

Received: 2016.01.18
Accepted: 2016.02.03
Published: 2016.03.21

Silencing of Transient Receptor Potential Channel 4 Alleviates oxLDL-induced Angiogenesis in Human Coronary Artery Endothelial Cells by Inhibition of VEGF and NF- κ B

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

BCDE 1 **Wen Qin***
BCDE 2 **Wei Xie***
AEG 3 **Ning Xia**
BCF 4 **Qinglin He**
BDF 4 **Tianwei Sun**

1 Department of Pathology, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, P.R. China
2 Department of Reproductive Medicine, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, P.R. China
3 Department of Endocrinology and Metabolism, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi, P.R. China
4 Guangxi Medical University, Nanning, Guangxi, P.R. China

* These authors contributed equally to this work

Corresponding Author: Ning Xia, e-mail: xianing1612@126.com

Source of support: This research was supported by National Natural Science Foundation of China (No.81060070) and The Health Department Self-Raised Funds Projects of Guangxi Province (No. Z2014056)

Background: Transient receptor potential channel 4 (TRPC4) plays central roles in endothelial cell function. The aim of this study was to investigate the silencing effects of TRPC4 on oxidized low-density lipoprotein (oxLDL)-induced angiogenesis in human coronary artery endothelial cells (HCAECs), as well as the underlying molecular mechanism involved in this process.

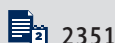
Material/Methods: HCAECs were transfected with small interfering RNA (siRNA) targeting TRPC4 (TRPC4-siRNA) or with a negative control (NC)-siRNA. The expression of TRPC4 was confirmed by real-time polymerase chain reaction (RT-PCR) and Western blotting. After the siRNA transfection, oxLDL was added to the medium. Cell proliferation, migration, and *in vitro* angiogenesis were determined by bromodeoxyuridine (BrdU) enzyme-linked immunosorbent assay (ELISA), Transwell assay and scratch-wound assay, respectively, and tube formation on Matrigel. Expression of vascular endothelial growth factor (VEGF) and nuclear factor (NF)- κ B p65 were assessed by Western blotting.

Results: Both the mRNA and protein levels of TRPC4 were significantly reduced by transfection with TRPC4-siRNA compared to the control group or NC-siRNA group ($P < 0.05$). Silencing of TRPC4 significantly decreased the cell proliferation, migration, and tube formation (all $P < 0.05$). Furthermore, the expression levels of VEGF and NF- κ B p65 were markedly lowered by silencing of TRPC4 in HCAECs.

Conclusions: These results suggest that silencing of TRPC4 alleviates angiogenesis induced by oxLDL in HCAECs through inactivation of VEGF and NF- κ B. Suppression of TRPC4 might be an alternative therapeutic strategy for atherosclerotic neovascularization.

MeSH Keywords: **Coronary Vessels • Endothelial Cells • Neovascularization, Pathologic • NF-kappa B • Transient Receptor Potential Channels • Vascular Endothelial Growth Factor A**

Full-text PDF: <http://www.medscimonit.com/abstract/index/idArt/897634>



2351



5



37



Background

Atherosclerosis and related cardiovascular diseases have been consistently regarded as a major cause of disability and mortality, contributing to one-third of deaths worldwide yearly [1,2]. Pathological neovascularization is a prominent feature of atherosclerotic lesions [3,4], and angiogenesis is the predominant form of neovascularization involved in atherosclerosis [5]. The pathological mechanisms responsible for angiogenesis in atherosclerosis are related predominantly to promotion of plaque expansion, plaque vulnerability, endothelialization, intraplaque hemorrhage (IPH), and rupture [6–9]. Low concentrations of oxidized low-density lipoprotein (oxLDL) have been reported to promote angiogenesis in many human endothelial cells, including human coronary artery endothelial cells (HCAECs) [10–12], leading to plaque vulnerability and intravascular thrombosis [13]. Therefore, prevention and treatment of angiogenesis might enable development of innovative and alternative atherosclerosis therapies.

Recently, the transient receptor potential channel (TRPC) superfamily has gained great attention in normal and pathophysiological vascular function [14–16]. TRPCs are expressed in endothelial cells (ECs) and vascular smooth muscle cells (SMCs) and are responsible for vasomotor control. It has been well documented that TRPCs are involved in vascular tone (e.g., TRPC4, TRPV1, and TRPV4), regulation of vascular permeability (e.g., TRPC1, TRPC4, TRPC6, and TRPV1), hypoxia-induced vascular remodeling (e.g., TRPC4), angiogenesis (e.g., TRPC4 and TRPC6), endothelial cell proliferation, and apoptosis [17]. Thus, TRPC4 plays significant roles in the TRPC superfamily. Song et al. suggested that suppression of TRPC4 inhibited vascular endothelial growth factor (VEGF)-induced retinal neovascularization and concluded that suppression of TRPC4 might be an alternative therapeutic strategy for VEGF-induced retinal neovascularization [18]. However, little information is available regarding the silencing effect of TRPC4 on HCAECs.

Therefore, in the present study, we investigated the silencing effect of TRPC4 on oxLDL-induced angiogenesis in HCAECs as well as the underlying molecular mechanisms. Our study may provide new insights into the effective treatment of atherosclerosis.

Material and Methods

Cell culture

HCAECs were obtained from Cell Applications, Inc (USA). These cells were maintained in endothelial basal medium-2 (EBM-2; Lonza, cat. no. CC-3156) supplemented with 10% fetal bovine serum (FBS; GIBCO, Grand Island, NY, USA), 100 units/ml penicillin (GIBCO), 100 µg/ml streptomycin (GIBCO), and endothelial

growth supplement (BD Biosciences). The cultures were maintained in a humidified incubator containing 5% CO₂ at 37°C.

Transfection

The siRNA targeting TRPC4 (TRPC4-siRNA) and negative control (NC)-siRNA were chemically synthesized and produced by Shanghai GenePharma Corporation (Shanghai, China). The TRPC4-siRNA or NC-siRNA was transfected into HCAECs using Lipofectamine 2000 (Invitrogen, USA) according to the manufacturer's instructions. Briefly, after culturing in 10% FBS/EBM-2 for 24 h at an appropriate concentration (about 70%), the HCAECs (1.2×10⁶ cells) were seeded in 100-mm culture dishes and transfected at the final concentration of 100 nM siRNA. After 48 h of transfection, the cells were harvested for further analysis. The efficacy of knockdown was assessed by real-time polymerase chain reaction (RT-PCR) and Western blotting.

RT-PCR

Expression of TRPC4 mRNA in HCAECs transfected with TRPC4-siRNA or NC-siRNA for 48 h was analyzed using RT-PCR. Total RNA was extracted from HCAECs using TRIzol reagent (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized from each RNA sample using the Reverse Transcription System (Promega Corporation, Madison, WI, USA). RT-PCR was performed using the 7900HT Fast Real-Time System (Applied Biosystems) according to the manufacturer's instructions. The mRNA levels of TRPC4 were normalized to GAPDH gene using 2^{-ΔΔCT} method. Reactions were run in triplicate.

Cell proliferation assay

Cell proliferation was assessed using bromodeoxyuridine (BrdU) enzyme-linked immunosorbent assay (ELISA) (Roche). Briefly, HCAECs were seeded at a density of 5×10⁵/well in 24-well plates coated with 0.01% gelatin. The cells were cultured in fully supplemented growth medium for 16 h, and then were maintained in basal medium for another 8 h. After the siRNA transfection, 5 µg/ml oxLDL (Sigma, USA) was added to the medium and incubated for 24 h. The supernatants were then harvested for detecting cell proliferation by using BrdU ELISA (R&D, USA) as previously described [19].

Cell migration assay

Cell migration assay was performed to assess and quantify the silencing effects of TRPC4 on HCAECs migration using a Transwell Boyden Chamber (8.0-µm membrane pores, Corning Costar, Cambridge, MA, USA). After transfection with TRPC4-siRNA or NC-siRNA, 5 µg/ml oxLDL was added and incubated for 8 h. The cells were subjected to starvation in EBM-2 medium

supplemented with 0.5% bovine serum albumin (BSA) but without FBS and endothelial growth supplements. After starvation for 16 h, HCAECs were seeded onto the upper chamber at a density of 2×10^4 cells. The lower membrane was pre-coated with 1 mg/ml fibronectin (Sigma-Aldrich). After 24 h of migration at 37°C, non-migrated cells were scraped from the upper surface of the membrane with a scraper. The remaining cells were fixed with methanol, stained with crystal violet, and visualized by an inverted fluorescence microscope (Olympus IX51). The number of stained cells was counted by 10 random fields at $\times 200$ magnification.

Scratch-wound assay

Scratch-wound assay was performed to evaluate the migration rate of HCAECs as previously described [20]. Briefly, HCAECs transfected with TRPC4-siRNA or NC-siRNA were seeded in 6-well culture plates and incubated with oxLDL, allowing growth until confluent state. Thereafter, the monolayer of HCAECs was scratched using sterile tips and washed with phosphate-buffered saline (PBS) 3 times to remove detached cells. Photographs of the wounded areas were obtained by using an Olympus microscope (Olympus, Japan). The size of the wounded areas was then quantitated using ImageJ Version 1.41o software (National Institutes of Health).

In vitro angiogenesis-tube formation on matrigel

Matrigel endothelial-like tube formation was examined after transfection with TRPC4-siRNA or NC-siRNA. Briefly, Matrigel (50 μ l, BD Biosciences) was added to 96-well plates and the plates were incubated for 30 min at 37°C to allow Matrigel to gel. HCAECs were starved in basal medium (without endothelial growth supplements) for 8 h. After transfection, oxLDL was added to the medium for 24 h. Subsequently, the cells were seeded at a density of 2×10^4 cells per well onto the Matrigel and incubated for 6 h at 37°C in a 5% CO₂ incubator. Tube formation was analyzed by an inverted phase-contrast microscope and quantified by measuring the tube lengths in 5 randomly chosen low-power (100 \times) fields using ImagePro Plus (version 6.0, Media Cybernetics).

Western blotting analysis

After 48 h of transfection with TRPC4-siRNA or NC-siRNA, total protein was extracted from the HCAECs, and the quantity of protein was determined using the BCA assay kit (Beyotime, Shanghai, China). The protein samples were then separated on 10–12% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were then blocked with 5% non-fat milk for 2 h at room temperature and incubated overnight at 4°C with the following antibodies: anti-TRPC4

antibody (Millipore, Bedford, MA, USA, #AB15302), anti-nuclear factor (NF)- κ B p65 antibody (Santa Cruz, SC-7151), or anti-VEGF antibody (Abcam, Cambridge, MA, USA, ab46154). The membranes were then incubated with horseradish peroxidase (HRP)-conjugated secondary antibody (Abcam, ab97023). GAPDH was used as loading control. Immunoreactive proteins were visualized using an enhanced chemiluminescence (ECL) chemiluminescent detection system (Pierce ECL Western Blotting Substrate detection system; Thermo Scientific).

Statistical analysis

All data are expressed as the mean \pm standard deviation (SD). All statistical analyses were performed using SPSS version 17.0 (Chicago, IL) software. The data were analyzed using the *t* test or ANOVA. A statistical significance was defined when $P < 0.05$.

Results

Expression of TRPC4 after transfection

To examine the role of TRPC4 in angiogenesis, TRPC4 siRNA was used to suppress the expression of TRPC4 in HCAECs. After 48 h of transfection, the influence on expression of TRPC4 was confirmed by RT-PCR and Western blotting. As indicated in Figure 1A and 1B, both the mRNA and protein levels of TRPC4 were significantly decreased by transfection with TRPC4-siRNA compared to the control group or NC-siRNA group ($P < 0.05$). No significant differences were observed between the control group and the NC-siRNA group. The results suggested that transfection was efficient.

Silencing effect of TRPC4 on HCAECs proliferation

We first examined the silencing effect of TRPC4 on HCAECs proliferation. The proliferation of HCAECs was induced by oxLDL. The BrdU ELISA demonstrated that there were no significant differences between the control group and the NC-siRNA group. However, silencing of TRPC4 markedly decreased the cell proliferation ($P < 0.05$), indicating that silencing of TRPC4 may exhibit the antiangiogenic activity by inhibition of vascular endothelial cell proliferation (Figure 2).

Silencing effect of TRPC4 on HCAECs migration

The silencing effect of TRPC4 on HCAECs migration induced by oxLDL was next determined using the Transwell Boyden chamber assay and scratch-wound assay. As shown in Figure 3A, the results demonstrated that the number of migrated cells was obviously reduced by silencing of TRPC4 ($P < 0.05$); however, no significant differences were found between the control group and the NC-siRNA group. We also assessed the migration rate of

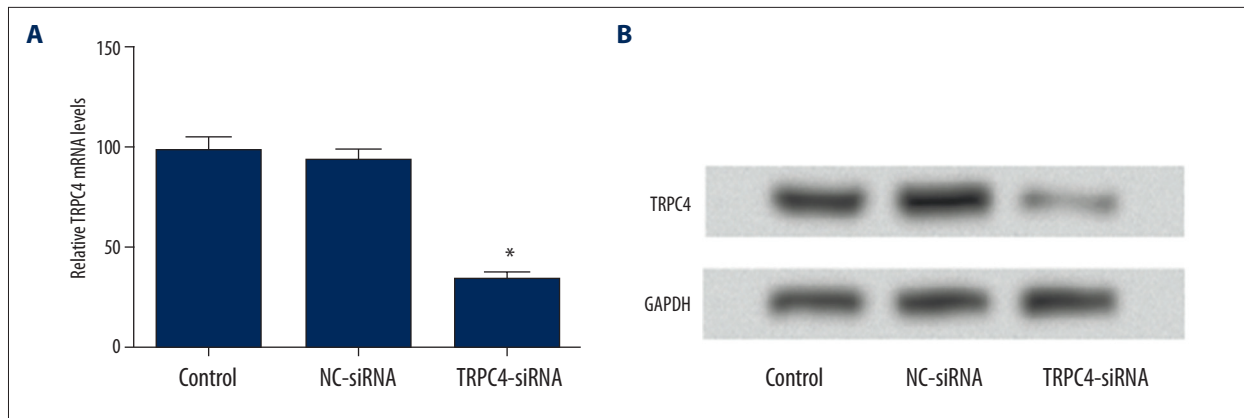


Figure 1. Expression of TRPC4 in HCAECs after transfection. After transfection, the expression of TRPC4 was confirmed by RT-PCR and Western blotting. **(A)** The relative mRNA levels of TRPC4 after transfection with TRPC4-siRNA or NC-siRNA; **(B)** The relative protein levels of TRPC4 after transfection with TRPC4-siRNA or NC-siRNA. TRPC4 – transient receptor potential channel 4; siRNA – small interfering RNA; HCAECs – human coronary artery endothelial cells; RT-PCR – real-time polymerase chain reaction; NC – negative control; * $P < 0.05$ compared to the control group or NC-siRNA group.

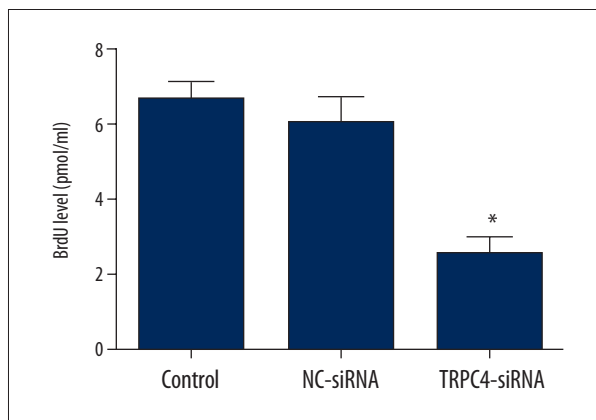


Figure 2. Silencing effect of TRPC4 on HCAECs proliferation. After transfection, the silencing effect of TRPC4 on HCAECs proliferation induced by oxLDL was evaluated by BrdU ELISA. TRPC4 – transient receptor potential channel 4; siRNA – small interfering RNA; HCAECs – human coronary artery endothelial cells; NC – negative control; BrdU – bromodeoxyuridine; ELISA – enzyme-linked immunosorbent assay; * $P < 0.05$ compared to the control group or NC-siRNA group.

HCAECs transfected with TRPC4-siRNA or NC-siRNA. We found that the migration rates of HCAECs were significantly lowered by transfection with TRPC4-siRNA (Figure 3B, 3C). The results suggested that silencing of TRPC4 may have an antiangiogenic effect by suppression of vascular endothelial cell migration.

Silencing effect of TRPC4 on tube formation

We investigated the silencing effect of TRPC4 on tube formation using Matrigel assay. As shown in Figure 4A, and 4B, the Matrigel assay indicated that silencing of TRPC4 significantly

suppressed the tube formation compared to the control group and the NC-siRNA group ($P < 0.05$), suggesting silencing of TRPC4 may have antiangiogenic activity by inhibition of tube formation.

Silencing effect of TRPC4 on expression of VEGF and NF- κ B p65

To examine the underlying molecular mechanism involved in silencing effects of TRPC4 on HCAECs, the expression levels of VEGF and NF- κ B p65 were determined (Figure 5). The Western blotting results showed that the protein levels of VEGF were significantly decreased by transfection with TRPC4-siRNA compared to the control group or NC-siRNA group, suggesting that the activation of VEGF was markedly suppressed by silencing of TRPC4. No significant differences were found in the levels of NF- κ B p65 in the cytoplasm among the 3 groups. However, the levels of NF- κ B p65 in the cell nucleus were significantly lower in the TRPC4-siRNA group compared to the control group or NC-siRNA group, demonstrating that silencing of TRPC4 inhibited the activation of NF- κ B.

Discussion

In the present study, the silencing effects of TRPC4 on oxLDL-induced *in vitro* angiogenesis were investigated, as well as the underlying molecular mechanisms involved in these effects. Our data showed that silencing of TRPC4 significantly attenuated angiogenesis induced by oxLDL in HCAECs by reducing cell proliferation, migration, and tube formation. The underlying mechanisms might be associated with modulation of the expression levels of VEGF and NF- κ B p65. These results suggest that suppression of TRPC4 might be an alternative therapeutic strategy for atherosclerotic neovascularization.

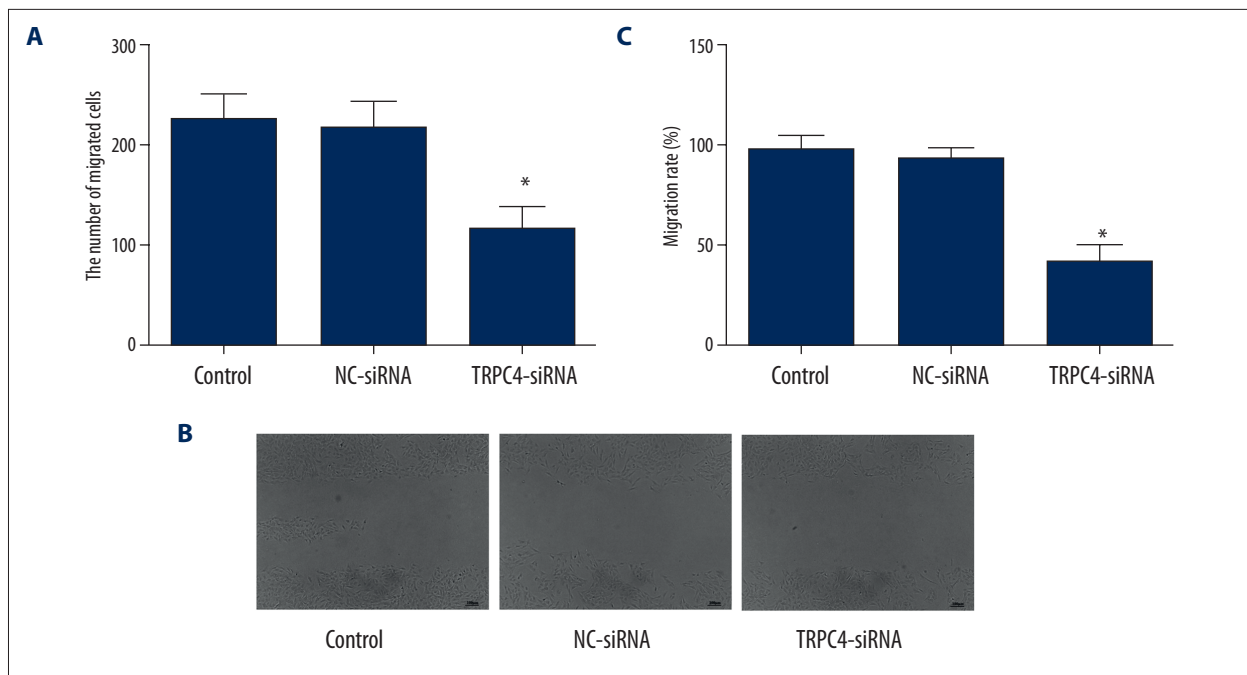


Figure 3. Silencing effect of TRPC4 on HCAECs migration. After transfection, the silencing effect of TRPC4 on HCAECs migration induced by oxLDL was determined using the Transwell Boyden chamber assay and scratch-wound assay. **(A)** The number of migration cells; **(B)** Image of scratch-wound assay; **(C)** Migration rate. TRPC4 – transient receptor potential channel 4; siRNA – small interfering RNA; HCAECs – human coronary artery endothelial cells; NC – negative control; * $P < 0.05$ compared to the control group or NC-siRNA group.

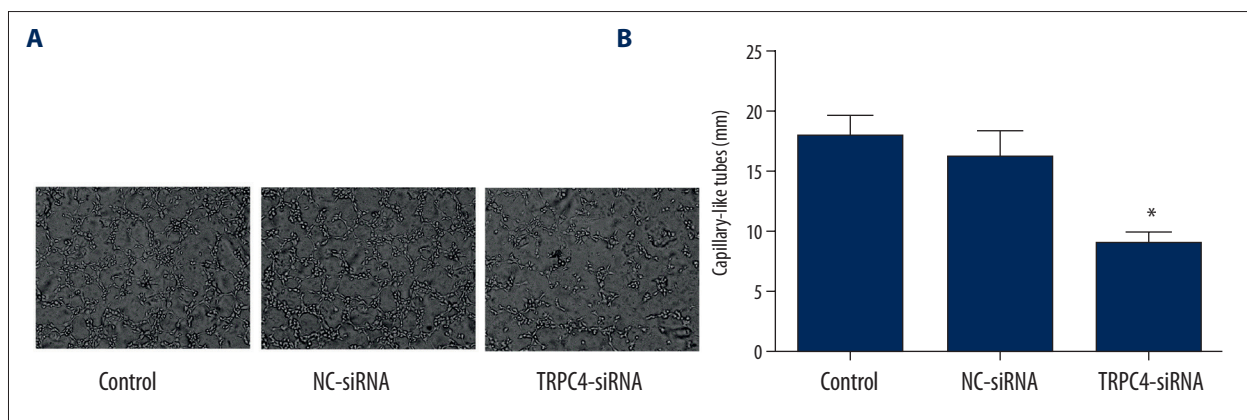


Figure 4. Silencing effect of TRPC4 on tube formation. After transfection, the silencing effect of TRPC4 on tube formation was determined using Matrigel assay. **(A)** Picture of *in vitro* tube formation; **(B)** Capillary-like tubes. TRPC4 – transient receptor potential channel 4; siRNA – small interfering RNA; NC – negative control; * $P < 0.05$ compared to the control group or NC-siRNA group.

TRPC proteins, as non-selective cation channels, are ubiquitously expressed throughout the cardiovascular system [21,22]. They significantly contribute to various human physiological and pathophysiological cardiovascular diseases by the functioning of $\text{Ca}^{2+}/\text{Na}^{+}$ permeable channels and/or signaling platforms [23–25]. Endothelial cytosolic Ca^{2+} plays a significant role in the process of angiogenesis. Nilius et al. have confirmed the potential roles of TRP-mediated Ca^{2+} influx in the angiogenic

process [26,27]. These pathologies are directly or indirectly related to the development of atherosclerotic lesions. With the development of atherosclerotic lesion, new vessels sprouting from the adventitia neovascularize the growing plaque. Inflammatory cells could be recruited through neovascularization, leading to exacerbation of inflammatory state and acceleration of plaque growth [25], even in-stent restenosis (ISR) [28]. Plaque neovascularization is thought to contribute to the

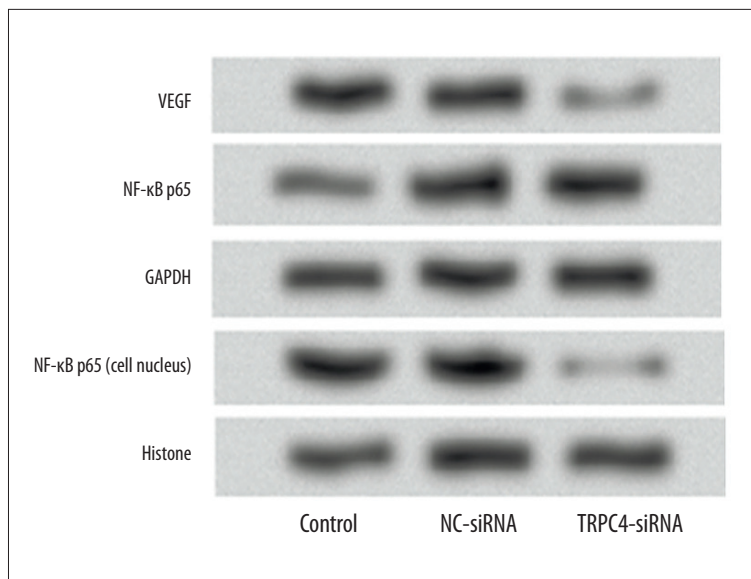


Figure 5. Silencing effect of TRPC4 on expression of VEGF and NF-κB p65. The expression levels of VEGF and NF-κB p65 were determined to examine the underlying molecular mechanism. TRPC4 – transient receptor potential channel 4; siRNA – small interfering RNA; NC – negative control; VEGF – vascular endothelial growth factor.

plaque instability, and ultimately results in thromboembolic complications [29]. Therefore, the functional roles of TRPCs in neovascularization have received great attention in recent years [27]. Previous studies have suggested that TRPC6 plays key roles in growth-factor-induced angiogenesis during plaque neovascularization [15,30]. In addition to TRPC6, TRPC4 has recently been attracting increased research attention. TRPC4, an essential component of the nonselective calcium-permeable cation channel, is assumed to be activated by Gq/phospholipase C-coupled receptors and tyrosine kinases [31], and it is associated with multiple processes, including cell proliferation, endothelial permeability, vasodilation, and neurotransmitter release [17]. Song et al. confirmed that suppression of TRPC4 inhibits retinal neovascularization [18,32]. Given the multiple functions of TRPC4, we hypothesized that TRPC4 might play a critical role in angiogenesis involved in atherosclerosis.

To investigate the functional role of TRPC4 in atheromatous angiogenesis, we first silenced the expression of TRPC4 using siRNA. The silencing effect of TRPC4 on cell proliferation, migration, and tube formation was explored. For the angiogenesis in HCAECs, we applied oxLDL, which is widely used to induce angiogenesis in multiple human endothelial cells. Our data demonstrate that silencing the expression of TRPC4 markedly reduced the cell proliferation, migration, and tube formation induced by oxLDL compared to the control group or NC group, indicating that silencing of TRPC4 could be a specific treatment to inhibit angiogenesis by reducing the number of proliferating cells. Studies have demonstrated that VEGF is required for adventitial angiogenesis and neovascularization [33]. VEGF blocking suppresses physiological and pathological angiogenesis in various diseases [33,34]. In the present study,

we found that silencing of TRPC4 significantly reduced the expression of VEGF; therefore, silencing of TRPC4 decreased the development of angiogenesis partly by reduction of VEGF. In addition, previous studies have suggested that suppression of TRPC4 inhibited activation of many signaling pathways that act downstream to VEGF to control angiogenesis, such as extracellular signal-regulated kinase (ERK), p38 mitogen-activated protein kinase (MAPK), and AKT signaling pathways [18]. The present study shows that TRPC4 inhibits the activation of NF-κB. NF-κB, an important transcription factor, is responsible for a variety of biological processes. Activation of constitutive NF-κB has been noted to mediate different biological functions, such as cell proliferation and angiogenesis [35]. Blockade of NF-κB signaling has been reported to inhibit angiogenesis by suppressing expression of VEGF in tumor cells [36]. Furthermore, inhibition of NF-κB protects against atherosclerosis [37]. Our study verifies that suppression of TRPC4 prevents angiogenesis by inhibition of NF-κB in HCAECs, perhaps by modulating VEGF expression. However, further research is needed to confirm this.

Conclusions

We demonstrated that suppressing the expression of TRPC4 inhibited angiogenesis induced by oxLDL in HCAECs. Silencing of TRPC4 might be a potential treatment for angiogenesis in atherosclerotic plaque.

Conflicts of interest

There are no conflicts of interest.

References:

- Hotamisligil GS: Endoplasmic reticulum stress and atherosclerosis. *Nat Med*, 2010; 16: 396–99
- Pescetelli I, Zimarino M, Ghirarduzzi A, De Caterina R: Localizing factors in atherosclerosis. *J Cardiovasc Med*, 2015; 16: 824–30
- Jeziorska M, Woolley DE: Neovascularization in early atherosclerotic lesions of human carotid arteries: Its potential contribution to plaque development. *Hum Pathol*, 1999; 30: 919–25
- Moreno PR, Purushothaman M, Purushothaman KR: Plaque neovascularization: Defense mechanisms, betrayal, or a war in progress. *Ann NY Acad Sci*, 2012; 1254: 7–17
- Carmeliet P: Angiogenesis in health and disease. *Nat Med*, 2003; 9: 653–60
- Chistiakov DA, Orekhov AN, Bobryshev YV: Contribution of neovascularization and intraplaque haemorrhage to atherosclerotic plaque progression and instability. *Acta Physiol*, 2015; 213: 539–53
- Cheng C, Chrifi I, Pasterkamp G, Duckers HJ: Biological mechanisms of microvessel formation in advanced atherosclerosis: the big five. *Trends Cardiovasc Med*, 2013; 23: 153–64
- Di Stefano R, Felice F, Balbarini A: Angiogenesis as risk factor for plaque vulnerability. *Curr Pharm Des*, 2009; 15: 1095–106
- Christ T, Dohmen PM, Holinski S et al: Suitability of the rat subdermal model for tissue engineering of heart valves. *Med Sci Monit Basic Res*, 2014; 20: 194–99
- Yu S, Wong SL, Lau CW et al: Oxidized LDL at low concentration promotes *in-vitro* angiogenesis and activates nitric oxide synthase through PI3K/Akt/eNOS pathway in human coronary artery endothelial cells. *Biochem Biophys Res Commun*, 2011; 407: 44–48
- Khaidakov M, Mitra S, Wang X et al: Large impact of low concentration oxidized LDL on angiogenic potential of human endothelial cells: A microarray study. *PLoS One*, 2012; 7: e47421
- Dandapat A, Hu C, Sun L, Mehta JL: Small concentrations of oxLDL induce capillary tube formation from endothelial cells via LOX-1-dependent redox-sensitive pathway. *Arterioscler Thromb Vasc Biol*, 2007; 27: 2435–42
- Wang L, Li G, Chen Q, Ke D: Octanoylated ghrelin attenuates angiogenesis induced by oxLDL in human coronary artery endothelial cells via the GHSR1a-mediated NF-kappaB pathway. *Metabolism*, 2015; 64: 1262–71
- Earley S, Reading S, Brayden JE: Functional significance of transient receptor potential channels in vascular function. In: Liedtke WB, Heller S (eds.), *TRP Ion Channel Function in Sensory Transduction and Cellular Signaling Cascades*. Boca Raton (FL): 2007
- Earley S, Brayden JE: Transient receptor potential channels and vascular function. *Clin Sci*, 2010; 119: 19–36
- Inoue R, Hai L, Honda A: Pathophysiological implications of transient receptor potential channels in vascular function. *Curr Opin Nephrol Hypertens*, 2008; 17: 193–98
- Yao X, Garland CJ: Recent developments in vascular endothelial cell transient receptor potential channels. *Circ Res*, 2005; 97: 853–63
- Song HB, Jun HO, Kim JH et al: Suppression of transient receptor potential canonical channel 4 inhibits vascular endothelial growth factor-induced retinal neovascularization. *Cell Calcium*, 2015; 57: 101–8
- Ajeawung NF, Maltais R, Jones C et al: Viability screen on pediatric low grade glioma cell lines unveils a novel anti-cancer drug of the steroid biosynthesis inhibitor family. *Cancer Lett*, 2013; 330: 96–105
- Hamada S, Satoh K, Hirota M et al: Bone morphogenetic protein 4 induces epithelial-mesenchymal transition through MSX2 induction on pancreatic cancer cell line. *J Cell Physiol*, 2007; 213: 768–74
- Earley S, Brayden JE: Transient receptor potential channels in the vasculature. *Physiol Rev* 2015; 95: 645–690.
- Mehta D, Malik AB: Signaling mechanisms regulating endothelial permeability. *Physiol Rev*, 2006; 86: 279–367
- Dietrich A, Gudermann T: TRP channels in the cardiopulmonary vasculature. *Adv Exp Med Biol*, 2011; 704: 781–810
- Randhawa PK, Jaggi AS: TRPV4 channels: Physiological and pathological role in cardiovascular system. *Basic Res Cardiol*, 2015; 110: 54
- Vazquez G: TRPC channels as prospective targets in atherosclerosis: Terra incognita. *Front Biosci (Schol Ed)*, 2012; 4: 157–66
- Nilius B, Droogmans G: Ion channels and their functional role in vascular endothelium. *Physiol Rev*, 2001; 81: 1415–59
- Nilius B, Droogmans G, Wondergem R: Transient receptor potential channels in endothelium: Solving the calcium entry puzzle? *Endothelium*, 2003; 10: 5–15
- Yagi H, Amiya E, Ando J et al: In-stent restenosis exacerbated by drug-induced severe eosinophilia after second-generation drug-eluting stent implantation. *Am J Case Rep*, 2014; 15: 397–400
- Virmani R, Burke AP, Farb A, Kolodgie FD: Pathology of the vulnerable plaque. *J Am Coll Cardiol*, 2006; 47: C13–18
- Ge R, Tai Y, Sun Y et al: Critical role of TRPC6 channels in VEGF-mediated angiogenesis. *Cancer Lett*, 2009; 283: 43–51
- Freichel M, Tsvilovskyy V, Camacho-Londono JE: TRPC4- and TRPC4-containing channels. *Handb Exp Pharmacol*, 2014; 222: 85–128
- Song HB, Jun HO, Kim JH, Kim JH: Suppression of transient receptor potential canonical (TRPC) channel 4 inhibits retinal neovascularization in the mouse model of oxygen induced retinopathy. *Invest Ophthalmol Vis Sci*, 2014; 55: 5401–9
- Ferrara N: Vascular endothelial growth factor. *Arterioscler Thromb Vasc Biol*, 2009; 29: 789–91
- Shibuya M: Vascular endothelial growth factor and its receptor system: Physiological functions in angiogenesis and pathological roles in various diseases. *J Biochem*, 2013; 153: 13–19
- De Martin R, Hoeth M, Hofer-Warbinek R, Schmid JA: The transcription factor NF-kappa B and the regulation of vascular cell function. *Arterioscler Thromb Vasc Biol*, 2000; 20: E83–88
- Huang S, Robinson JB, Deguzman A et al: Blockade of nuclear factor-kappaB signaling inhibits angiogenesis and tumorigenicity of human ovarian cancer cells by suppressing expression of vascular endothelial growth factor and interleukin 8. *Cancer Res*, 2000; 60: 5334–39
- Gareus R, Kotsaki E, Xanthoulea S et al: Endothelial cell-specific NF-kappaB inhibition protects mice from atherosclerosis. *Cell Metab*, 2008; 8: 372–83