Atherosclerosis causes stroke and coronary heart disease and is associated with a high mortality rate worldwide. However, the pathogenesis of atherosclerosis remains unclear. Endothelial cell apoptosis is one of the early changes observed in atherosclerosis. Previous studies have found that microRNA (miR)-616-3p may be involved in the development of atherosclerosis, but the specific mechanism is not clear. The present study aimed to investigate whether miR-616-3p is involved in endothelial cell apoptosis and its underlying mechanism. The present study demonstrated that compared with normal HUVECs, HUVECs treated with oxidized low-density lipoprotein expressed higher miR-616-3p and lower X-linked inhibitor of apoptosis protein (XIAP) levels. In the present study, HUVECs were transfected with miR-616-3p mimic and Cell Counting Kit-8 (CCK-8), flow cytometry and TUNEL staining assays demonstrated that compared with miR-616-3p mimic control, the miR-616-3p mimic promoted HUVEC apoptosis. In addition, using StarBase 3.0 for bioinformatics analysis it was predicted that miR-616-3p may bind to the 3' untranslated region (UTR) of XIAP mRNA. The present study performed the CCK-8, flow cytometry, TUNEL staining and dual-luciferase reporter assays and demonstrated that miR-616-3p binds to the 3'UTR of the XIAP mRNA and inhibits its expression and that this further promotes apoptosis in HUVECs. In addition, western blotting demonstrated that compared with miR-616-3p mimic control, the miR-616-3p mimic increases the level of cleaved caspase-3 in HUVECs. In summary, the present study demonstrated that miR-616-3p can directly inhibit the expression of XIAP mRNA by targeting its 3'UTR which promoted apoptosis in HUVECs. miR-616-3p and XIAP may be used as therapeutic targets of atherosclerosis in the future.

Introduction

Atherosclerosis is a disease in which atherosclerotic plaques are deposited in arterial wall and cause arterial stenosis (1). Atherosclerosis may lead to coronary artery disease, stroke and peripheral artery disease (2). Globally, 7.4 million deaths are caused by coronary heart disease each year and 6.7 million patients die from stroke in 2015 (3). The exact mechanism of atherosclerosis is still not fully understood, but endothelial cell apoptosis is known to be one of the important mechanisms underlying atherosclerosis progression (4).

Endothelial cell injury, which is one of the earliest pathophysiological changes in atherosclerosis promotes the production of inflammatory mediators, such as interleukin-1β (5) and free radicals including reactive oxygen species and reactive nitrogen species (6) to form an inflammatory and an oxidative stress environment (7). Normal endothelium serves a role in regulating vascular tone, cell adhesion, smooth muscle cell proliferation and in maintaining vascular homeostasis (8). A previous have found a significant increase in endothelial cell apoptosis in atherosclerotic blood vessels and plaques (9). Apoptosis of endothelial cells allows leukocytes and low-density lipoprotein (LDL) to pass through the blood vessel wall more easily and continuous accumulation of LDL in the endothelium, which results in plaque formation and subsequently, atherosclerosis (10). In accordance, some factors that cause atherosclerosis, such as high levels of LDL, elevated blood glucose levels, reduced nitric oxide levels and increased oxidative stress levels have been associated with an increase in endothelial cell apoptosis (11). Apoptosis of endothelial cells results in activation of the coagulation system, which is followed by destruction of the vascular endothelium and even, local thrombosis that can eventually lead to vascular occlusion, unstable angina pectoris, heart attack and stroke (12). Based on all these findings, a growing number of researchers have been focusing on how endothelial cell apoptosis can be inhibited.

MicroRNA (miR) is a ribonucleic acid molecule with a length of 21-23 nucleotides that is widely present in eukaryotes and can regulate the expression of other genes (13). Various miRNAs have been found to serve an important role in the pathogenesis of atherosclerosis. For example, miR-34 has a protective effect against oxidative stress in endothelial cells (14) and miR-155 has the ability to destroys tight junctions and the integrity of endothelial barriers, leading
to an increased endothelial permeability and enhanced atherosclerotic progression (15). In addition, miR-345-3p, miRNA-26a-5p, miR-142-3p were found to regulate endothelial cell apoptosis (16-19). Additionally, previous studies found that miR-616-3p may be involved in the development of coronary atherosclerosis. For example, miR-616-3p was found to participate in the development of atherosclerosis by directly acting on the 3’UTR of paraoxonase 1 (PON-1), but the specific mechanism is still unclear (20). Another study demonstrated that miR-616-3p single nucleotide polymorphisms at PON1 could affect genetic expression and that this was associated with an elevated risk for ischemic stroke and subclinical atherosclerosis (21). Hence, the precise role of miR-616-3p in the pathogenesis of coronary atherosclerosis is not clear (21). It may be beneficial to explore the mechanisms of miR-616-3p in endothelial cell injury, as it may prove to be a potential treatment target for coronary atherosclerosis in the future.

Based on all the previously reported findings, the present study hypothesized that miR-616-3p is involved in the pathogenesis of coronary atherosclerosis via its effect on endothelial cell apoptosis. In addition, the present study aimed to explore the potential mechanisms via which miR-616-3p may play a role in endothelial cell apoptosis. The purpose of this study was to elucidate the mechanism of atherosclerosis and to find new therapeutic targets for atherosclerosis. miR-616-3p and XIAP may be used as future therapeutic targets of atherosclerosis.

Materials and methods

Cell culture and treatment. HUVECs (ATCC® PCS-100-013™) were purchased from ATCC and cultured in RPMI-1640 medium (Thermo Fisher Scientific Inc.) containing 10% FBS (Hyclone; GE Healthcare Life Sciences) and 1% penicillin-streptomycin solution. The cells were cultured at 37°C in a humidified incubator containing 5% CO₂ and passaged when they reached 90% confluence. Subsequently, HUVECs were planted in 6-well plates at a density of 1x10⁶ per well and were treated with RPMI-1640 medium (with 10% FBS and 1% penicillin-streptomycin solution) containing 60 µg/ml oxidized low-density lipoprotein (ox-LDL) for 48 h at 37°C in a humidified incubator containing 5% CO₂ (22). Untreated HUVECs served as the control group. RNA was extracted from the treated cells for subsequent reverse transcription-quantitative (RT-q) PCR.

Reverse transcription-quantitative (RT-q) PCR. According to the manufacturer’s protocol, RNAiso (Takara Bio, Inc.) was used to extract total RNA from cells, and NanoDrop2000 was used to measure RNA concentration and purity. PrimeScript™ RT Master Mix (Takara Bio, Inc.) was used for reverse transcription according to the manufacturer’s instructions. The following protocol was used: 37°C for 15 min (reverse transcription reaction) and 85°C for 5 sec (reverse transcriptase inactivation reaction). The cDNA obtained by reverse transcription was amplified on StepOne Plus (Thermo Fisher Scientific Inc.) using the TB Green® Premix Ex Taq™ kit (Takara Bio, Inc.) according to the manufacturer’s protocol. The thermocycling conditions used were as follows: Initial denaturation at 95°C for 30 sec, followed by 40 cycles of denaturation at 95°C for 5 sec and finally annealing and extension at 60°C for 30 sec. The relative expression level was calculated using the 2^ΔΔCq method (23). The following primers were used: GAPDH forward, 5’-GCCAAAGGGCTAT-3’ and reverse, 5’-GAGTCCTTTCAGGATACAA-3’; X-linked inhibitor of apoptosis protein (XIAP) forward, 5’-GTGACTAGATGTCCACAGG-3’ and reverse, 5’-GTGAGGAGGTGTCTGTAAG-3’; U6 forward, 5’-CTCGTCTCGGCAGCACA-3’ and reverse, 5’-AAGCGT TCACGATTGGTCCGT-3’; and miR-616-3p forward, 5’-ACCCACTGCTGGAAGCTATGTTGGTT-3’ and reverse, 5’-TGGTGTCGTTGAGTCGTC-3’. GAPDH was used as the internal control for XIAP and U6 was used as the internal control for miR-616-3p.

Cell counting kit-8. HUVECs were seeded into 96-well plates at a density of 4,000 cells per well and the culture plate was placed in the incubator for 24 h at 37°C. Cell counting Kit-8 (CCK-8; Dojindo Molecular Technologies Inc.) solution (10 µl) was added to each well and the culture plate was placed in the incubator for 1 h at 37°C. Absorbance was measured at 450 nm with a microplate reader (iMark™ Microplate Absorbance Reader; Bio-Rad Laboratories Inc.), and the measured optical density (OD) value was used as an indicator of cell viability.

Transfection. miR-616-3p mimic, miR-616-3p mimic non-targeting control, XIAP overexpression plasmid and non-targeting control (empty vector) were designed and synthesized by Shanghai GenePharma, Co. Ltd. miR-616-3p mimic (100 nM), miR-616-3p mimic non-targeting control (100 nM), XIAP overexpression plasmid (4 µg) and non-targeting control (4 µg) were transfected into HUVECs with Lipofectamine 2000® (Invitrogen; Thermo Fisher Scientific Inc.) at 37°C for 48 h. Untransfected cells were used as negative control (NC). RNA and total protein were extracted for subsequent experiments 48 h after transfection. miR-616-3p mimic, 5’-AGUCAUUGGAGGUUGAGCA G-3’; and miR-616-3p mimic non-targeting control, 5’-ACU ACUGAGUGACAGUAGA-3’.

Flow cytometry analysis. HUVECs (1x10⁶/well) from negative control, miR-616-3p and miR-616-3p mimic control were collected and washed with pre-cooled PBS and centrifuged at 725 x g for 10 min at 4°C. The supernatant was discarded and the cells were resuspended in 200 µl binding buffer. Subsequently, 10 µl of Annexin V-FITC and 10 µl propidium iodide (PI) was added (Dead Cell Apoptosis Kit with Annexin V FITC and PI; Thermo Fisher Scientific Inc.) and mixed for 15 min at room temperature in the dark. Finally, 300 µl of binding buffer was added and flow cytometry analysis was performed within 1 h on the FACSCalibur Flow Cytometry System (BD Biosciences), and the results were analyzed using FlowJo v.8.0 software (Tree Star, Inc.). Both early and late apoptosis were assessed.

TUNEL staining. HUVECs were seeded into 48-well plates at a density of 10⁴ cells per well. First, the cells were washed with saline. Subsequently, the cells were fixed in PBS with
luciferase activity was tensit/-. All results are expressed as mean ± SD. Statistical analysis was performed using SPSS 19.0 (IBM Corp.). Paired Student's t-test was used for comparison between 2 groups. ANOVA followed by a post hoc Tukey's test was used for comparison between multiple groups. P<0.05 was considered to indicate a statistically significant difference.

Results

ox-LDL treatment increases miR-616-3p levels in HUVECs. RT-qPCR analysis of HUVECs treated with ox-LDL revealed that compared with normal HUVECs, ox-LDL treatment resulted in an increase in the expression of miR-616-3p (Fig. 1).

miR-616-3p inhibits viability and promotes apoptosis of HUVECs. Firstly, HUVECs were transfected with miR-616-3p mimic and the corresponding non-targeting control. The results confirmed that compared with miR-616-3p mimic non-targeting control, miR-616-3p mimic increased miR-616-3p expression (Fig. 2A). Subsequently, the viability of HUVECs was assessed using the CCK-8 assay. The results demonstrated that compared with miR-616-3p mimic non-targeting control, miR-616-3p mimic significantly inhibited the viability of HUVECs (Fig. 2B). Flow cytometry analysis and TUNEL staining were used to analyze apoptosis of HUVECs. Flow cytometry analysis demonstrated that compared with miR-616-3p mimic control, miR-616-3p mimic significantly increased cell apoptosis (Fig. 2C). The results of the TUNEL staining experiment were consistent with the results of flow cytometry analysis (Fig. 2D).

miR-616-3p directly inhibits XIAP expression. Next, the present study explored the mechanism via which miR-616-3p inhibits viability and promotes apoptosis in HUVECs. Based on the StarBase 3.0 prediction, miR-616-3p was found to have a potential binding site for the 3'UTR of XIAP mRNA (Fig. 3A). Subsequently, whether ox-LDL treatment would cause changes in XIAP expression was assessed. Using RT-qPCR, it was demonstrated that contrary to its effect on miR-616-3p, ox-LDL treatment inhibited the expression of XIAP (Fig. 3B). Next, the effect of miR-616-3p on Western blotting. HUVECs (1x10⁴/well) were washed once with ice cold PBS and lysed with RIPA lysis buffer (cat. no. P0013B; Beyotime Institute of Biotechnology) on ice for 30 min. The homogenate was collected and centrifuged at 14,000 x g for 10 min at 4˚C. The supernatant was the total protein and was used to determine the protein concentration with the bicinchoninic acid (BCA) method. Next, 40 μg of the extracted protein was added to each well for 10% SDS-PAGE electrophoresis and the protein was transferred to a PVDF membrane. The PVDF membrane was blocked with 5% skimmed milk containing TBS-0.1% Tween-20 (TBS-T) at room temperature for 1 h and incubated with diluted primary antibodies (all Abcam) against β-actin (1:1,000; cat. no. ab8227), XIAP (1:1,000; cat. no. ab229050), cleaved caspase-3 (1:1,000; cat. no. ab32042) and total caspase-3 (1:1,000; cat. no. ab32150) at 4˚C overnight. The PVDF membrane was then washed with TBST and incubated with the corresponding diluted secondary antibodies (1:5,000; cat. no. ab205718; Abcam) at room temperature, the cells were washed twice with PBS. Then, the slides were incubated with TUNEL reaction mixture (One Step TUNEL Apoptosis Assay kit; cat. no. C1090; Beyotime Institute of Biotechnology) for 60 min at 37˚C. The cells were then immediately observed under a fluorescence microscope to observe the red fluorescence (magnification, x400).

Dual-luciferase reporter assay. Based on the StarBase 3.0 prediction, miR-616-3p was found to have a potential binding site for the 3'UTR of XIAP mRNA. Luciferase reporter plasmids containing the wild-type (wt) or mutant (mut) 3' untranslated region (UTR) sequence of XIAP. pmirGLO-XIAP-wt and pmirGLO-XIAP-mut plasmids were constructed by Shanghai Gene Pharma Co. Ltd. HUVECs were seeded in 24-well plates at a density of 2x10⁴ cells/well. miR-616-3p mimic (5'-AGU CAU UGG AGG GUUUGACGAC-3') and miR-616-3p non-targeting control (5'-ACUACUGAGUGACAGUGA-3') were synthesized by Shanghai Gene Pharma Co. Ltd. According to the manufacturer's instructions, Lipofectamine 2000® (Invitrogen; Thermo Fisher Scientific Inc.) was used to transfect miR-616-3p mimic, miR-616-3p non-targeting control, pmirGLO-XIAP-wt and pmirGLO-XIAP-mut into cells. After 48 h of transfection, the medium was removed and fluorescence intensity was detected using the Dual Luciferase Reporter Gene Assay kit (Beyotime Institute of Biotechnology). Renilla luciferase activity was used as the normalization control.

Statistical analysis. All experiments were repeated 3 times. All results are expressed as mean ± SD. Statistical analysis
XIAP in HUVECs was assessed using western blotting. The results demonstrated that compared with miR-616-3p mimic non-targeting control, miR-616-3p mimic inhibited XIAP protein expression (Fig. 3C). Finally, the direct interaction between miR-616-3p and XIAP was demonstrated through dual-luciferase experiments. The results revealed that compared with co-transfection of miR-616-3p mimic and pmirGLO-XIAP-mut, the fluorescence intensity of cells treated with pmirGLO-XIAP-wt and the miR-616-3p mimic decreased significantly (Fig. 3D). This indicated that miR-616-3p directly acts on XIAP mRNA to inhibit XIAP gene expression (Fig. 3D).

Inhibition of miR-616-3p expression inhibits the expression of cleaved caspase-3 protein in HUVECs. Cleaved caspase-3 is an apoptosis-related protein (24). Since miR-616-3p was found to promote apoptosis in HUVECs, western blotting analysis was used to determine whether miR-616-3p affects the expression of cleaved caspase-3 protein. Compared with non-targeting control, XIAP
overexpression plasmid significantly increase the expression of XIAP (Fig. 4A). Co-transfection of miR-616-3p mimic and XIAP overexpression plasmid inhibited cleaved caspase-3 protein expression in HUVECs (Fig. 4B). This indicated that the miR-616-3p mimic promoted apoptosis of HUVECs by inhibiting XIAP.

Discussion
The present study investigated the role of miR-616-3p in endothelial cell apoptosis and the potential mechanisms that may be involved in the context of atherosclerosis. The results of flow cytometry and TUNEL staining in the present study...
demonstrated that miR-616-3p significantly promoted HUVEC apoptosis. This finding of the present study confirmed the role of miR-616-3p in atherosclerosis via promotion of apoptosis in endothelial cells. Using StarBase3.0, the present study predicted that miR-616-3p may bind to the XIAP mRNA to induce its effects on HUVECs. Shin et al (25) demonstrated that miR-513a-5p mediates tumor necrosis-α and lipopolysaccharide induced apoptosis via downregulation of XIAP in HUVECs. Another study by Li et al (26) demonstrated that miR-122 promotes endothelial cell apoptosis by targeting XIAP.

Hence, the present study examined whether miR-616-3p promoted apoptosis of endothelial cells by directly acting on the 3'UTR of XIAP and inhibiting the expression of XIAP. In the present study, ox-LDL treatment resulted in an increase in miR-616-3p expression and decrease in XIAP expression in HUVECs. In addition, dual-luciferase experiments performed in the present study demonstrated that miR-616-3p mimic can directly target the XIAP 3'UTR. Flow cytometry and TUNEL staining experiments performed in the present study also confirmed that miR-616-3p mimic can promote apoptosis.
of HUVECs and this effect can be partially reversed by the XIAP overexpression plasmid.

Caspase-3 is the most important terminal cleavage enzyme in the process of apoptosis and cleaved-caspase-3 is the activated form of caspase-3 (27). Through western blotting changes in the expression of the apoptosis-related protein cleaved caspase-3 were found in the present study. In the present study, compared with miR-616-3p non-targeting control, miR-616-3p mimic increased the expression of cleaved caspase-3 protein and this effect was partially reversed by the XIAP overexpression plasmid.

The present study had several limitations. Firstly, the specificity of miR-166-3p and the causal relationship between miR-166-3p and endothelial cell apoptosis need to be further verified. Secondly, only in vitro cell experiments were conducted in the present study and future in vivo experiments are needed to verify the findings of the present study.

In summary, the present study found that miR-616-3p can directly act on the 3'UTR of XIAP to promote apoptosis of HUVECs. The present study provides a new basis for the pathogenesis of atherosclerosis and indicates that miR-616-3p may have potential as a treatment target in the future.

Acknowledgements

Not applicable.

Funding

This study was supported by a grant from the Natural Science Foundation of Inner Mongolia (grant no. 2018MS08069). The funding body did not play a role in the design of the study; collection, analysis, and interpretation of data and manuscript writing.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

XZ designed the experiments. HC, XL, YW, XWu, XWen and YL performed the experiments. HC collected and analyzed the data. All authors confirmed the authenticity of the raw data. HC and XZ wrote the manuscript. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Boucher P, Matz RL and Terrand J: atherosclerosis: Gone with the Wmt? Atherosclerosis 301: 15-22, 2020.
2. Shao C, Wang J, Tian J and Tang YD: Coronary artery disease: From mechanism to clinical practice. Adv Exp Med Biol 1177: 1-36, 2020.
3. Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, Tokgözoglu L and Lewis EF: Atherosclerosis. Nat Rev Dis Primers 5: 56, 2019.
4. Grechowa I, Horke S, Walrath A, Vahl CF and Dorweiler B: Human neutrophil elastase induces endothelial cell apoptosis by activating the PERK-CHOP branch of the unfolded protein response. FASEB J 31: 3868-3881, 2017.
5. Gomez D, Baylis RA, Durgin BG, Newman AAC, Alencar GF, Mahan S, St HC, Müller W, Waisman A, Francis SE, et al: Interleukin-1β has atheroprotective effects in advanced atherosclerotic lesions of mice. Nat Med 24: 1418-1429, 2018.
6. Singh R, Devi S and Gollen R: Role of free radical in atherosclerosis, diabetes and dyslipidemia: Larger-than-life. Diabetes Metab Res Rev 31: 113-126, 2015.
7. Gimbrone MA Jr and García-Cardeña G: Endothelial cell dysfunction and the pathobiology of atherosclerosis. Circ Res 118: 620-636, 2016.
8. Rajendran P, Renganaraj T, Thangavel J, Nishigaki Y, Sakhthisekaran D, Sethi G and Nishigaki I: The vascular endothelium and human diseases. Int J Biol Sci 9: 1057-1069, 2013.
9. Stoeneman VE and Bennett MR: Role of apoptosis in atherosclerosis and its therapeutical implications. Clin Sci (Lond) 107: 343-354, 2004.
10. Werner N, Wassmann S, Ahlers P, Kossiols S and Nickeng E: Circulating CD31+/annexin V+ apoptotic microparticles correlate with coronary endothelial function in patients with coronary artery disease. Arterioscler Thromb Vasc Biol 26: 112-116, 2006.
11. Libby P, Okamoto Y, Rocha VZ and Folco E: Inflammation in atherosclerosis: Transition from theory to practice. Circ J 74: 213-220, 2010.
12. Paone S, Baxter AA, Hulett MD and Poon IKH: Endothelial cell apoptosis and the role of endothelial cell-derived extracellular vesicles in the progression of atherosclerosis. Cell Mol Life Sci 76: 1093-1106, 2019.
13. Krol J, Loedige I and Filipowicz W: The widespread regulation of microRNA biogenesis, function and decay. Nat Rev Genet 11: 597-610, 2010.
14. Zhong X, Li P, Li J, He R, Cheng G and Li YL: Downregulation of microRNA-34a inhibits oxidized low-density lipoprotein-induced apoptosis and oxidative stress in human umbilical vein endothelial cells. Int J Mol Med 42: 1134-1144, 2018.
15. Zheng B, Yin WN, Suzuki T, Zhang XH, Zhang Y, Song LL, Jin LS, Zhan X, Zhang H, Li JS and Wen JK: Exosome-mediated miR-155 transfer from smooth muscle cells to endothelial cells induces endothelial injury and promotes atherosclerosis. Mol Ther 25: 1279-1294, 2017.
16. Wei Q, Tu Y, Zuo L, Zhao J, Chang Z, Zou Y and Qiu J: miR-345-3p attenuates apoptosis and inflammation caused by oxidized low-density lipoprotein by targeting TRAF6 via TAK1/p38/NF-kB signaling in endothelial cells. Life Sci 241: 117142, 2020.
17. Jing R, Zhong QQ, Long TY, Pan W and Qian ZX: Downregulated miRNA-26a-5p induces the apoptosis of endothelial cells in coronary heart disease by inhibiting PI3K/AKT pathway. Eur Rev Med Pharmacol Sci 23: 4940-4947, 2019.
18. Zhong X, Zhang L, Li Y, Li P, Li J and Cheng G: Kaempferol alleviates ox-LDL-induced apoptosis by up-regulation of miR-26a-5p via inhibiting TLR4/NF-kB pathway in human endothelial cells. Biomed Pharmacother 108: 1783-1789, 2018.
19. Qin B, Shu Y, Long L, Li H, Men X, Feng L, Yang H and Lu Z: MicroRNA-142-3p induces atherosclerosis-associated endothelial cell apoptosis by directly targeting rictor. Cell Physiol Biochem 47: 1589-1605, 2018.
20. Wang Z, Chen S, Zhu M, Zhang W, Zhang H, Li H and Zou C: Functional SNP in the 3’UTR of PON1 is associated with the risk of calcific aortic valve stenosis via MiR-616. Cell Physiol Biochem 45: 1390‑1398, 2018.
21. Liu ME, Liao YC, Lin RT, Wang YS, His E, Lin HF, Chen KC and Juo SH: A functional polymorphism of PON1 interferes with microRNA binding to increase the risk of ischemic stroke and carotid atherosclerosis. Atherosclerosis 228: 161-167, 2013.
22. Feng Y, Cai ZR, Tang Y, Hu G, Lu J, He D and Wang S: TLR4/NF-κB signaling pathway-mediated and oxLDL-induced up-regulation of LOX-1, MCP-1, and VCAM-1 expressions in human umbilical vein endothelial cells. Genet Mol Res 13: 680-695, 2014.

23. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.

24. Zhuo EQ, Cai CQ, Liu WZ, Li KS and Zhao WZ: Downregulated microRNA-140-5p expression regulates apoptosis, migration and invasion of lung cancer cells by targeting zinc finger protein 800. Oncol Lett 20: 390, 2020.

25. Shin S, Moon KC, Park KU and Ha E: MicroRNA-513a-5p mediates TNF-α and LPS induced apoptosis via downregulation of X-linked inhibitor of apoptotic protein in endothelial cells. Biochimie 94: 1431-1436, 2012.

26. Li Y, Yang N, Dong B, Yang J, Kou L and Qin Q: MicroRNA-122 promotes endothelial cell apoptosis by targeting XIAP: Therapeutic implication for atherosclerosis. Life Sci 232: 116590, 2019.

27. Jiang M, Qi L, Li L and Li Y: The caspase-3/GSDME signal pathway as a switch between apoptosis and pyroptosis in cancer. Cell Death Discov 6: 112, 2020.