSMCs. qRT-PCR assay of PTEN mRNA expression *(A)* and Western blot of PTEN protein expression *(B)* in serum of AS patients (n=38) and healthy volunteers (n=38). ***C,*** HA-VSMCs were treated with different concentrations (0, 25, 50, 75 and 100 μg/mL) of ox-LDL for 24 h, followed by the detection of PTEN mRNA expression by qRT-PCR. ***D,*** Correlation between PTEN expression and miR-647 level was detected in serum of AS patients (n=38). * $p<0.05$ *vs.* corresponding control.

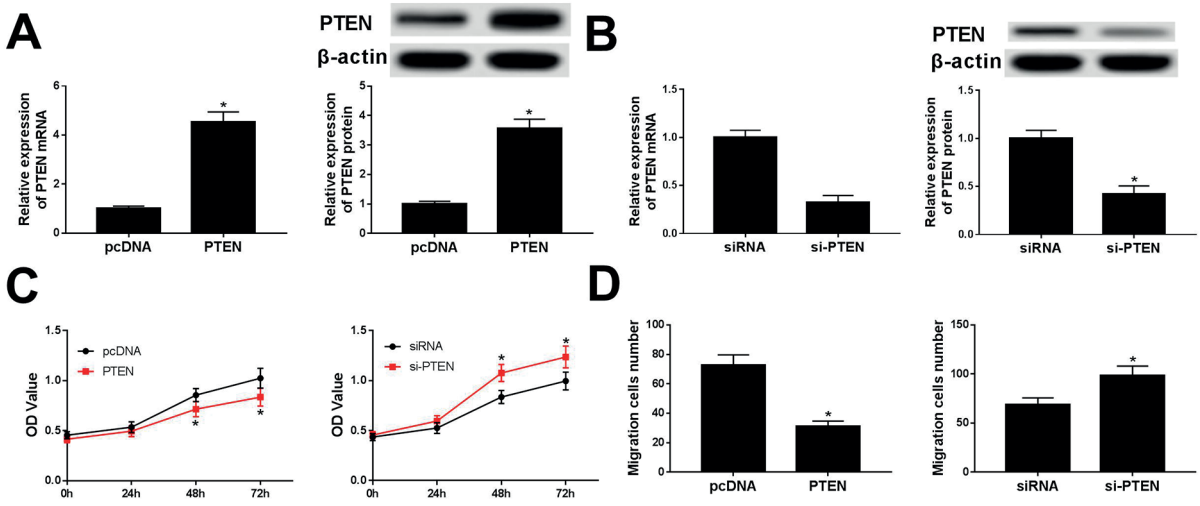


**Figure 5.** The role of PTEN on the proliferation and migration of ox-LDL-treated HA-VSMCs. pcDNA-PTEN, si-PTEN or corresponding control was transfected into HA-VSMCs prior to ox-LDL treatment, followed by the measurement of PTEN mRNA and protein levels *(A)* and *(B)*, cell proliferation by CCK-8 assay *(C)* and cell migration by transwell assay *(D)*. * $p<0.05$ *vs.* pcDNA or siRNA.

### *MiR-647 Exerted its Regulatory Effects on Proliferation and Migration by PTEN in ox-LDL-Treated HA-VSMCs*

To provide further mechanistic insight into the link between miR-647 and PTEN on AS, miR-647 mimics+pcDNA-PTEN or anti-miR-647+si-PTEN was transfected into HA-VSMCs prior to ox-LDL treatment. These data showed that the co-transfection of pcDNA-PTEN significantly abrogated miR-647 mimics-mediated inhibition on PTEN expression (Figure 6A). Likewise, si-PTEN co-transfection also affected the expression of PTEN in anti-miR-647-transfected HA-VSMCs under the ox-LDL treatment (Figure 6A). Subsequent functional experiments showed that the restoration of PTEN expression substantially abated miR-647-mediated regulatory effects on the proliferation and migration of ox-LDL-treated HA-VSMCs (Figure 6B and 6C).

### *PI3K/AKT Signaling Pathway was Involved in MiR-647/PTEN-Mediated Regulation in ox-LDL-Treated HA-VSMCs*

PI3K/AKT signaling is demonstrated to function as a crucial regulator in various human diseases, including AS. Also, it regulates cell proliferation, differentiation, and metabolism[12]. PTEN was validated to be a potent negative regulator of PI3K/AKT pathway[13]. We determined whether the PI3K/AKT pathway was involved in the regulation of miR-647/PTEN axis. We transfected miR-647 mimics, miR-647 mimics+pcDNA-PTEN, anti-miR-647 or anti-miR-647+si-PTEN into HA-VSMCs prior to ox-LDL treatment. These data revealed that the transfection of miR-647 mimics significantly activated the PI3K/AKT pathway, presented as an increase of PI3K and AKT phosphorylation levels (Figure 7A). On the other hand, miR-647 knockdown blockaded the PI3K/AKT pathway in ox-LDL-treated HA-VSMCs (Figure 7B). Moreover, these results showed that PTEN expression restoration highly abrogated miR-647-mediated regulatory effect on the PI3K/AKT signaling in ox-LDL-treated HA-VSMCs (Figure 7A and 7B).

## Discussion

Increasing evidence has suggested that miRNAs play key roles in cellular and molecular processes related to the development and progression of AS. For example, upregulated miR-21, miR-210, miR-34a, and miR-146a/b in human atherosclerotic plaques were involved in processes known to be connected to AS[14]. MiR-712 was an upregulated miRNA induced by disturbed flow in endothelial cells and its silencing repressed

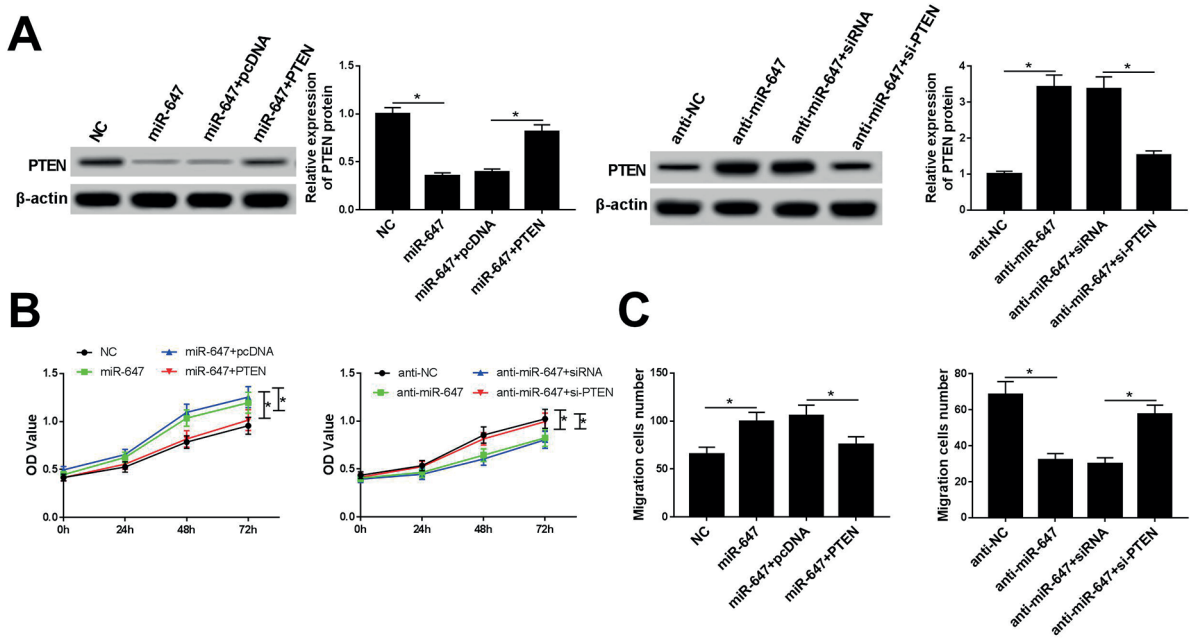


**Figure 6.** MiR-647 regulated cell proliferation and migration by PTEN. HA-VSMCs were transfected with miR-647 mimics, miR-647 mimics+pcDNA-PTEN, anti-miR-647, anti-miR-647+si-PTEN or respective control and then were treated with 100 μg/mL of ox-LDL for 24 h, followed by the determination of PTEN protein level by Western blot ***(A)***, cell proliferation by CCK-8 assay ***(B)*** and cell migration by transwell assay ***(C)***. * $p<0.05$ *vs.* respective control.

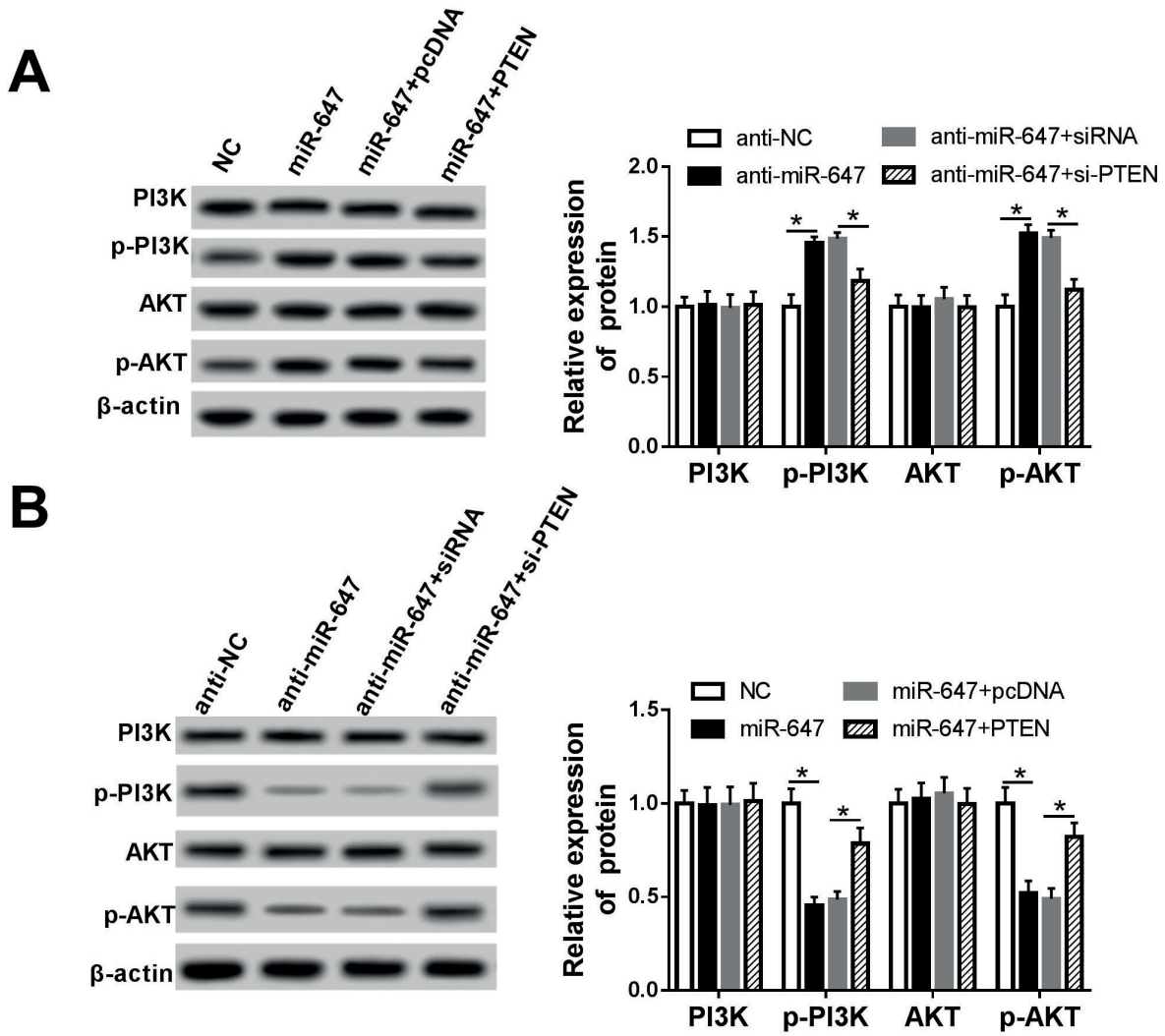


**Figure 7.** MiR-647/PTEN regulated PI3K/AKT signaling pathway in ox-LDL-treated HA-VSMCs. HA-VSMCs were transfected with miR-647 mimics, miR-647 mimics+pcDNA-PTEN ***(A)***, anti-miR-647, anti-miR-647+si-PTEN ***(B)*** or respective control prior to ox-LDL treatment, followed by the measurement of PI3K, AKT, p-PI3K and p-AKT levels by Western blot. * $p<0.05$ *vs.* NC or miR-647 mimics+pcDNA.

AS progression by rescuing tissue inhibitor of metalloproteinase 3 (TIMP3) expression in AS murine models[15]. Moreover, miR-145 deficiency alleviated AS partly by targeting the transporter ATP-binding cassette A1 (ABCA1) expression[16]. In the present report, we validated that miR-647 was highly upregulated in the serum of AS patients and ox-LDL-treated HA-VSMCs, in accordance with previous work[9].

MiR-647 has been identified as a tumor suppressor in a series of human cancers, such as gastric cancer[17]. On the other hand, it was demonstrated as an oncomiR in some cancers, including colorectal cancer[18]. Besides, miR-647 was showed to have good predictability of clinical recurrence of prostate cancer and prognosis of ovarian cancer[19,20]. Chen and Li[21] reported that a high miR-647 expression was found by miRNA arrays in pulmonary arterial hypertension tissues. Moreover, miR-647 was demonstrated to be involved in APPAT-mediated regulation in AS[9]. In this research, we verified that the proliferation and migration of ox-LDL-treated HA-VSMCs were promoted by miR-647 overexpression, while they were repressed following the miR-647 knockdown. These results implied that miR-647 might play a destructive role in AS.

The online software TargetScan was performed to predict the targets of miR-647. Among the predicted candidates, PTEN was well-studied in this work, considering the vital roles of PTEN in the progression of AS[22]. Subsequently, we manifested that PTEN was a direct target of miR-647. PTEN has been identified as an indispensable regulator of the differentiated VSMCs phenotype[10,11]. Yuan et al[23] have demonstrated that PTEN participates in the pathological development of AS. PTEN was manifested to have the capacity of inhibition of VSMCs proliferation and migration[24]. It was reported that the AS progression was significantly accelerated in VSMC-specific PTEN-null/ApoE$^{-/-}$ double-knockout mice[10]. This research detected that PTEN was downregulated in the serum of AS patients and ox-LDL-treated HA-VSMCs, consistent with the previous studies[10,11]. Moreover, our data supported that PTEN suppressed the proliferation and migration of ox-LDL-treated HA-VSMCs. Further, we demonstrated that miR-647 exerted its regulatory effects on proliferation and migration by PTEN in ox-LDL-treated HA-VSMCs.

The PI3K/AKT signaling pathway, one of the most important intracellular pathways, plays a critical role in AS. Also, a selective inhibition of this pathway was manifested to repress the AS progression[25,26]. PTEN was validated to be a potent negative regulator of PI3K/AKT pathway[13]. Moreover, PTEN selectively suppressed the expression of vascular cell adhesion molecule (VCAM)-1 by regulating the PI3K/AKT/GSK-3β/GATA-6 signaling in tumor necrosis factor (TNF)-α-treated human endothelial cells[27]. In the study, we found that the PI3K/AKT signaling pathway was involved in the regulation of miR-647/PTEN axis in ox-LDL-treated HA-VSMCs. Therefore, more researches about the regulatory relation between miR-647 and PTEN/PI3K/AKT signaling in ox-LDL-treated HA-VSMCs are needed in further works.

## Conclusions

We supported that miR-647 was upregulated and PTEN was downregulated in the serum of AS patients and ox-LDL-treated HA-VSMCs. Furthermore, we first demonstrated that miR-647 accelerated the proliferation and migration of ox-LDL-treated HA-VSMCs at least partly by targeting the PTEN/PI3K/AKT signaling pathway. Targeting miR-647 may be a promising method for the AS treatment.

### Conflict of Interests

The Authors declare that they have no conflict of interests.

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