Roles of miR-4463 in H$_2$O$_2$-induced oxidative stress in human umbilical vein endothelial cells

XUEQIN WANG$^1$, XUEMEI HE$^2$, XIAN DENG$^{1,3}$, YANZHENGE HE$^1$ and XIANGYU ZHOU$^1$

$^1$Department of Vascular and Thyroid Surgery; $^2$Medical Research Center, The Affiliated Hospital of Southwest Medical University, Jiangyang, Luzhou, Sichuan 646000; $^3$State Key Laboratory of Quality Research in Chinese Medicine, Faculty of Chinese Medicine, Macau University of Science and Technology, Taipa, Macau SAR 519020, P.R. China

Received July 23, 2016; Accepted April 28, 2017

DOI: 10.3892/mmr.2017.7001

Abstract. Oxidative stress is implicated in the pathophysiology of vascular diseases, including atherosclerosis, aneurysm and arteriovenous fistula. A previous study from our lab suggested that microRNA (miR)-4463 may be involved in the pathogenesis of vascular disease; however, the roles of oxidative stress in the molecular mechanisms underlying the actions of miR-4463 in vascular disease have yet to be elucidated. The aim of the present study was to investigate the role of miR-4463 in hydrogen peroxide (H$_2$O$_2$)-induced oxidative stress in human umbilical vein endothelial cells (HUVECs). Reverse transcription-quantitative polymerase chain reaction was used to assess the expression levels of miR-4463 in HUVECs treated with various concentrations of H$_2$O$_2$. Flow cytometry was used to evaluate the percentage of apoptotic cells, and the protein expression levels of the apoptotic markers cleaved (C)-caspase3, poly (adenosine diphosphate-ribose) polymerase 1 (PARP1), B cell lymphoma-2 (Bcl-2), Bcl-2-associated X protein (Bax) and X-linked inhibitor of apoptosis protein (XIAP) were determined using western blot analysis. The results demonstrated that the apoptotic rate of HUVECs was increased following treatment with H$_2$O$_2$ in a concentration-dependent manner, and the expression of miR-4463 was also upregulated in a dose-dependent manner. Following transfection with miR-4463 mimics, the levels of malondialdehyde and reactive oxygen species were increased in HUVECs, with a corresponding increase in the apoptotic rate. Furthermore, western blot analysis revealed that the protein expression levels of C-caspase3, PARP1 and Bax were upregulated, whereas the levels of Bcl-2 and XIAP were downregulated. In conclusion, the present findings suggested that the upregulation of miR-4463 may enhance H$_2$O$_2$-induced oxidative stress and promote apoptosis in HUVECs in vitro.

Introduction

Oxidative stress is an imbalance between oxidative and antioxidative processes in vivo and is involved in the pathogenesis of numerous diseases. Vascular disease is an inflammatory process, and oxidative stress has been implicated in vascular pathology (1,2). A previous study demonstrated that treatment with fucoidan, which is an aggregate name for algal fucose-enriched sulfated polysaccharides extracted from the extracellular matrix of seaweeds, may increase oxidative stress production by activating endothelial nitric oxide synthase and promoting Akt phosphorylation in myocardial disease and stroke (3). A recent study suggested that oxidative stress may be involved in high-fat diet-induced aortic remodeling, which may be exacerbated by Zn$^{2+}$ deficiency, as the anti-oxidative molecule resveratrol was demonstrated to prevent high-fat diet-induced vascular inflammation, oxidative stress and pathological remodeling (4). Therefore, investigating the implications of oxidative stress in the pathogenesis of vascular diseases and exploring the underlying molecular mechanisms are imperative for the development of effective preventive and therapeutic strategies aimed at patients with vascular diseases.

Signaling pathways related to oxidative stress are associated with endothelial cells that line the inner walls of blood vessels, which are continuously exposed to various stresses owing to the mechanical force exerted by blood flow and pressure, and are sensitive to oxidative damage (5,6). A recent study demonstrated that the natural antioxidant allicin protected human umbilical vein endothelial cells (HUVECs) from oxidized low-density lipoprotein-induced injury by preventing apoptosis through the inhibition of caspase-3 and nicotinamide-adenine dinucleotide phosphate oxidase-mediated proapoptotic signaling (7). Another recent study reported that resveratrol (polyphenolic compounds) treatment ameliorated high glucose-induced HUVEC injury through the activation of 5’ adenosine monophosphate-activated protein kinase α, leading to an increase in reductive reactions and a corresponding decrease in oxidative stress (8). These studies
suggested that oxidative stress may serve crucial roles during the development of vascular endothelial cell injury. Therefore, the present study aimed to investigate the effects of oxidative stress in HUVECs in vitro.

MicroRNAs (miRNAs) are small (~22 nucleotides long) non-coding RNA molecules, which modulate the stability and the translational efficiency of target mRNA transcripts (9). miRNAs are involved in the post-transcriptional regulation of gene expression and are implicated in numerous biological processes, including proliferation, differentiation, senescence and death (10). Previous studies have suggested that miRNAs may be involved in the regulation of oxidative stress during vascular disease pathogenesis, serving important roles in oxidative stress-induced endothelial dysfunction and mitochondrial metabolism dysregulation (1,11). Several miRNAs have been implicated in endothelial development, including miRNA (miR)-20a, miR-23b-3p, miR-150, miR-195 and miR-200b (11,12). Previous studies have also revealed crucial roles for miRNAs in endothelial physiology and pathology, thus suggesting that miRNAs may have potential as novel therapeutic targets for the treatment of patients with vascular diseases (13,14).

miR-4463 has garnered attention as a prognostic and diagnostic biomarker in patients with polycystic ovary syndrome (15). In addition, our previous studies have demonstrated the aberrant expression of miR-4463 in vascular diseases, including arteriosclerosis obliterans (16) and carotid artery stenosis (17), which suggested that miR-4463 may also have potential as a biomarker for the early diagnosis of vascular diseases. However, the roles of miR-4463 in the regulation of oxidative stress in endothelial cells, and the molecular mechanisms underlying its actions, have yet to be elucidated. Therefore, the present study aimed to investigate the putative relationship between miR-4463 and oxidative stress, and explore the molecular mechanisms that may be involved in HUVECs.

Materials and methods

Cell culture and oxidative stress model. The HUVEC cell line was purchased from ScienCell Research Laboratories, Inc. (Carlsbad, CA, USA) and cultured in low-glucose Dulbecco's modified Eagle's medium (DMEM; HyClone, GE Healthcare Life Sciences, Logan, UT, USA), supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Inc., Waltham, MA, USA) and maintained in a humidified 5% CO₂ atmosphere. Cells between passages 4 and 6, in the logarithmic phase of growth, were used in the subsequent experiments. The morphology of the cells was observed using a phase-contrast microscope (cellsens standard software version 1.6, DP72; Olympus Corporation, Tokyo, Japan).

Hydrogen peroxide (H₂O₂) is commonly used in models of oxidative stress-induced apoptosis (18). In the present study, HUVECs were treated with various concentrations of H₂O₂ (200, 500, 700 and 1,000 µmol/l; Table I) using 0.3% H₂O₂ stock solution, or 0 µl H₂O₂ (low-glucose DMEM alone containing 10% FBS) for 16 h to induce oxidative stress, as previously described (19-21).

Transfection. The intracellular level of miR-4463 was upregulated or downregulated by the transfection of miRNA mimics and inhibitor respectively, according to the manufacturer's protocol (Guangzhou RiboBio Co., Ltd., Guangzhou, China). The miRNA mimics synthesized by the chemical method induces a high level of expression of mature miRNA in the cell, and it enhances the regulation of endogenous miRNA and gains certain functions in cells. miRNA inhibitors inhibit the effects of mature miRNA, and weaken the role of endogenous gene regulation and cell function. When the cell density reached 50-70%, the transfection efficiency was optimal (Table II). Transfection was performed using 100 µmol/l miR-4463 mimic or inhibitor for 15 min at room temperature, and the efficiency of transfection was determined by RT-qPCR. Finally, 100 nmol/l NC, miR-4463 mimics and miR-4463 inhibitor was chosen for the next step after 3 days transfection. NC, miR-4463 mimics and miR-4463 inhibitor sequences are listed on the Guangzhou RiboBio Co., Ltd. Website: http://ribobio.bioon.com.cn.

| Table I. Various concentrations of H₂O₂. | Low-glucose DMEM with 10% FBS |
|----------------------------------------|-------------------------------|
| H₂O₂ concentration | 0.3% H₂O₂ | 500.0 µl |
| 0 µmol/l | 0.0 µl | 500.0 µl |
| 200 µmol/l | 11.4 µl | 488.6 µl |
| 500 µmol/l | 28.5 µl | 471.5 µl |
| 700 µmol/l | 39.9 µl | 460.1 µl |
| 1,000 µmol/l | 57.0 µl | 443.0 µl |

RNA extraction and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Following treatment with H₂O₂, total RNA was extracted from HUVECs (2-3x10⁶ cells) using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Total RNA (500 ng) was reverse transcribed into cDNA using an miScript II RT kit (catalog no. 218160 and 21816; Qiagen GmbH, Hilden, Germany), according to the manufacturer's protocol. The specific reaction components of reverse-transcription are mentioned in Table III. The mix was incubated for 60 min at 37°C, incubated for 5 min at 95°C and stored at -20°C. Acquired cDNA was used for RT-qPCR according to the manufacturer's protocol using the miScript SYBR-Green PCR kit (catalog no. 208054; Qiagen GmbH). Mature has-miR-4463 primer (catalog no. MIMAT0018987) and U6 primer (catalog no. MS00044996) were purchased from Qiagen GmbH. The specific reaction components of RT-qPCR are mentioned in Table IV, and the cycling conditions are mentioned in Table V. qPCR was performed using the StepOnePlus version 2.2.3 real-time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Relative expression levels were quantified using the 2^(-ΔΔCt) method (22) and normalized to the expression of U6; Ct values >35 were excluded. Experiments were performed in triplicate.

Apoptosis assay. Apoptosis and necrosis were assessed using the fluorescein isothiocyanate (FITC) Annexin V and...
propidium iodide (PI) detection kit (BD Pharmingen; BD Biosciences, Franklin Lakes, NJ, USA), according to the manufacturer’s protocol. Following treatment with H₂O₂, HUVECs (5x10⁵ cells) were washed with 10X Annexin V binding buffer, and stained for 10 min with 5 µl FITC Annexin V and 5 µl PI at room temperature in the dark. Stained cells were analyzed by flow cytometry using a BD FACSVersa and BD FACSuite™ software version 1.0.3 (BD Biosciences), and the experiment was repeated three times.

Oxidative stress assays. Reactive oxygen species (ROS) production and lipid peroxidation were measured in order to assess oxidative stress in HUVECs, using ROS and malondialdehyde (MDA) detection kits (Beyotime Institute of Biotechnology, Haimen, China). Experiments were repeated >3 times. To detect ROS production, H₂O₂-treated HUVECs (1x10⁵ cells) were incubated with the fluorescent probe 2’,7’-dichlorodihydrofluorescein diacetate (chloromethyl derivative) at 37°C for 30 min, as previously described (23-25). Subsequently, samples were digested by trypsin-EDTA solution (Beyotime Institute of Biotechnology), centrifuged (1,000 x g for 5 min at room temperature) and transferred to black 96-well plates. The fluorescence was measured using an excitation wavelength of 488 nm and an emission wavelength of 525 nm using a microplate reader. MDA is a natural product of lipid oxidation in organisms. The level of lipid oxidation may be measured by detecting MDA contents in HUVECs (1x10⁵ cells), and the experiment was repeated three times.

Cell proliferation assay. Cellular proliferation was evaluated using a Cell Counting Kit-8 (CCK-8; Beyotime Institute of Biotechnology), as previously described (28,29). Following treatment with H₂O₂ for 16 h, HUVECs (1x10⁵ cells/well) were seeded in 96-well plates (5 wells/experimental group) in 100 µl serum-free DMEM (Hyclone; GE Healthcare Life Sciences) and 10 µl CCK-8 solution were added to each well and cells were incubated at 37°C for 35 min. The absorbance of each sample was measured at 532 nm using a microplate reader, as previously described (26,27).

Western blot analysis. Following treatment with H₂O₂, total protein was extracted from HUVECs (2x10⁵ cells) using Radioimmunoprecipitation Assay Lysis buffer (Beyotime Institute of Biotechnology) as previously described (2,3). Equal amounts (20 µg) of protein in each sample (extracted using a bicinchoninic acid assay) were separated by 12% SDS-PAGE and transferred onto polyvinylidene difluoride membranes. Then, membranes were blocked for 2 h in 0.5% nonfat milk at room temperature, and washed three times for 5 mins with PBS containing 0.1% Tween-20. Next, membranes were incubated with the following primary antibodies at 4°C overnight: Anti-poly (adenosine diphosphate-ribose) polymerase
Table V. Cycling conditions of miScript SYBR Green polymerase chain reaction.

| Step                   | Time | Temperature | Additional comments                                                                 |
|------------------------|------|-------------|--------------------------------------------------------------------------------------|
| Initial activation step| 15 min| 95°C        | HotStarTaq® DNA polymerase is activated by this heating step.                         |
| Denaturation            | 15 sec| 94°C        |                                                                                     |
| Annealing               | 30 sec| 55°C        |                                                                                     |
| Extension               | 30 sec| 70°C        | Perform fluorescence data collection.                                               |
| Cycle number            | 40 cycles |               | Cycle number depends on the amount of template cDNA and abundance of the target.   |

Statistical analysis. Statistical analysis was performed using SPSS software version 19.0 (IBM Corp., Armonk, NY, USA).
Data are expressed as the mean ± standard deviation. The statistical significance of the differences between groups was assessed using one-way analysis of variance followed by a post hoc Dunnett's two-tailed test for multiple comparisons. P<0.05 was considered to indicate a statistically significant difference.

Results

Alterations in HUVEC morphology and miR-4463 expression following treatment with H₂O₂. When examined under an inverted phase-contrast microscope, HUVECs in the untreated control group exhibited physiological morphology, characterized by spindle- or round-shaped cell architecture, cells that were adherent and formed paving stone-like cultures (32,33).

Conversely, the number of HUVECs in culture appeared to be markedly reduced following H₂O₂ treatment, with undefined boundaries and observable necrotic and disintegrative phenomena among the cells, which concurred with previously published data (34-36) (Fig. 1).

The expression levels of miR-4463 were assessed in HUVECs treated with various concentrations of H₂O₂. The results demonstrated that miR-4463 expression levels were significantly upregulated in H₂O₂-treated HUVECs in a dose-dependent manner compared with in untreated control cells (Fig. 2). miR-4463 expression appeared to be the highest following treatment with H₂O₂ at a concentration of 700 µmol/l. The high concentration of H₂O₂ (1,000 µmol/l) may promote cell death and inhibit the expression of the cells under oxidative stress.

Figure 3. H₂O₂ induces apoptosis in HUVECs. HUVECs were treated with 200, 500 and 700 µmol/l H₂O₂ for 16 h. Control cells were not exposed to H₂O₂. (A) Cellular apoptosis was assessed using flow cytometry following staining with FITC Annexin V and propidium iodide. (B) Treatment with H₂O₂ significantly increased the apoptotic rate of HUVECs. Data are expressed as the mean ± standard deviation (n=3). *P<0.05 vs. Control. FITC, fluorescein isothiocyanate; H₂O₂, hydrogen peroxide; HUVEC, human umbilical vein endothelial cell.
miR-4463 promotes H$_2$O$_2$-induced increases in oxidative stress. The apoptotic rate of HUVECs pretreated with miR-4463 mimics prior to H$_2$O$_2$ exposure was significantly increased compared with negative control-treated H$_2$O$_2$-exposed cells (Fig. 5), thus suggesting that miR-4463 may serve a role in promoting apoptosis following H$_2$O$_2$ treatment. Detecting NC-treated H$_2$O$_2$ as the control group eliminates any background effects of the transfection reagent. The roles of miR-4463 in H$_2$O$_2$-induced oxidative stress were also investigated, and the results revealed that intracellular ROS and MDA levels were significantly increased in H$_2$O$_2$-treated HUVECs following transfection with miR-4463 mimics; however, the miR-4463 inhibitor did not induce a significant difference. This suggested that miR-4463 overexpression enhanced H$_2$O$_2$-induced oxidative stress (Fig. 6A and B). However, the miR-4463 mimics did not have an exact effect on cell proliferation (Fig. 6C). These findings suggested that miR-4463 may enhance oxidative stress on HUVECs but have no apparent effect on the proliferation of HUVECs.

miR-4463 promotes proapoptotic signaling in H$_2$O$_2$-treated HUVECs. The protein expression levels of pro- and antiapoptotic markers, including C-caspase-3, Bax, Bcl-2, XIAP and PARP1, were detected in H$_2$O$_2$-treated HUVECs by western blot analysis (Fig. 7). The results demonstrated that following H$_2$O$_2$ treatment, the expression levels of the antiapoptotic proteins Bcl-2 and XIAP were downregulated, whereas the expression of the proapoptotic C-caspase3, PARP1 and Bax was enhanced. Notably, HUVECs pretreated with miR-4463

---

$H_2O_2$ induces apoptosis in HUVECs. Flow cytometric analysis revealed that treatment with $H_2O_2$ was able to induced apoptosis in HUVECs (Fig. 3). Annexin V-positive cells and cellular uptake of PI were evaluated in order to distinguish between living, apoptotic and necrotic cells, as previously described. The proapoptotic effects of $H_2O_2$ treatment appeared to be concentration-dependent (Fig. 3B): No significant difference in the apoptotic rate was detected following treatment with 200 µmol/l $H_2O_2$; however, HUVEC apoptosis was significantly increased following treatment with 500 and 700 µmol/l $H_2O_2$ compared with control untreated cells (P<0.05). As $H_2O_2$ at a concentration of 700 µmol/l appeared to produce the greatest increase in apoptotic rate (43%) and necrotic rate (8.25%), this concentration was selected for subsequent experiments.

$H_2O_2$ induces oxidative stress in HUVECs. To confirm the induction of oxidative stress following $H_2O_2$ treatment in HUVECs, oxidative stress products were detected and cellular viability was evaluated, using commercially available kits. MDA is an end product of lipid oxidation that is involved in the oxidative damage of the cellular membrane; therefore, MDA contents may be measured as an indication of the degree of lipid peroxidation and the extent of membrane damage. In HUVECs treated with 700 µmol/l $H_2O_2$, MDA levels were increased by 1.5-fold compared with the untreated control cells (P<0.05; Fig. 4A); whereas no significant differences were indicated in HUVECs treated with 200 or 500 µmol/l $H_2O_2$. In addition, intracellular ROS production was significantly enhanced in $H_2O_2$-treated cells, as indicated by the 3.75-fold increase in ROS levels following treatment with 700 µmol/l $H_2O_2$ compared with the control. Treatment with 500 µmol/l $H_2O_2$ increased ROS levels by ~2-fold compared with the control, and there was no significant increase after treatment with 200 µmol/l $H_2O_2$ (Fig. 4B). Notably, no significant difference in cellular proliferation was detected between 200 µmol/l $H_2O_2$-treated HUVECs and control cells (Fig. 4C). However, cell proliferation and viability was significantly reduced following treatment with 500 or 700 µmol/l $H_2O_2$ (Fig. 4C). These findings suggested that treatment with $H_2O_2$ may induce oxidative stress in HUVECs and suppress their viability in a concentration-dependent manner.

---

Figure 4. $H_2O_2$ induces oxidative stress in HUVECs. HUVECs were treated with 200, 500 and 700 µmol/l $H_2O_2$ for 16 h: Control cells were not exposed to $H_2O_2$. (A) MDA levels and (B) ROS production were assessed in HUVECs using commercially available kits. (C) A Cell Counting Kit-8 assay was used to evaluate cell viability. Data are expressed as the mean ± standard deviation (n=3). *P<0.05 vs. Control. H$_2$O$_2$, hydrogen peroxide; HUVEC, human umbilical vein endothelial cell; MDA, malondialdehyde; ROS, reactive oxygen species.
mimics prior to H\textsubscript{2}O\textsubscript{2} exposure exhibited significantly increased C-caspase3, cleaved PARP and Bax protein expression levels, whereas the expression of Bcl-2 and XIAP was significantly suppressed. The present findings suggested that miR-4463 overexpression may increase the proapoptotic effects of H\textsubscript{2}O\textsubscript{2} on HUVECs.

**Discussion**

miRNAs are able to combine with complementary sequences in the 3′-untranslated region of target mRNA transcripts, and thus regulate the transcription of target genes and ultimately inhibit the translation of specific proteins (9). miRNAs have been demonstrated previously to be involved in the regulation of HUVEC proliferation, migration and apoptosis (12). In addition, miRNAs have been associated with processes involved in the development of oxidative stress (24). However, the roles of miRNAs in the pathophysiology of vascular diseases, as well as their clinical value, have yet to be elucidated. Our previous study reported that miR-4463 has a greater change in the plasma levels of vascular patients (16). Therefore, the present study hypothesized that miR-4463 may have an effect on the pathophysiological processes associated with vascular diseases.

H\textsubscript{2}O\textsubscript{2} is one of the main ROS, which has been reported to decrease cellular proliferation and induce apoptosis, in a process involving several miRNAs (28). Exposure of endothelial cells to H\textsubscript{2}O\textsubscript{2} has been reported to cause cell dysfunction

Figure 5. miR-4463 enhances H\textsubscript{2}O\textsubscript{2}-induced HUVEC apoptosis. HUVECs were pretreated with a miR-4463 mimic, a miR-4463 inhibitor or NC miRNA, and then exposed to 700 µmol/l H\textsubscript{2}O\textsubscript{2} for 16 h. Control cells did not receive treatment. (A) Cellular apoptosis was assessed using flow cytometry following staining with FITC Annexin V and propidium iodide. (B) miR-4463 overexpression enhanced the proapoptotic effects of H\textsubscript{2}O\textsubscript{2}. Data are expressed as the mean ± standard deviation (n=3). *P<0.05 vs. 700+NC group. miR, microRNA; H\textsubscript{2}O\textsubscript{2}, hydrogen peroxide; HUVEC, human umbilical vein endothelial cell; NC, negative control; FITC, fluorescein isothiocyanate.
miR-4463 enhances H2O2-induced oxidative stress in HUVECs. HUVECs were pretreated with miR-4463 mimics, a miR-4463 inhibitor or a NC miRNA, and then exposed to 700 µmol/l H2O2 for 16 h; Control cells did not receive treatment. (A) ROS production and (B) MDA levels were assessed using commercially available kits. (C) A Cell Counting Kit-8 assay was used to evaluate cell viability. Data are expressed as the mean ± standard deviation (n=3). *P<0.05 vs. 700+NC group. H2O2, hydrogen peroxide; HUVEC, human umbilical vein endothelial cell; MDA, malondialdehyde; miR, microRNA; NC, negative control; ROS, reactive oxygen species.

Figure 6. miR-4463 enhances H2O2-induced oxidative stress in HUVECs.
required to confirm the exact roles of miR-4463 in HUVECs under oxidative stress and to determine the possible underlying molecular mechanisms that are involved.

Oxidative stress is a harmful mechanism for self-protection, therefore, excessive activation may enhance this effect. The results of the present study revealed that H₂O₂ treatment altered miR-4463 expression in HUVECs, and miR-4463 overexpression enhanced H₂O₂-induced oxidative stress. Therefore, the molecular mechanisms underlying the involvement of miR-4463 in apoptotic pathways were investigated in HUVECs, by assessing the expression levels of apoptosis-associated marker proteins. Following miR-4463 overexpression in H₂O₂-treated HUVECs, the proapoptotic proteins C-caspase3, PARP1 and Bax were significantly upregulated, whereas the protein expression of the anti-apoptotic effectors Bcl-2 and XIAP was significantly reduced. These findings suggested that miR-4463 may be implicated in the regulation of apoptosis-related protein expression in HUVECs treated with H₂O₂. However, further studies are required to fully elucidate the roles of miR-4463 and its target genes in apoptotic pathways.

In conclusion, the present results suggested that miR-4463 may be implicated in the pathogenesis of cardiovascular disease through the regulation of oxidative processes. Future studies will address the roles of specific target genes during the regulation of HUVEC oxidative stress and apoptosis, and

![Figure 7](image-url)
the effects of miRNAs in the pathophysiological mechanisms involved in oxidative processes will be explored. The results of the present study suggested that miR-4463 may have potential for the development of novel strategies for the diagnosis, prognosis and treatment of cardiovascular diseases.

References

1. Ono K: MicroRNA-133a in the development of arteriosclerosis obliterans. J Atheroscler Thromb 22: 342-343, 2015.
2. Rajput C; Tauseef M, Farazuddin M, Yazbeck P, Amin MR, Avin Br V, Sharma T and Mehta D: MicroRNA-150 suppression of alpha-smooth muscle actin expression decreases cell proliferation in vitro. Med Sci Monit Basic Res 20: 82-92, 2014.
3. Csdoria A, Kreuttmayer S, Ploner C, Braun PR, Karlas A, Backovic A, Wick G and Bernhard D: Cigarette smoke extract induces prolonged endoplasmic reticulum stress and autophagic cell death in human umbilical vein endothelial cells. Cardiovasc Res 92: 141-148, 2011.
4. Jeon BK, Kwon K, Kang JI and Choi YH: Csk-induced phosphorylation of Src at tyrosine 530 is essential for H2O2-mediated suppression of ERK1/2 in human umbilical vein endothelial cells. Sci Rep 5: 12725, 2015.
5. Chen X, Pang S, Liu J, Xia J and Wang Y: Allicin prevents oxidized low-density lipoprotein-induced endothelial cell injury by inhibiting apoptosis and oxidative stress pathway. BMC Complement Altern Med 16: 133, 2016.
6. Zhou DY, Su Y, Guo P, Yang QH, Wang Z and Xu Q: Resveratrol ameliorates high glucose-induced oxidative stress injury in human umbilical vein endothelial cells by activating AMPK. Life Sci 136: 94-99, 2015.
7. Zheng D, Yu Y, Li M, Wang G, Chen R, Fan GC, Martin C, Xiong S and Peng T: Inhibition of microRNA 195 prevents apoptosis and multiple-organ injury in mouse models of sepsis. J Infect Dis 215: 1661-1670, 2016.
8. Hecker M, Thamilarasan M, Koczan D, Schröder I, Flechtnar B, Freiesleben S, Füllen G, Thiesen HJ and Zettl U: MicroRNA-1298 is regulated by DNA methylation and affects vascular smooth muscle cell function of antivirally active tricyclic analog of acyclovir. J Med Chem 62: 137-145, 2015.
9. Liu X, Tong Q, Wang W, Xiong W, Shi C and Fang J: Dihydromycrictin prevents endothelial cells from hydrogen peroxide-induced oxidative stress damage by regulating mitochondrial pathways. Life Sci 130: 38-46, 2015.
10. Wu W, Zhang D, Pan D, Zuo G, Ren X and Chen S: Downregulation of vascular endothelial growth factor receptor-2 under oxidative stress conditions is mediated by b-transduction repeat-containing protein via glycosyn synthesis kinase-3b signaling. J Int Med 37: 911-920, 2016.
37. Yin YL, Li P, Yang J, Liu DW, Sun RL, Pan GP, Wan GM and Wan GR: Protective effect of ultrafiltered XinMaiJia extract against H$_2$O$_2$-induced injury in human umbilical vein endothelial cells through NHE1 downregulation. Genet Mol Res 13: 8436-8449, 2014.

38. Zhang L, Zhou M, Qin G, Weintraub NL and Tang Y: miR-92a regulates viability and angiogenesis of endothelial cells under oxidative stress. Biochem Biophys Res Commun 446: 952-958, 2014.

39. Kojima H, Otani A, Oishi A, Makiyama Y, Nakagawa S and Yoshimura N: Granulocyte colony-stimulating factor attenuates oxidative stress-induced apoptosis in vascular endothelial cells and exhibits functional and morphologic protective effect in oxygen-induced retinopathy. Blood 117: 1091-1100, 2011.

40. Yu DS, Lv G, Mei XF, Cao Y, Wang YF, Wang YS and Bi YL: miR-200c regulates ROS-induced apoptosis in murine BV-2 cells by targeting FAP-1. Spinal cord: Dec 2, 2014 (Epub ahead of print).

41. Kong BS, Cho YH and Lee EJ: G protein-coupled estrogen receptor-1 is involved in the protective effect of protocatechuic aldehyde against endothelial dysfunction. PLoS One 9: e113242, 2014.