Hsa_circ_0054633 regulates PDGF-BB-induced proliferation, migration and oxidative stress of vascular smooth muscle cells through miR-107/TXNIP axis

Type
Research paper

Keywords
Oxidative stress, miR-107, hsa_circ_0054633, TXNIP, HA-VSMCs

Abstract

Introduction
Hsa_circ_0054633 has been found to be elevated in the blood of coronary artery disease (CAD) patients. However, the molecular mechanism and the role of hsa_circ_0054633 in the pathogenesis of CAD have not been reported in detail.

Material and methods
The expression of hsa_circ_0054633, microRNA (miR)-107 and thioredoxin-interacting protein (TXNIP) mRNA was measured using quantitative real-time polymerase chain reaction. Human artery vascular smooth muscle cell (HA-VSMC) proliferation, cell cycle, and migration were detected by cell counting kit-8 assay, flow cytometry and transwell assay, respectively. The generation of reactive oxygen species (ROS) was analyzed by dichlorofluorescein diacetate (DCFH-DA) assay. Western blot was utilized to determine the levels of proliferating cell nuclear antigen (PCNA), cyclin D1, matrix metalloproteinase 9 (MMP-9), Mn-superoxide dismutase (SOD2) and TXNIP protein. The interaction between miR-107 and hsa_circ_0054633 or TXNIP was confirmed by dual-luciferase reporter, RNA immunoprecipitation assay or pull-down assay.

Results
Hsa_circ_0054633 was elevated in the plasma of CAD patients, and might be a potential blood biomarker for CAD prediction. Hsa_circ_0054633 silencing reversed PDGF-BB-induced promotion on HA-VSMC proliferation, cell cycle, migration and ROS production. MiR-107 directly interacted with hsa_circ_0054633 and TXNIP, and hsa_circ_0054633 regulated TXNIP expression by sponging miR-107. Besides, rescue assay indicated that the action of hsa_circ_0054633 silencing on PDGF-BB-treated HA-VSMCs could be attenuated by miR-107 inhibition or TXNIP overexpression, respectively.

Conclusions
Hsa_circ_0054633 knockdown protected HA-VSMCs against PDGF-BB-induced dysfunction through regulating miR-107/TXNIP axis, suggesting a potential therapeutic target for coronary atherosclerosis.
**Hsa_circ_0054633 regulates PDGF-BB-induced proliferation, migration and oxidative stress of vascular smooth muscle cells through miR-107/TXNIP axis**

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**Keywords:** hsa_circ_0054633, miR-107, TXNIP, oxidative stress, HA-VSMCs
Introduction

Coronary artery disease (CAD) is a leading cause of mortality in the world. Despite great improvements in prevention, medical and interventional management, the incidence and mortality of CAD remain increasing in recent years [1, 2]. Atherosclerosis (AS) is a chronic inflammatory disease of the arterial wall, characterized by plaque formation, and is the well-recognized primary cause of CAD [3]. The pathogenesis of AS lesion formation is complex, and the initiating phase of AS plaques formation is the result of vascular smooth muscle cells (VSMCs) dysfunction [4]. Increasing researches have revealed VSMCs damage is associated with the regulation of atherosclerosis at tissue and molecular levels [5, 6]. Thus, better understanding the molecular mechanism underlying abnormal VSMCs function is of great significance for the development of new therapeutics.

Circular RNAs (circRNAs) are a type of endogenous non-coding RNAs derived from reverse splicing of exons, introns or both [7, 8]. They have covalently closed loop structures, which confer increasing stability relative to their linear transcripts [9]. Besides, many circRNAs are highly conserved and have tissue-specific expression patterns. Importantly, growing evidence has documented that circRNAs play significant roles in a variety of physiological and pathological processes, including cell cycle, proliferation, migration, invasion, tumorigenesis, immune responses and oxidative stress [10, 11]. Thus, circRNAs are ideal candidates for future diagnostic biomarkers and therapeutic interventions [12]. Hsa_circ_0054633 is a novel identified circRNA. Zhao et al. demonstrated that hsa_circ_0054633 was up-regulated in the peripheral
blood of type 2 diabetes mellitus (T2DM) patients, and was a potential blood biomarker for pre-diabetes and T2DM prediction [13]. Pan et al. revealed that hsa_circ_0054633 down-regulation enhanced high glucose-mediated inhibition on proliferation, migration and angiopoiesis of endothelial cells [14]. Importantly, Li et al. exhibited that hsa_circ_0054633 expression was up-regulated in the blood of CAD patients [15]. However, the role of hsa_circ_0054633 on VSMCs dysfunction remains unclear.

Emerging studies have reported that platelet-derived growth factor-BB (PDGF-BB) is one of the most potent stimulants for the dysfunction of VSMCs [16, 17]. Thus, PDGF-BB was employed to induce VSMC dedifferentiation to mimic VSMC dysfunction in coronary atherosclerosis in vitro. This study aimed to explore the molecular mechanism and the role of hsa_circ_0054633 in PDGF-BB-induced VSMCs dysfunction, thus investigating the potential roles of hsa_circ_0054633 in the presence and progression of CAD.

Materials and methods

Clinical samples

A total of 33 patients with CAD were recruited from The Second Affiliated Hospital of Xi'an Medical University. There were 19 males and 14 females, ranging in age from 30 to 75 years. All CAD patients were diagnosed by coronary angiography in line with the guidelines established by American College of Cardiology/American Heart Association. The exclusion criteria were as follows: (1) cancer and other severe diseases; (2) serious infection within six weeks of the start of this work; (3) clinically acute or
active chronic inflammatory disease. In the same period, a total of 33 healthy individuals were recruited to serve as the control group, and there were 18 females and 15 males with age ranging from 27 to 70 years.

Whole blood (20 ml) was extracted from each participant on the day of admission to the study and transferred to anticoagulant tubes. Blood samples were centrifuged at 3000 g for 15 min, and plasma was collected in a plastic tube, followed by centrifuging a second time at 3000 g for 15 min at room temperature. Subsequently, the plasma was collected and stored at -80 °C before used. This study was approved by the Ethics Committee of The Second Affiliated Hospital of Xi'an Medical University and written informed consent was collected from all subjects.

**Cell culture and treatment**

Human artery vascular smooth muscle cells (HA-VSMCs) obtained from Chinese Academy of Sciences (Shanghai, China) were maintained in Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Waltham, MA, USA), which was supplemented with 10% fetal bovine serum (FBS) (Gibco, Carlsbad, CA, USA), at 37°C with 5% CO2. For the PDGF-BB group, HA-VSMCs were treated with 30 ng/mL PDGF-BB (Sigma, St. Louis, MO, USA) for 6 h at 37°C.

**Quantitative real-time polymerase chain reaction (qRT-PCR)**

The extraction of total RNA from plasma and cultured HA-VSMCs was performed using TRIzol reagent (Invitrogen). First-strand complementary DNA (cDNA) was synthesized by using the Reverse Transcription System Kit (Takara, Dalian, China), then cDNA amplification was conducted using SYBR Green I (Takara) on the ABI7500
system. The relative fold change in expression was assessed by $2^{\Delta\Delta Ct}$ method and normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or U6 small nuclear B noncoding RNA (U6). The primers obtained from Qiagen (Valencia, CA, USA) were listed as followed: hsa_circ_0054633, F 5’-TTGCTTTCTACACTTTACAGGTGAC-3’ and R 5’-GCTTTTTTGCTGTAGTCAACCACCA-3’; miR-107, F 5’-GTTAAGTCAGAGCGGGCTT-3’, and R 5’-CACCTCGCTTTTTCAGTGCC-3’; TXNIP, F 5’-GCCACACTTACCTTGCAAT-3’, and R 5’-TGGATCCAGGAACGCTAAC-3’; GAPDH, F 5’-GTCAACGGATTGGTCTGTATT-3’, and R 5’-AGTCTTCTGGGTGGCAGTGAT-3’; U6, F 5’-CTCGCTTCGGGCAGCACA-3’, and R 5’-AACGCTTCAGAATTTGCCT-3’.

**Cell transfection**

The mimic or inhibitor targeting miR-107 (miR-107 or anti-miR-107), and their corresponding negative control (miR-NC and anti-NC) were purchased from RIBOBIO (Guangzhou, China). Two forms of small interfering RNA (siRNA) were designed by Invitrogen to target hsa_circ_0054633 covalent closed junction (si-circ#1, si-circ#2), the same vector harboring a scrambled sequence was used as a negative control (si-NC). Also, pcDNA-TXNIP overexpression vector (TXNIP) and pcDNA negative control (vector) were synthesized by Invitrogen. Subsequently, the transfection was performed by Lipofectamine 2000 (Invitrogen).

**Cell counting kit-8 (CCK-8) assay**

After transfection and/or treatment, cells (5000/well) were cultivated into a 96-well
plate overnight, and then interacted with 10 μL CCK-8 solution (Dojindo Molecular Technologies, Japan) at 37°C for another 2 h. Finally, the optical density of each well at 450 nm was analyzed by a microplate reader.

**Flow cytometry**

Following transfection and/or treatment, cells (1×10⁶) were trypsinized and resuspended to obtain single-cell suspensions, then detached cells were fixed overnight in 70% ethanol, followed by staining with propidium iodide (Cell Cycle Detection kit; BD Biosciences, San Jose, CA, USA). Finally, quantitation of cell cycle distribution was analyzed with a FACScan flow cytometer (BD Biosciences). The percentage of the cells in G0/G1, S, and G2/M phases was counted with FlowJo software.

**Transwell assay**

After transfection and/or treatment, cells in 200 mL serum-free DMEM were seeded into the upper chamber of a transwell insert (Cell Biolabs, Inc. Santiago, CA, USA), then lower chamber was filled with 600 mL medium supplementing with 10% FBS. Following incubation at 37°C with 5% CO₂ for 24 h, cells on the lower face of the membranes were fixed and stained. Finally, migrated cells from 10 randomly selected fields were counted by a microscope.

**Detection of reactive oxygen species (ROS)**

The production of ROS was measured by Dichlorofluorescein diacetate (DCFH-DA) assay. Transfected and/or treated HA-VSMCs were suspended using 20 mM 2',7'-Dichlorodihydrofluorescein diacetate (DCFH-DA; Sigma) for 30 min at 37°C in the dark. After washing with PBS three times, the fluorescence intensity of the samples was
detected using a fluorescence microplate reader at 488 nm excitation wavelength and 525 nm emission wavelength, respectively.

**Western blot**

Total protein was extracted using RIPA buffer (Beyotime, Shanghai, China) from cells, and protein concentrations were determined using the bicinchoninic acid (BCA) Protein Assay kit (Takara). Protein extractions were separated by 12% sodium lauryl sulfate-polyacrylamide gels (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (PVDF; Millipore, Billerica, MA, USA). Then membranes were interacted with primary antibodies against proliferating cell nuclear antigen (PCNA) (1:5000, ab29), cyclin D1 (1:10000, ab134175), matrix metallopeptidase 9 (MMP-9) (1:2000, ab38898), Mn-superoxide dismutase (SOD2) (1:5000, ab13533), TXNIP (1:2000, ab188865) (Abcam, Cambridge, MA, USA), and the secondary antibody anti-rabbit IgG-horseradish peroxidase at a 1:1000 dilution (Sangon, Shanghai, China). β-actin (1:2000, 4967, Cell Signaling Technology, Boston, MA, USA) was used as an internal control.

**Dual-luciferase reporter assay**

The sequences of TXNIP 3'-UTR or hsa_circ_0054633 containing the wild-type (wt) or mutant (mut) potential binding sites of miR-107 were amplified and subcloned into pRL-TK luciferase reporter vector (Promega, Madison, WI, USA), respectively. Then cells were placed in 24-well plates and co-transfected with 10 ng wt or mut constructed luciferase vectors, and 40 nM of miR-107 mimic or miR-NC mimic. The luciferase activity was measured by a dual luciferase assay kit (Promega) and
normalized by renilla luciferase activity.

**RNA immunoprecipitation (RIP) assay**

HA-VSMCs were lysed with RIP lysis buffer (Millipore, Billerica, MA, USA). Cell lysis was co-immunoprecipitated with magnetic beads containing human anti-Ago2 antibody (Abcam). Normal mouse IgG (Millipore) was used as a negative control. Finally, immunoprecipitated RNA was purified and subjected to qRT-PCR analysis.

**Pull-down assay**

Biotin (Bio)-miR-107 and Bio-NC were synthesized by GenePharma Company (Shanghai, China), and then transfected into HA-VSMCs for 48 h. Subsequently, cells were lysed, and the extracts were incubated with streptavidin-coated magnetic beads (Invitrogen). After elution, the bead-bound RNA complex was isolated and subjected for reverse transcription using qRT-PCR analysis.

**Statistical analysis**

All experiments were performed three times. Data were expressed as mean ± standard deviation (SD). Statistical differences in different groups were analyzed by Student’s *t* test or one-way analysis of variance (ANOVA) with GraphPad Prism 7 software. Receiver operating characteristic (ROC) curves were plotted to analyze the diagnostic value of hsa_circ_0054633. *P*<0.05 indicated statistically significant.

**Results**

**Demographic characteristics of CAD patients and controls**

In this study, the demographic data, laboratory parameters of CAD patients and
controls were summarized in Table 1. As expected, there were significant differences in hypertension, total cholesterol, triglyceride, LDL-cholesterol (LDL-C), and high-sensitivity C-reactive protein (Hs-CRP) between these two subgroups ($P < 0.01$). However, other factors, including sex, age, body mass index, cigarette smoking, drinking, diabetes mellitus, and HDL-C, were not significantly different ($P > 0.05$).

**Hsa_circ_0054633 expression is elevated in CAD patients and PDGF-BB-induced HA-VSMCs**

The expression of hsa_circ_0054633 was detected and results showed hsa_circ_0054633 expression was up-regulated in the plasma of CAD patients relative to the plasma of healthy people (Fig. 1A). Moreover, ROC curves revealed that the area under the ROC curve (AUC) was 0.762 (95% confidence interval (CI): 0.637-0.887), the cut-off value was 1.615, and the sensitivity and specificity were 63.64% and 96.97%, respectively (Fig. 1B). Furthermore, highly expressed hsa_circ_0054633 were associated with smoking history, with symptoms of hypertension and diabetes mellitus, and with total cholesterol $\geq$ 5.1 mmol/L and triglyceride $\geq$ 1.8 mmol/L ($P < 0.05$; Table 2). Besides that, hsa_circ_0054633 expression was also up-regulated in PDGF-BB-induced HA-VSMCs compared with the cells untreated with PDGF-BB (Fig. 1C).

**Hsa_circ_0054633 knockdown reverses PDGF-BB-induced HA-VSMCs dysfunction**

To investigate the role of hsa_circ_0054633 in CAD, we knocked down hsa_circ_0054633 in HA-VSMCs by transfecting with two forms of constructed si-hsa_circ_0054633 plasmid (si-circ#1, si-circ#2). Then the effects of si-circ#1 and si-
circ#2 were determined, and qRT-PCR analysis showed both si-circ#1 and si-circ#2 remarkably declined hsa_circ_0054633 expression compared with the si-NC group (Fig. 2A). Subsequently, HA-VSMCs were treated with PDGF-BB, PDGF-BB + si-NC, or PDGF-BB + si-circ#1 to assay cell cycle, proliferation, migration and ROS production, and we found hsa_circ_0054633 silence overturned PDGF-BB-mediated enhancement of hsa_circ_0054633 expression (Fig. 2B). After that, CCK-8 assay indicated hsa_circ_0054633 knockdown attenuated PDGF-BB-induced promotion on HA-VSMC proliferation (Fig. 2C). Flow cytometric analysis showed PDGF-BB reduced the number of HA-VSMCs in the G0/G1 phase and increased the number of HA-VSMCs in the S phase, suggesting PDGF-BB promoted cell cycle progression, while this promotion was abated by hsa_circ_0054633 knockdown (Fig. 2D). Transwell assay exhibited that the increase of migrated HA-VSMCs induced by PDGF-BB was mitigated by the silencing of hsa_circ_0054633 (Fig. 2E). Besides that, we found PDGF-BB induced the generation of ROS, while hsa_circ_0054633 knockdown inhibited the production of ROS in HA-VSMCs (Fig. 2F). In addition, western blot showed hsa_circ_0054633 down-regulation also decreased PDGF-BB-mediated up-regulation of PCNA, cyclin D1, MMP9, and SOD2 (Fig. 2G), further indicating hsa_circ_0054633 knockdown reversed PDGF-BB-induced proliferation, cell cycle, migration and oxidative damage in HA-VSMCs.

**Hsa_circ_0054633 is a sponge of miR-107**

The molecular mechanisms underlying the action of hsa_circ_0054633 on HA-VSMC properties were explored. According to the prediction of starBase online
program, we found miR-107 had the potential binding sites of hsa_circ_0054633 (Fig. 3A). The expression of miR-107 was detected and results showed miR-107 expression was down-regulated in the plasma of CAD patients compared with the plasma from healthy people (Fig. 3B), moreover, lowly expressed miR-107 was related to smoking history, symptoms of hypertension, total cholesterol \( \geq 5.1 \) mmol/L and triglyceride \( \geq 1.8 \) mmol/L \( (P < 0.05; \) Table 2). Also, PDGF-BB decreased the expression of miR-107 in HA-VSMCs (Fig. 3C). Thus, miR-107 might be a potential biomarker for the development of CAD. Subsequently, we verified miR-107 mimic transfection could up-regulate miR-107 expression in HA-VSMCs relative to miR-NC mimic transfection (Fig. 3D). Then the interaction between hsa_circ_0054633 and miR-107 was analyzed. The dual luciferase reporter assay showed miR-107 mimic reduced the luciferase activity in HA-VSMCs cells transfected with the hsa_circ_0054633-wt (circ-wt) (Fig. 3E). RIP assay demonstrated that hsa_circ_0054633 and miR-107 were enriched in Ago2 immunoprecipitates compared with control IgG immunoprecipitates (Fig. 3F). Moreover, HA-VSMCs transfected with the bio-miR-107 showed elevated hsa_circ_0054633 levels but exhibited no changes in bio-NC (Fig. 3G). Besides that, we also observed that hsa_circ_0054633 knockdown up-regulated miR-107 expression in HA-VSMCs (Fig. 3H). Taken together, hsa_circ_0054633 was a sponge of miR-107 and negatively regulated its expression.

Hsa_circ_0054633 knockdown alleviates PDGF-BB-induced HA-VSMCs dysfunction via targeting miR-107

We further studied whether miR-107 involved in hsa_circ_0054633-mediated HA-
VSMC dysfunction. First, HA-VSMCs were transfected with anti-NC or anti-miR-107, and anti-miR-107 transfection significantly reduced the level of miR-107 (Fig. 4A). Next, HA-VSMCs were transfected with si-NC, si-circ#1, si-circ#1 + anti-NC, or si-circ#1 + anti-miR-107 after treatment with PDGF-BB, and qRT-PCR analysis showed hsa_circ_0054633 knockdown elevated miR-107 expression, while this elevation was reduced by miR-107 inhibition (Fig. 4B). After that, functional experiments were conducted and results exhibited that miR-107 inhibition reversed hsa_circ_0054633 knockdown-induced reduction of the number of migratory (Fig. 4C) and proliferating cell (Fig. 4D), cell cycle arrest (Fig. 4E), and ROS production (Fig. 4F) in PDGF-BB-treated HA-VSMCs. Western blot analysis displayed that hsa_circ_0054633 down-regulation also decreased PDGF-BB-mediated up-regulation of PCNA, cyclin D1, MMP9, and SOD2, while these down-regulations were rescued by miR-107 inhibition in HA-VSMCs (Fig. 4G). Altogether, hsa_circ_0054633 alleviated PDGF-BB-induced HA-VSMC dysfunction by targeting miR-107.

**TXNIP is a target of miR-107**

Through searching the starBase program, we found TXNIP contained the binding sites of miR-107 (Fig. 5A). TXNIP expression was found to be up-regulated in the plasma from CAD patients relative to the plasma from healthy people (Fig. 5B), and high expression of TXNIP was linked with symptoms of hypertension, and total cholesterol $\geq 5.1$ mmol/L as well as triglyceride $\geq 1.8$ mmol/L ($P < 0.05$; Table 2), suggesting TXNIP increase might be associated with the development of CAD. Similarly, TXNIP expression at mRNA and protein levels also increased in PDGF-BB-
induced HA-VSMCs (Fig. 5C, D). Afterwards, a decline of luciferase activity in HA-VSMCs cells co-transfected with TXNIP 3’UTR-wt and miR-107 mimic confirmed the direct interaction between TXNIP and miR-107 (Fig. 5E). Subsequent western blot analysis displayed that miR-107 overexpression decreased the level of TXNIP (Fig. 5F, G). These data verified that miR-107 targetedly suppressed TXNIP expression.

Importantly, we also observed that hsa_circ_0054633 silence decreased the level of TXNIP in HA-VSMCs, while this decrease was rescued by miR-107 inhibition (Fig. 5H, I), thus hsa_circ_0054633 could indirectly regulate TXNIP expression through miR-107.

Hsa_circ_0054633 moderates PDGF-BB-induced HA-VSMCs damage by regulating TXNIP

Given the relationship between hsa_circ_0054633 and TXNIP, we further investigated whether TXNIP participated in the action of hsa_circ_0054633 on HA-VSMCs. First, HA-VSMCs were transfected with TXNIP or vector, by contrast to vector transfection, TXNIP transfection significantly elevated the expression of TXNIP at mRNA and protein levels (Fig. 6A, B). Then HA-VSMCs were transfected with si-NC, si-circ#1, si-circ#1 + vector, or si-circ#1 + TXNIP after treatment with PDGF-BB, we found TXNIP rescued hsa_circ_0054633 knockdown-induced down-regulation of TXNIP expression (Fig. 6C, D), further indicating hsa_circ_0054633 could regulate TXNIP expression. Subsequently, rescued assay showed TXNIP up-regulation reversed hsa_circ_0054633 knockdown-induced migration suppression (Fig. 6E), decreased ROS production (Fig. 6F), and proliferation inhibition (Fig. 6G) and cell cycle arrest.
in PDGF-BB-treated HA-VSMCs. Besides that, TXNIP up-regulation also elevated the levels of PCNA, cyclin D1, MMP9, and SOD2 mediated by hsa_circ_0054633 knockdown in PDGF-BB-treated HA-VSMCs (Fig. 6I). In all, hsa_circ_0054633 moderated PDGF-BB-induced HA-VSMC dysfunction by regulating TXNIP.

Discussion

VSMC phenotypic switching is an early event in atherosclerosis and neointimal formation, and the involvement of circRNAs in the regulation of phenotypic switching of VSMCs has been identified [18, 19]. For example, circ-Sirt1 suppressed NF-κB activation by up-regulating SIRT1 expression via sponging miR-132/212 to hinder inflammatory phenotypic switching of VSMCs [20]. Circ-SATB2 promoted VSMC phenotypic proliferation, apoptosis, differentiation and migration through up-regulating STIM1 expression [21]. CircRNA_0020397 enhanced VSMC proliferation via regulating miR-138/KDR axis to alleviate intracranial aneurysm progression [22]. Circ_RUSC2 overexpression affected phenotypic modulation of VSMCs by contributing to proliferation, migration and suppressing apoptosis through increasing miR-661-mediated SYK expression [23]. Thus, circRNAs may be important factors in the dysfunction of VSMCs. In this study, hsa_circ_0054633 expression was higher in the plasma of CAD patients than that in healthy individuals, besides, hsa_circ_0054633 showed a higher ROC AUC, and might be a potential diagnostic biomarker for early prediction of CAD. Additionally, hsa_circ_0054633 expression was also up-regulated
in PDGF-BB-induced cells, suggesting that hsa_circ_0054633 elevation might be associated with the development of CAD. Then functional experiments showed hsa_circ_0054633 silence reversed PDGF-BB-induced promotion on HA-VSMC proliferation, cell cycle, and migration, thereby impeding the formation of atherosclerosis. PCNA is a gene on chromosome 20pter-p12 that encodes a homotrimeric nuclear protein that acts as a processivity factor for DNA polymerase delta in eukaryotic cells and is essential for DNA replication, repair, and recombination [24]. Cyclin D1 (CCND1) is a proto-oncogene located on chromosome 11q13 that is an essential regulator of the G1-S transition in cell cycle control progression [25]. MMP-9 is a matrixin, a class of enzymes that belong to the zinc-metalloproteinases family involved in the degradation of collagens IV and V, gelatins I and V, and fibronectin, and plays a important role in local proteolysis of the extracellular matrix and in leukocyte migration [26]. In this study, western blot analysis indicated hsa_circ_0054633 down-regulation also decreased PDGF-BB-mediated up-regulation of PCNA, cyclin D1 and MMP9 expression, further revealing the involvement of hsa_circ_0054633 in PDGF-BB-induced HA-VSMC dysfunction. ROS always is generated during the inflammatory response, which oxidizes low-density lipoproteins and results in structural damage and dysfunction of endothelial cells, ultimately leads to vascular remodeling [27-29]. Also, the antioxidant system, such as SOD2 enzyme, a mitochondrial enzyme that catalyzes the conversion of O2− to hydrogen peroxide (H2O2), and the amount of ROS are kept in a certain state of homeostasis [30-32]. In this study, we also found hsa_circ_0054633 silence reversed PDGF-BB-induced ROS
production and SOD2 elevation in HA-VSMCs. Thus, hsa_circ_0054633 was important in VSMC homeostasis.

MicroRNAs (miRNAs) are a class of small noncoding RNA molecules, which have been revealed to play important roles in multiple cellular processes, including cell proliferation, apoptosis, migration, tumorigenesis, oxidative stress and differentiation [33-35]. Increasing evidence suggests that many miRNAs involve in VSMC phenotypic modulation [36]. MiR-107 is a functional miRNA. Gao et al. revealed that miR-107 overexpression reduced the level of blood lipid and atherosclerotic index, suppressed vascular endothelial cell (VEC) apoptosis, inflammation and endoplasmic reticulum stress through KRT1-dependent Notch signaling pathway in coronary atherosclerosis [37]. Besides that, miR-107 was found to be decreased in the blood of AS patients, and involved in circRNA-0044073-mediated promotion of the proliferation and invasion of human VSMCs and human VECs [38]. In this study, miR-107 was confirmed to be a target of hsa_circ_0054633. MiR-107 was decreased in HA-VSMCs, and was down-regulated by PDGF-BB and hsa_circ_0054633. What’s more, miR-107 inhibition reversed hsa_circ_0054633 silence-mediated inhibition of the proliferation, cell cycle, migration and ROS generation in PDGF-BB-induced HA-VSMCs.

TXNIP is an endogenous inhibitor and regulator of thioredoxin (TRX), and modulate oxidative stress via repressing antioxidant activity of TRX [39]. TXNIP is abundant in the vascular wall [40], and involves in controlling vascular neointimal lesion formation [41]. TXNIP promotes Ox-LDL exposure-induced inflammatory
injuries of human aortic endothelial cells (HAECs) to contribute to atherosclerotic
development [42]. This study found TXNIP was up-regulated in the plasma of CAD
patients and PDGF-BB-induced HA-VSMCs. TXNIP was a target of miR-107, and
hsa_circ_0054633 served as a competing endogenous RNA for miR-107 to regulate
TXNIP expression in HA-VSMCs. Additionally, TXNIP overexpression overturned the
inhibitory action of hsa_circ_0054633 knockdown on PDGF-BB-induced HA-VSMC
dysfunction.

However, although some interesting results have been drawn from our study, there
are still some limitations. First, we mainly performed our research work in PDGF-BB-
induced HA-VSMC. Further researches should be carried out in vivo and a larger cohort
of the disease. People may put efforts in making use of animal models with high or low
hsa_circ_0054633 expression in mice in the future. Besides that, the involvement of
circRNAs in regulating cell functions is very complex, new study should be conducted
to explore other potential molecular mechanisms underlying hsa_circ_0054633 in HA-
VSMC dysfunction.

In conclusion, this study demonstrated that hsa_circ_0054633 knockdown
protected HA-VSMCs from PDGF-BB-induced dysfunction through up-regulating
miR-107 and subsequent down-regulating TXNIP expression, suggesting a useful
strategy for coronary atherosclerosis therapy.
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Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Authors’ contribution

Conceptualization, Methodology, Formal analysis and Data curation: Yi Ding and Ye Tian; Validation and Investigation: Xiaochun Lei and Peng Ding; Writing - original draft preparation and Writing - review and editing: Xiaochun Lei, Yi Ding and Peng Ding; Approval of final manuscript: all authors

Ethics approval and consent to participate

The present study was approved by the ethical review committee of the Second Affiliated Hospital of Xi'an Medical University.

Patient consent for publication

Not applicable.
Competing interests

The authors declare that they have no competing interests.

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**Figure legends**

**Fig. 1 Hsa_circ_0054633 expression is elevated in CAD patients and PDGF-BB-induced HA-VSMCs.** (A) The expression of hsa_circ_0054633 was detected in the plasma from CAD patients and healthy people using qRT-PCR. (B) ROC curves were plotted to determine the diagnostic value of hsa_circ_0054633 in plasma of CAD...
patients. (C) The expression of hsa_circ_0054633 was measured in HA-VSMCs treated with or without PDGF-BB by qRT-PCR. *P<0.05, **P<0.01.

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**Fig. 5** TXNIP is a target of miR-107. (A) The putative binding sequences of miR-107 on TXNIP 3’UTR. (B) qRT-PCR analysis of TXNIP expression in the plasma from CAD patients and healthy people. (C, D) TXNIP expression measurement in HA-VSMCs treated with or without PDGF-BB using qRT-PCR and western blot. (E) Dual-luciferase reporter assay in HA-VSMCs co-transfected with TXNIP 3’UTR-wt or -mut and miR-107 or miR-NC. (F, G) TXNIP expression detection in HA-VSMCs transfected with miR-NC or miR-107 using qRT-PCR and western blot. (H, I) TXNIP expression detection in HA-VSMCs transfected with si-NC, si-circ#1, si-circ#1 + anti-NC, or si-circ#1 + anti-miR-107 using qRT-PCR and western blot. *P<0.05, **P<0.01.

**Fig. 6** Hsa_circ_0054633 moderates PDGF-BB-induced HA-VSMCs damage by regulating TXNIP. (A, B) TXNIP expression detection in HA-VSMCs transfected with vector or TXNIP with qRT-PCR and western blot. HA-VSMCs were transfected with...
si-NC, si-circ#1, si-circ#1 + vector, or si-circ#1 + TXNIP after treatment with PDGF-BB. After transfection, (C, D) TXNIP expression detection in HA-VSMCs using qRT-PCR and western blot. (E) transwell assay of HA-VSMC migration; (F) ROS generation analysis of HA-VSMCs with DCFH-DA assay; (G) CCK-8 assay of proliferation in HA-VSMCs; (H) flow cytometric analysis of cell cycle in HA-VSMCs; (I) western blot analysis of PCNA, cyclin D1, MMP9, and SOD2 levels in HA-VSMCs. *P<0.05, **P<0.01.

Table 1. The clinical and demographic characteristics of the patients with CAD and Controls.

Table 2. Correlation of the expression of hsa_circ_0054633/miR-107/TXNIP with clinicopathologic features in patients of CAD.
Table 1. The clinical and demographic characteristics of the patients with CAD and Controls.

<table>
<thead>
<tr>
<th></th>
<th>Normal (n=33)</th>
<th>CAD (n=33)</th>
<th>P value</th>
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<td>Males/females</td>
<td>15/18</td>
<td>19/14</td>
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<tr>
<td>Age (years)</td>
<td>56.1 ± 5.6</td>
<td>57.5 ± 6.1</td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>22.4 ± 2.2</td>
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<td>Smokers % (n)</td>
<td>32.3 (11)</td>
<td>48.5 (16)</td>
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<tr>
<td>Drinking % (n)</td>
<td>24.2 (8)</td>
<td>21.2 (7)</td>
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<tr>
<td>Hypertension % (n)</td>
<td>12.1 (4)</td>
<td>60.6 (20)</td>
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<tr>
<td>Diabetes mellitus % (n)</td>
<td>6.1 (2)</td>
<td>27.2 (9)</td>
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<td>Total cholesterol (mmol/L)</td>
<td>4.3 ± 1.1</td>
<td>5.1 ± 1.3</td>
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<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.3 ± 0.4</td>
<td>1.8 ± 0.6</td>
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<td>HDL-C (mmol/L)</td>
<td>1.2 ± 0.3</td>
<td>1.1 ± 0.4</td>
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<tr>
<td>LDL-C (mmol/L)</td>
<td>2.9 ± 0.8</td>
<td>2.1 ± 0.6</td>
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<tr>
<td>Hs-CRP (mg/L)</td>
<td>1.6 ± 0.7</td>
<td>3.2 ± 1.7</td>
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HDL-C, high-density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; Hs-CRP: high-sensitivity C-reactive protein.

Data are represented as mean ± SD or number (percentage) for category variables. 

*P*: CAD vs. Normal
Table 2. Correlation of the expression of hsa_circ_0054633/ miR-107/TXNIP with clinicopathologic features in patients of CAD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>hsa_circ_0054633 expression</th>
<th>miR-107 expression</th>
<th>TXNIP expression</th>
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