Tohoku J. Exp. Med., 2025, **265**, 271-278

MiR-24-3p Inhibits Migration and Proliferation of HUVECs by Downregulating CHI3L1

Guanjun Wu,[1] Lei Gao,[2] Xin Zhang,[1] Qi Xue,[1] Lifang Ye,[1] Yaru Zheng[1] and Jianlei Zheng[1,2,3]

[1]Department of Cardiovascular Medicine, Heart Center, Zhejiang Provincial People's Hospital/People's Hospital of Hangzhou Medical College, Hangzhou, China
[2]Graduate school, Bengbu Medical University, Bengbu, Anhui, China
[3]Department of Cardiology, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, China

Angiogenesis is regarded as a critical factor in the pathogenesis of unstable atherosclerotic plaques, and numerous proteins and microRNAs (miRNAs) were involved in this process. In our previous study, the overexpression of Chitinase 3-like 1 (CHI3L1) aggravates the neo-vessels in carotid plaques of apoE$^{-/-}$ mice fed with a high-fat diet. MiR-24-3p is one of target miRNAs adjusting the expression of CHI3L1. Extracellular signal-regulated kinase (ERK) signaling pathway is an important regulator related to cell proliferation and pathophysiological process of CHI3L1. This study aims to investigate whether the miR-24-3p plays a role in migration and proliferation of human umbilical vein endothelial cells (HUVECs) through influencing the expression of CHI3L1 and potential molecular mechanism. CCK8 assay, transwell and matrigel tests were used to determine the effects of miR-24-3p on proliferation, migration and tube formation of HUVECs by targeting CHI3L1. Luciferase assay was carried out to value the direct interaction between miR-24-3p and CHI3L1 3′-untranslated region (3′-UTR). Western blot was used to measure protein expression of CHI3L1, ERK and phosphorylation of ERK (p-ERK). This study demonstrated that miR-24-3p mimic inhibits the proliferation, migration and angiogenesis of HUVECs. The role of miR-24-3p affects the function of HUVECs through negative regulation of CHI3L1 expression targeting CHI3L1 3′-UTR. Furthermore, we found that p-ERK was accordant with CHI3L1 expression in HUVECs, and miR-24-3p mimics significantly diminished the CHI3L1 expression and the level of p-ERK. MiR-24-3p is one of miRNAs regulating the expression and function of CHI3L1, which may provide an efficient strategy for treatment of angiogenesis in atherosclerotic plaques.

Keywords: angiogenesis; chitinase 3-like 1; human umbilical vein endothelial cells; miR-24-3p; signaling pathway
Tohoku J. Exp. Med., 2025 April, **265** (4), 271-278.
doi: 10.1620/tjem.2024.J128

Introduction

Cardiovascular diseases are the leading cause of mortality all over the world. Atherosclerosis, especially the unstable plaque, is the main pathological prerequisite for cardiovascular death (Andrews et al. 2018). Inflammation, large lipid core and angiogenesis in atherosclerotic plaque are regarded as pivotal factors for unstable plaque (Virmani et al. 2005; Zhu et al. 2018; John Chapman and Preston Mason 2022). However, atherosclerosis is a complex and multifactorial pathology occurred in arterial walls and has not been completely elucidated. Recently, various studies showed that microRNAs (miRNAs) also play an important role in the process of atherosclerosis and cardiovascular events (Fasolo et al. 2019; Ren et al. 2024).

MiRNAs are single-stranded RNAs with about 20 nucleotides. As a subgroup of non-coding miRNAs, they play a key role in regulating the protein expression of post-transcription via targeting messenger RNA (mRNA) 3′-untranslated region (3′-UTR), causing the proteins degradation or suppressing mRNA translation (Krol et al. 2010; Corà et al. 2017). miRNAs, such as miR-24-3p, were proven to be involved in multiple physical and/or pathological process of different diseases, including the role of regulating proliferation, migration and angiogenesis (Yu et al. 2017; Yan et al. 2018; Mukherjee et al. 2022; Gerami et al.

Received February 6, 2024; revised and accepted October 28, 2024; J-STAGE Advance online publication November 7, 2024
Correspondence: Jianlei Zheng, Department of Cardiology, The Seventh Affiliated Hospital, Sun Yat-sen University, Shenzhen, China.
e-mail: zhengjianlei@sysush.com

2023). Previous studies have shown that miR-24-3p is implicated in cardiovascular diseases (Tan et al. 2018; Zhang et al. 2022), and it is reported that miR-24-3p targets the expression of mRNA of Chitinase 3-like 1 (CHI3L1) (Jin et al. 2015; Jingjing et al. 2017). CHI3L1 is an important cytokine promoting migration and angiogenesis of endothelial cells, and our previous studies has proved that increased CHI3L1 level significantly promotes tube formation of human umbilical vein endothelial cells (HUVECs) and neovascularization in carotid plaques of apoE$^{-/-}$ mice fed with western diets (Sun et al. 2021; Xue et al. 2021). In addition, extracellular signal-regulated kinase (ERK) signaling pathway plays an important role in regulating cell proliferation and migration, and it is demonstrated ERK pathway involves in pathophysiological process regulated by CHI3L1 (Xu et al. 2024). Hence, we are originally to explore whether miR-24-3p influences the migration and tubular formation of endothelial cells by regulating the expression of CHI3L1 and related signal pathway involved with miR-24-3p-CHI3L1 axis.

Materials and Methods

Cell culture and transfection

HUVECs were obtained from Lonza (Basel, Switzerland). HUVECs were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco, USA) with 10% fetal bovine serum (FBS) (Gibco, USA) and were incubated at 37°C in a humidified atmosphere containing 5% CO_2. In addition, 0.1 mg/mL streptomycin and 100 U/ mL penicillin (Gibco, USA) were also added to the culture media. MiR-24-3p mimic (5′-UGGCUCAGUUCAGCAGGAACAG-3′), miR-24-3p inhibitor (5′-CUGUUCCUGCUGAACUGAGCCA-3′) and negative control (5′-UUGUACUACACAAAA GUACUGUU-3′) were purchased from Guangzhou RiBoBio Co., Ltd. To conduct cell transfection, HVUECs were firstly cultured to about 30-50% confluence. The mimic, inhibitor and negative control of miR-24-3p were used and transfected into HVUECs with riboFECT™ CP transfection kit according to the manufacturer's manual. The group with phosphate buffer saline (PBS) solution was set as blank control. Cells were incubated in the conditions above for 48 h and collected for further experiments.

Total RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

According to the manufacturer's instructions, total RNAs from cells were extracted using TRIzol Reagent (Invitrogen, USA). The total RNA was reverse-transcribed to cDNA for CHI3L1 using a high capacity cDNA Reverse Transcription Kit from Genecopoeia (Genecopoeia, USA) with 42°C for 30 min followed by 85°C for 10 min. To analyze the expression of miRNA, cDNAs were synthesized by All-in-One miRNA First strand cDNA Synthesis kit of miRcute miRNA (KR211-1) (TianGen, China) using the temperature protocol consisting of 42°C for 60 min followed by 95°C for 3 min. qRT-PCR was then performed using SYBR® Green qPCR Master Mix (Lifeint, China.) with GAPDH (for mRNA) and U6 (for miRNA) as an endogenous control. The thermocycling conditions of the qRT-PCR reaction were: Initial denaturation at 95°C for 3 min, followed by 40 cycles of 95°C for 12 sec and 62°C for 40 sec. Relative expression levels of mRNAs and miRNAs were calculated using the $2^{-\Delta\Delta Ct}$ method (Huang et al. 2022). The primers sequences were listed as follows: CHI3L1 forward, 5′-GGACGGAGAGACAAACAGCA-3′ and reverse, 5′-CCATCACCAGCTTACTGGCA-3′; GAPDH forward, 5′-TGTGGGCATCAATGGATTTGG-3′ and reverse, 5′-ACACCATGTATTCCGGGTCAAT-3′; MiR-24-3p forward, 5′-GTCGTATCCAGTGCAGGGTCC-3′ and reverse, 5′-AATCGGCGTGGCTCAGTTCAG-3′; U6 forward, 5′-AAAGCAAATCATCGGACGACC-3′ and reverse, 5′-GTACAACACATTGTTTCCTCGGA-3′.

Cell proliferation assay

Proliferation of HUVECs transfected respectively with miR-24-3p mimic, inhibitor, negative control and blank control was measured at 0 h, 24 h, 48 h and 72 h using a Cell Counting kit 8 (CCK-8, Beyotime Biotechnology, Shanghai, China). Briefly, HUVECs respectively transfected with miR-24-3p mimic, inhibitor, negative control and blank control were seeded in 96-well plate (8,000 cells per well) with 100 μL complete medium and cultured for 0 h, 24 h, 48 h and 72 h. At last, 10 μl solutions of CCK-8 reagent were added to each well at corresponding terminal time of transfection. The cells were cultured with the CCK-8 solution for 2 h at 37°C. Absorbance value was measured using a microplate reader（DR-3518G, Wuxi, China) at 450 nm. Each group was measured in triplicate.

Migration assay

The migration of HUVECs was evaluated with transwell system (Corning Costar, USA). Of note, cell suspension (1.0×10^5 cells/ml) successfully transfected with miR-24-3p mimic, inhibitor, negative control and blank control was prepared in DMEM (Gibco, USA) without serum. For transwell migration assay, about 200 μl cell suspension was seeded into the upper chamber and 600 μl DMEM supplemented with 10% FBS (Gibco, USA) was added into the lower chamber. After culturing for 24 h at 37°C, the unattached cells were removed from the upper surface by gentle scrubbing. Migratory cells in lower chamber were fixed in 90% ethyl alcohol for 15 min at room temperature, then stained with 0.1% crystal violet (Beyotime Biotechnology, China) for 20 min at room temperature, observed and counted using a light microscope (Olympus Corporation, Japan) (magnification 100 ×). Each treatment was repeated three times.

Angiogenesis analysis

The tube formation assay was performed to evaluate the effect of miR-24-3p on the angiogenesis ability of HUVECs. In brief, the cells transfected with miR-24-3p

mimic, inhibitor, negative control and blank control were seeded into a 96-well plate at a density of 5×10^3 cells/well overlaid with matrigel substrate (BD Biosciences, USA), and were incubated at 37°C for 6 h. Cells were first stained with Calcein-AM (Beijing Solarbio Science Technology Co., Ltd, China), then the total tube length, the total number of nodes and total branch length representing the capacity of angiogenesis were determined using Image J software (software 1.53e, USA) with automated angiogenesis plug-in package. Representative images were captured using Leica Application Suite Program (Leica Microsystems Ltd., Heerbrugg, Switzerland) (magnification 100 ×).

Dual-luciferase reporter assay

HUVECs were seeded into 96-well plates and the confluence of HUVECs reached to 60-70% after 24 h incubation. pmirGLO/CHI3L1-3′-UTR wide type (wt) and pmirGLO/CHI3L1-3′-UTR mutant type (mt) reporter plasmids were constructed by Shanghai LMAI Bio Company. According to the manufacturer's instruction, HUVECs were transiently co-transfected with miR-24-3p mimic or negative control together with 0.1 μg pmirGLO/CHI3L1-3′-UTR wt or pmirGLO/CHI3L1-3′-UTR mt reporter plasmids using Lipofectamine 2000 transfection reagent (Invitrogen, USA). After 48 h, Pierce™ Cypridina-Firefly Luciferase Dual Assay Kit (Thermo-Fisher Scientific, USA) was used to perform the luciferase reporter assays according to the manufacturer's protocol.

Western blot

HVUECs (1×10^6 cells/each well in 6-well plates) were transfected with blank control, negative control, miR-24-3p mimic and miR-24-3p inhibitor for 48h. Cell lysates were collected using 1 mL radio immunoprecipitation assay lysis buffer (RIPA) (Beyotime Biotechnology, China) with 10 μL Phenylmethanesulfonyl fluoride (PMSF) (Beyotime Biotechnology, China) at 4°C for 30 min and subjected to 12,000 rpm centrifugation at 4°C for 5 min. Protein concentration of cell lysates was measured with a BCA Protein Assay Kit (Beyotime Biotechnology, China). Western blot was carried out by separating 25 μg of protein by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Beyotime Biotechnology, China), transferred to polyvinylidene fluoride (PVDF) membranes (Beyotime Biotechnology, China), blocked with 5% skimmed milk for 1 h at room temperature, washed with tris-buffered saline Tween-20 (TBST) (Beyotime Biotechnology, China) buffer and then incubated with primary antibody at 4°C overnight. The antibodies were used in this study including anti CHI3L1 (1:1,000, Abcam, UK), anti p-ERK, anti-ERK (1:1,000, Abcam, UK; 1:10,000, Abcam, UK, respectively), and anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH) (1:5,000, Affinity, China). Next, membranes treated with primary antibody were washed 3 times with TBST and incubated with an anti-rabbit horseradish peroxidase (HRP)-linked antibody (Affinity, China) at a 1:5,000 dilutions for 1 h at room temperature. Finally, the content of proteins was measured by an enhanced chemiluminescence system (Applygen, China).

Statistical analysis

Data are presented as means ± standard deviation (SD). Analyses were done using the statistical SPSS 23.0 software (SPSS, Inc., USA). A two-tailed Student's *t*-test was used to determine statistical significance between groups. A value of $p < 0.05$ indicated a significant difference.

Results

MiR-24-3p inhibits HUVECs proliferation in vitro

To investigate the biological function of miR-24-3p in HUVECs, CCK8 assay was performed to show the proliferation effect of miR-24-3p on HUVECs. The results indicated that miR-24-3p inhibitor significantly promotes the cell viability (0.80 ± 0.07 vs. 0.56 ± 0.07, $p < 0.01$) compared with negative control after 48 h transfection. On the other hand, the viability of HUVECs in miR-24-3p mimic group was remarkably decreased in contrast to groups of miR-24-3p inhibitor and negative control (0.33 ± 0.06 vs. 0.80 ± 0.07 and 0.56 ± 0.07, both $p < 0.01$) (Fig. 1).

MiR-24-3p inhibits migration of HUVECs

We studied cell migration abilities in HUVECs which were transfected with mimic or inhibitor of miR-24-3p. The results proved that cell counts in one field were significantly increased in HUVECs transfected with inhibitor of miR-24-3p in contrast with the HUVECs transfected with negative control ($1{,}101 \pm 89$ vs. 528 ± 68, $p < 0.01$). But the migration ability of HUVECs transfected with mimic of miR-24-3p was remarkably inhibited (146 ± 31 vs. 528 ± 68, $p < 0.01$) compared with negative control (Fig. 2.)

Inhibition of miR-24-3p promotes tube formation of HUVECs

We further investigated the antiangiogenic response of miR-24-3p on cells by examining the formation of tube-like capillary structures of HUVECs in matrix-gel. As expected, inhibitor of miR-24-3p increased the tube formation including the total tube length ($10{,}536 \pm 419$ pix vs. $8{,}494 \pm 1{,}041$ pix, $p < 0.05$), total length of the branches ($3{,}829 \pm 272$ pix vs. $2{,}812 \pm 284$ pix, $p < 0.05$), and total branching points (72 ± 7 vs. 46 ± 4, $p < 0.01$) of HUVECs in comparison with negative control. On the contrary, mimic of miR-24-3p significantly decreased the tube formation of HUVECs. It is shown that the differences about total tube length ($5{,}812 \pm 996$ pix vs. $8{,}494 \pm 1{,}041$ pix, $p < 0.05$), total length of the branches ($1{,}899 \pm 376$ pix vs. $2{,}812 \pm 284$ pix, $p < 0.05$) and total branching points (32 ± 6 vs. 46 ± 4, $p < 0.05$) between group of miR-24-3p mimic and negative control are statistically significant (Fig. 3).

MiR-24-3p inhibits the RNA expression of CHI3L1

In contrast to negative control, the level of miR-24-3p

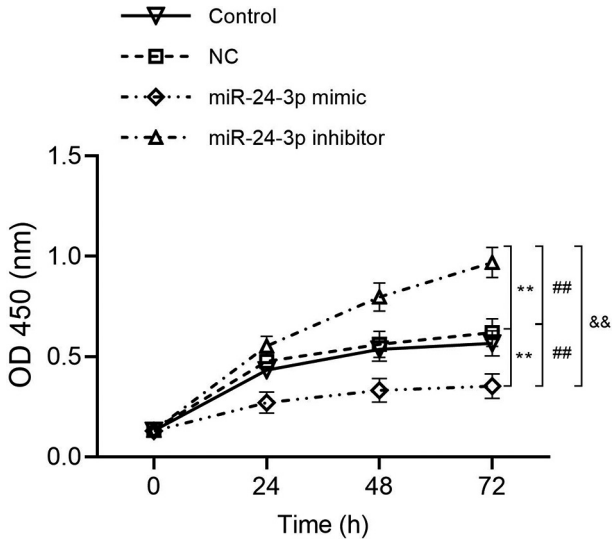

Fig. 1. The effect of miR-24-3p on the proliferation of HUVECs.
MiR-24-3p inhibits the proliferation of HUVECs at different time interval evaluated with CCK-8 assay. Data were presented as mean ± S.D. (n = 3 per group). **p < 0.01 compared with control; ##p < 0.01 compared with NC group (negative control of miR-24-3p); &&p < 0.01 compared with miR-24-3p mimic group.

in HUVECs was decreased significantly (0.47 ± 0.08 vs. 0.95 ± 0.15, p < 0.01) and increased notably (2.76 ± 0.25 vs. 0.95 ± 0.15, p < 0.01) when transfecting with miR-24-3p inhibitor and miR-24-3p mimic, respectively (Fig. 4A). Furthermore, compared with negative control, CHI3L1 mRNA level in HUVECs was also declined notably after transfection with miR-24-3p mimic (0.64 ± 0.08 vs. 1.08 ± 0.09, p < 0.01), while the expression content of CHI3L1 mRNA was promoted remarkably in HUVECs transfected with miR-24-3p inhibitor (1.55 ± 0.22 vs. 1.08 ± 0.09, p < 0.05) (Fig. 4B).

MiR-24-3p targets to 3′-UTR of CHI3L1 and regulates ERK signaling pathway in HUVECs

It has been reported that miR-24-3p may influence the expression of CHI3L1 (Jingjing et al. 2017), and the putative mechanisms of miR-24-3p regulating HUVECs was performed with dual-luciferase reporter assays. As a result, the relative luciferase activity in miR-24-3p mimic + CHI3L1 3′-UTR (wt) is remarkably suppressed compared with that in negative control miRNA + CHI3L1 3′-UTR (wt) (0.40 ± 0.04 vs. 1.00 ± 0.09, p < 0.01) (Fig. 5A). However, the mutation of the miR-24-3p binding site in CHI3L1 3′-UTR completely abolished the difference (Fig. 5A). To further understand whether miR-24-3p regulating the proliferation of HUVECs was involved in ERK signaling pathway, we performed western blot experiment to testify above hypothesis (Fig. 5B). The result showed that CHI3L1 (0.49 ± 0.07 vs. 0.94 ± 0.12, p < 0.01) levels and ratio of phosphorylation of ERK (p-ERK) to ERK (p-ERK/ERK) (0.63 ± 0.09 vs. 1.06 ± 0.11, p < 0.01) in miR-24-3p mimic group were decreased significantly compared with negative control group (Fig. 5C,D). It indicated that miR-24-3p mimic inhibited activation of ERK signaling pathway and suppressed cell proliferation and migration through downregulating the expression of CHI3L1.

Discussion

Cardiovascular and cerebrovascular diseases are the

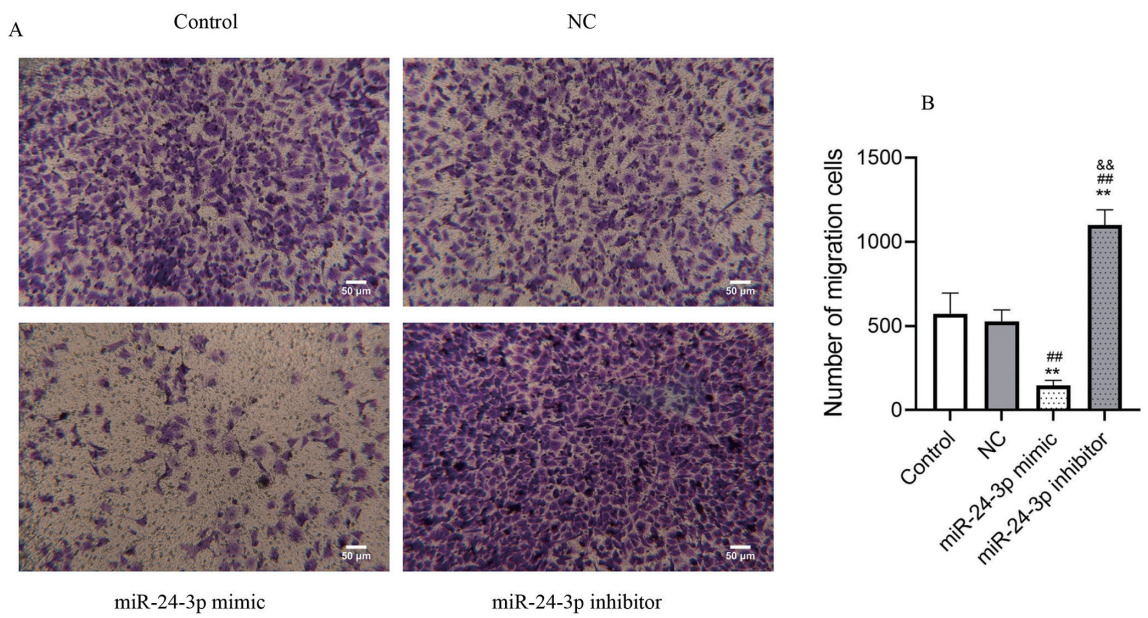

Fig. 2. MiR-24-3p mimic inhibits the migration of HUVECs (magnification × 100).
A. The effects of miR-24-3p mimic and inhibitor on HUVECs migration. B. Cell migration was calculated by mean cell counts in one field. Data were presented as mean ± S.D. (n = 3 per group). **p < 0.01 compared with control; ##p < 0.01 compared with NC group (negative control of miR-24-3p); &&p < 0.01 compared with miR-24-3p mimic group.

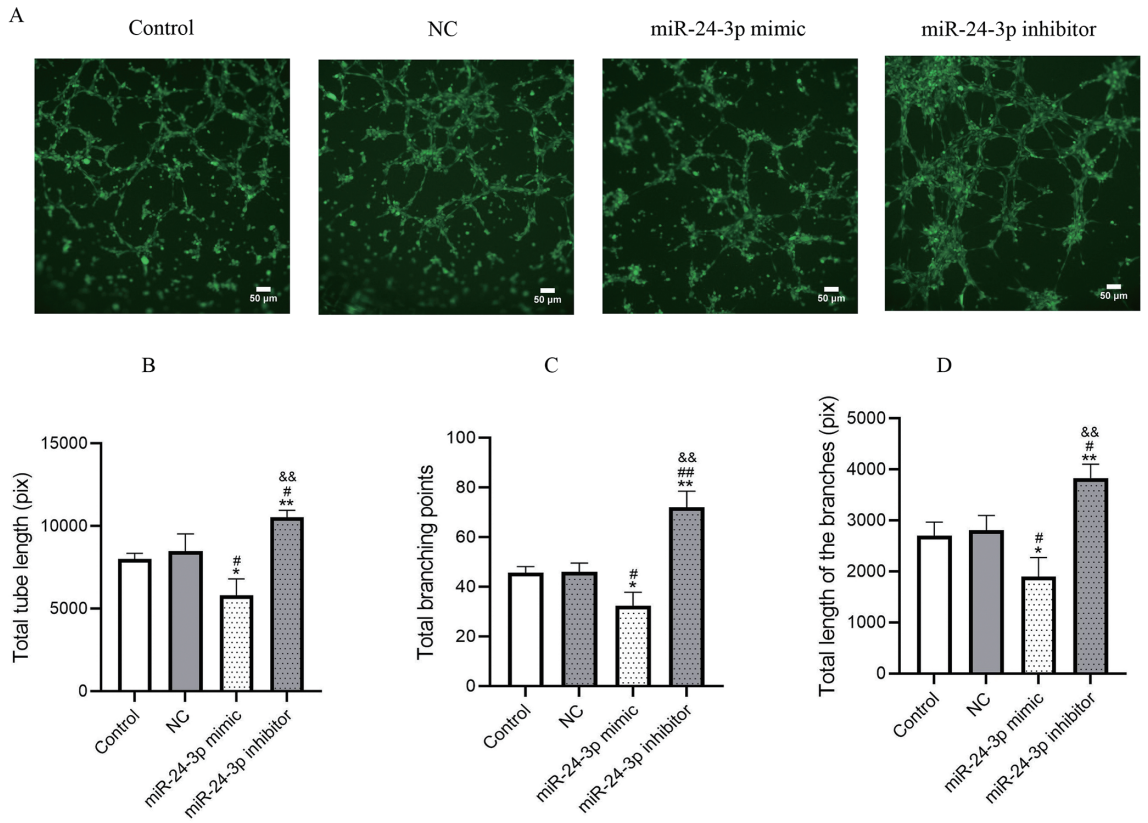

Fig. 3. MiR-24-3p inhibitor prompts angiogenesis of HUVECs *in vitro* (magnification × 100).
A. The tube formation of HUVECs including total tube length, total branch points and total length of the branches. B. The number of total tube length was calculated. C. The number of total branch points was measured. D. The number of total length of the branches was evaluated. Data were presented as mean ± S.D. (n = 3 per group). *p < 0.05, **p < 0.01 compared with control; #p < 0.05, ##p < 0.01 compared with NC group (negative control of miR-24-3p); &&p < 0.01 compared with miR-24-3p mimic group.

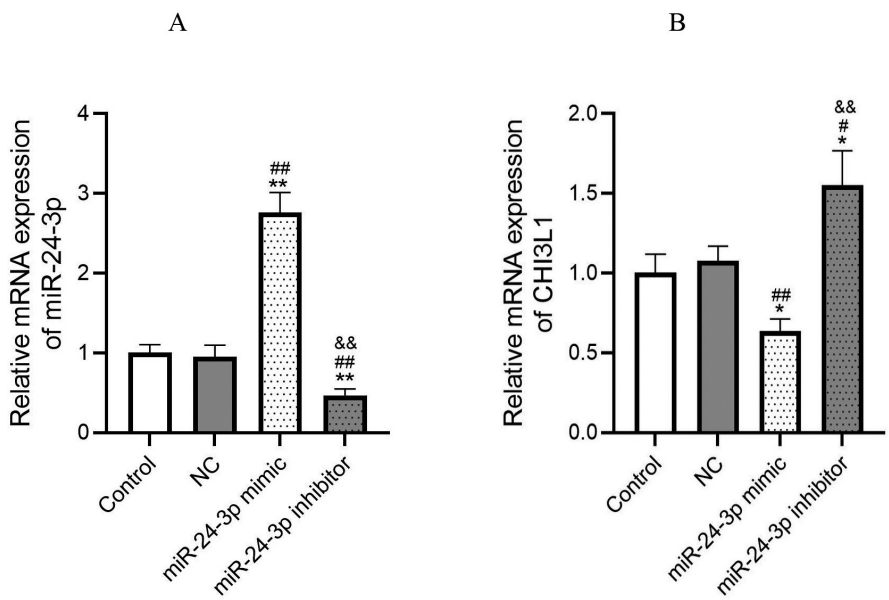

Fig. 4. The mRNA expression of miR-24-3p and CHI3L1 in HUVECs.
A. The mRNA expression of miR-24-3p in HUVECs transfected with miR-24-3p inhibitor, mimic, negative control and blank control. B. The mRNA expression of CHI3L1 in HUVECs transfected with miR-24-3p inhibitor, mimic, negative control and blank control. Data were presented as mean ± S.D. (n = 3 per group). *p < 0.05, **p < 0.01 compared with control; #p < 0.05, ##p < 0.01 compared with NC group (negative control of miR-24-3p); &&p < 0.01 compared with miR-24-3p mimic group.

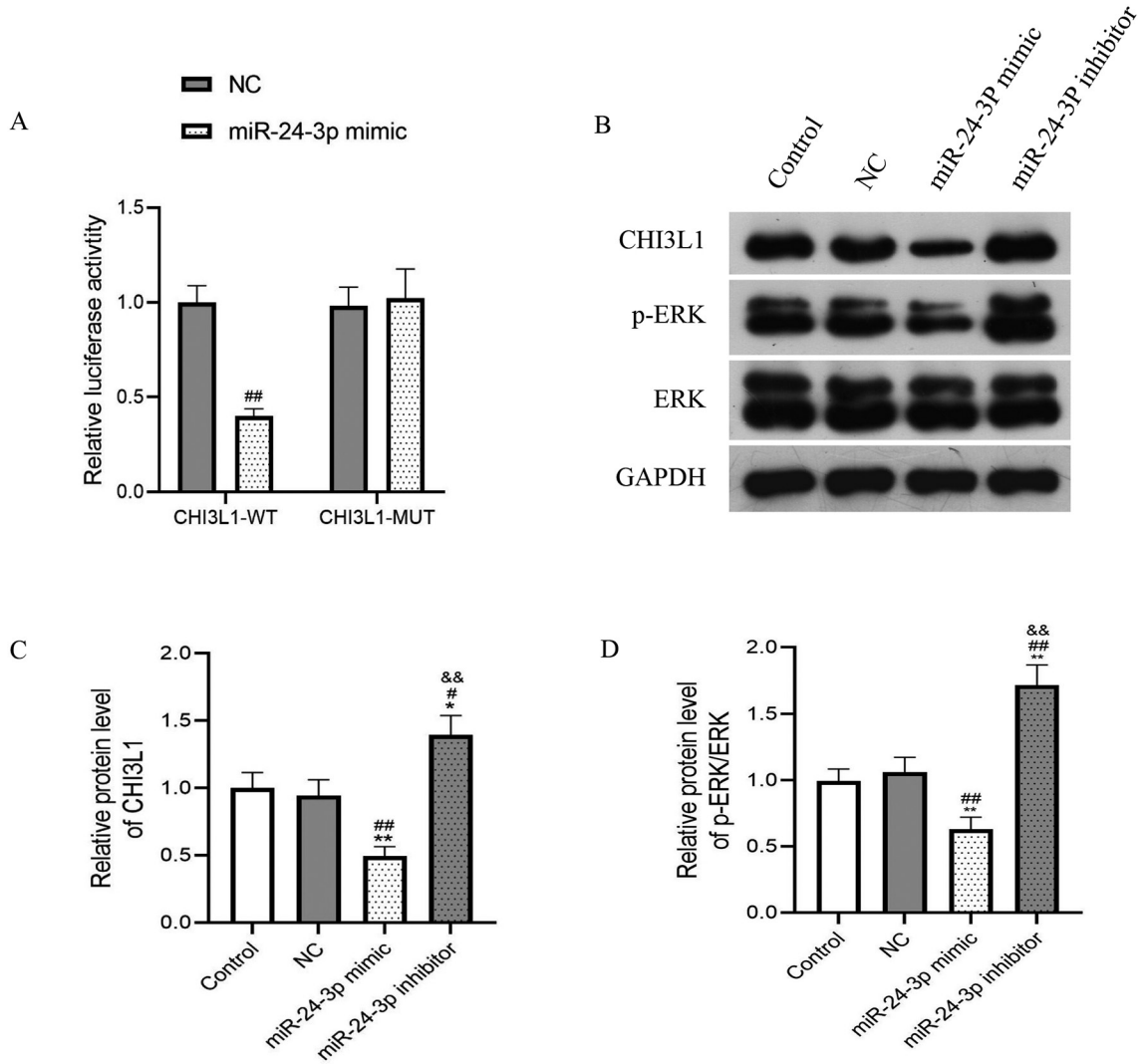

Fig. 5. MiR-24-3p suppressed the expression of p-ERK and CHI3L1 in HUVECs, and the molecular mechanism of miR-24-3p targeting CHI3L1.
A. The luciferase activity of miR-24-3p mimic on CHI3L1 3′-UTR. B. Protein blots of CHI3L1, p-ERK, ERK and GAPDH. C. Histogram of protein expression levels for CHI3L1. D. Histogram of protein expression levels for p-ERK/ERK. Data were presented as mean ± S.D. (n = 3 per group), *$p < 0.05$, **$p < 0.01$ compared with control; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with NC group (negative control of miR-24-3p); $^{\&\&}p < 0.01$ compared with miR-24-3p mimic group.

leading causes of death in the world. The main risk factors for atherosclerosis include hypertension, hyperlipidemia, heavy smoking, obesity and genetic factors (Turk-Adawi et al. 2018; O'Sullivan et al. 2022). The unstable atherosclerotic lesions commonly cause the lesion rupture or thrombosis formation, which is the fundamental factor for severe cardiovascular events. Although there are many mechanisms involved in the pathological process of atherosclerosis, recently, angiogenesis in atherosclerotic lesions is regarded as an important ingredient for unstable plaque. The neo-vessels in atherosclerotic lesions are usually immature and fragile due to incomplete basement membrane, which gives rise to inflammatory cells to infiltrate into the plaques; meanwhile, inflammatory state is a strong attractant for inflammatory cells releasing pro-angiogenic cytokines and miRNAs which facilitate the initiation and development of angiogenesis (Fleiner et al. 2004; Tiwari et al. 2018).

Recently, the miRNAs are regarded as one of pivotal molecular regulators involved in cardiovascular diseases, such as atherosclerotic plaque, lipid disorders, heart failure, angiogenesis and so on. It is speculated that miRNAs are able to regulate one-third of human genes (Hammond 2015). In fact, one miRNA may regulate expressions of multiple genes, and several miRNAs could regulate the expression of a single gene. As for the mechanism of miRNAs to target genes, it is well known, on one hand, miRNAs are capable of degrading mRNAs of the target genes through complementary base pairing in a complete way; On the other hand, miRNAs are endowed with the ability to

inhibit the translation of target genes in an incomplete way without affecting the stability of the mRNAs (Bartel 2009; Wang et al. 2021). Overexpression of miR-24-3p suppressed retinoblastoma cell migration and invasion (Luan et al. 2021). However, understanding the role of miR-24-3p in proliferation and angiogenesis of HUVECs is limited. In this study, to investigate the biological relevance of miR-24-3p in HUVECs, miR-24-3p inhibitor and mimic were transfected into HUVECs. Our results suggested that miR-24-3p inhibitor promotes cell proliferation, migration and angiogenesis *in vitro*. Meanwhile, miR-24-3p mimic remarkably decreases the content of mRNA and protein of CHI3L1 which is measured through qRT-PCR and western blot assay. The above results showed that the gene of CHI3L1 may be one of the target genes for miR-24-3p. In fact, the association between miR-24-3p and CHI3L1 has been mentioned in previous studies (Jin et al. 2015; Jingjing et al. 2017). As a result, we further demonstrated that miR-24-3p decreases the level of CHI3L1 in HUVECs through binding to 3′-UTR of CHI3L1 via dual-luciferase reporter assay. Dual-luciferase reporter assay showed that significant decrease in relative luciferase activity was noted when pmirGLO/CHI3L1-3′-UTR wt was co-transfected with the miR-24-3p mimic compared with negative control group. However, there is no significant difference in these two groups when pmirGLO/CHI3L1-3′-UTR mt co-transfected with negative control and miR-24-3p mimic, which suggests miR-24-3p specifically controls the expression of CHI3L1 through regulating 3′-UTR of CHI3L1.

Recently, it was reported that serum CHI3L1 (also called YKL-40) may be as a biomarker involved in cardiovascular diseases. Several clinical investigations demonstrated that elevated circulating YKL-40 levels are independently associated with the presence and extent of coronary heart disease (CHD) (Kucur et al. 2007; Schroder et al. 2020). Increasing level of YKL-40 has a prognostic role in the progression of coronary artery disease according to coronary angiography (Zheng et al. 2010). In addition, CHI3L1 mRNA and protein expression are found in the early development of the human musculoskeletal system, which reflects that CHI3L1 is, to some extent, associated with cell proliferation, differentiation and tissue morphogenesis (Johansen et al. 2007). It has been shown that CHI3L1 modulated the morphology of vascular endothelial cells by promoting the formation of branching tubules (Malinda et al. 1999). In our previous studies, we found that increased CHI3L1 level promoted the angiogenesis of HUVECs (Sun et al. 2021). We further demonstrated that adenovirus-packaged CHI3L1 overexpression vector injected into tail veins enhanced neovascularization in carotid plaques of apoE$^{-/-}$ mice with western diet (Xue et al. 2021). The activation of ERK is an important signaling pathway related to migration, proliferation and angiogenesis in various studies (Asl et al. 2021; Wang et al. 2022). Overexpression of CHI3L1 promotes the level of p-ERK (Sun et al. 2021; Xue et al. 2021). In this study, miR-24-3p mimic inhibits the expression of CHI3L1, and meanwhile decreases the content of p-ERK. However, the inhibitor of miR-24-3p promotes the content of CHI3L1 in HUVECs and increases the level of p-ERK.

Taken together, we identified miR-24-3p is an important regulator for expression of CHI3L1, and influences cell proliferation, migration and angiogenesis. Moreover, miR-24-3p inhibits the expression of CHI3L1 is partly mediated by ERK signaling pathway. In fact, it is sophisticated how to control the expression of miRNAs in their own metabolism. In the current view, the expression of miRNAs is partially regulated by corresponding long non-coding RNAs (lncRNAs) and RNA-binding proteins (RBPs) (Janakiraman et al. 2018). So how to intervene the expression of miR-24-3p in the microenvironment of atherosclerotic plaques is possibly an important way to affect the development of unstable plaques. In this present study, we slightly disclose the role of miR-24-3p in HUVECs by *in vitro* assays. The detailed mechanism should be investigated more deeply in the future researches including experiments *in vivo*. Thus, these results should be further demonstrated in future studies through transfecting mimic and inhibitor of miR-24-3p into apoE$^{-/-}$ mice and then monitoring the characters of atherosclerotic plaques and the contents of neo-vessels in plaques.

Funding

This work was supported by Medical Science and Technology Project of Zhejiang Province (Project code: 2020KY049); Traditional Chinese Medicine Administration of Zhejiang Province (Project code: 2020ZA006).

Conflict of Interest

The authors declare no conflict of interest.

References

Andrews, J.P.M., Fayad, Z.A. & Dweck, M.R. (2018) New methods to image unstable atherosclerotic plaques. *Atherosclerosis*, **272**, 118-128.

Asl, E.R., Amini, M., Najafi, S., Mansoori, B., Mokhtarzadeh, A., Mohammadi, A., Lotfinejad, P., Bagheri, M., Shirjang, S., Lotfi, Z., Rasmi, Y. & Baradaran, B. (2021) Interplay between MAPK/ERK signaling pathway and MicroRNAs: A crucial mechanism regulating cancer cell metabolism and tumor progression. *Life Sci.*, **278**, 119499.

Bartel, D.P. (2009) MicroRNAs: target recognition and regulatory functions. *Cell*, **136**, 215-233.

Corà, D., Re, A., Caselle, M. & Bussolino, F. (2017) MicroRNA-mediated regulatory circuits: outlook and perspectives. *Phys. Biol.*, **14**, 045001.

Fasolo, F., Di Gregoli, K., Maegdefessel, L. & Johnson, J.L. (2019) Non-coding RNAs in cardiovascular cell biology and atherosclerosis. *Cardiovasc. Res.*, **115**, 1732-1756.

Fleiner, M., Kummer, M., Mirlacher, M., Sauter, G., Cathomas, G., Krapf, R. & Biedermann, B.C. (2004) Arterial neovascularization and inflammation in vulnerable patients: early and late signs of symptomatic atherosclerosis. *Circulation*, **110**, 2843-2850.

Gerami, M.H., Khorram, R., Rasoolzadegan, S., Mardpour, S., Nakhaei, P., Hashemi, S., Al-Naqeeb, B.Z.T., Aminian, A. &

Samimi, S. (2023) Emerging role of mesenchymal stem/stromal cells (MSCs) and MSCs-derived exosomes in bone- and joint-associated musculoskeletal disorders: a new frontier. *Eur. J. Med. Res.*, **28**, 86.

Hammond, S.M. (2015) An overview of microRNAs. *Adv. Drug Deliv. Rev.*, **87**, 3-14.

Huang, J., Li, Y., Jiang, Z., Wu, L., Liu, Y., Ma, S., Li, L. & Wang, H. (2022) IL-1beta promotes hypoxic vascular endothelial cell proliferation through the miR-24-3p/NKAP/NF-kappaB axis. *Biosci. Rep.*, **42**, BSR20212062.

Janakiraman, H., House, R.P., Gangaraju, V.K., Diehl, J.A., Howe, P.H. & Palanisamy, V. (2018) The Long (lncRNA) and Short (miRNA) of It: TGFbeta-Mediated Control of RNA-Binding Proteins and Noncoding RNAs. *Mol. Cancer Res.*, **16**, 567-579.

Jin, T., Lu, Y., He, Q.X., Wang, H., Li, B.F., Zhu, L.Y. & Xu, Q.Y. (2015) The Role of MicroRNA, miR-24, and Its Target CHI3L1 in Osteomyelitis Caused by Staphylococcus aureus. *J. Cell Biochem.*, **116**, 2804-2813.

Jingjing, Z., Nan, Z., Wei, W., Qinghe, G., Weijuan, W., Peng, W. & Xiangpeng, W. (2017) MicroRNA-24 Modulates Staphylococcus aureus-Induced Macrophage Polarization by Suppressing CHI3L1. *Inflammation*, **40**, 995-1005.

Johansen, J.S., Hoyer, P.E., Larsen, L.A., Price, P.A. & Mollgard, K. (2007) YKL-40 protein expression in the early developing human musculoskeletal system. *J. Histochem. Cytochem.*, **55**, 1213-1228.

John Chapman, M. & Preston Mason, R. (2022) Cholesterol crystals and atherosclerotic plaque instability: Therapeutic potential of Eicosapentaenoic acid. *Pharmacol. Ther.*, **240**, 108237.

Krol, J., Loedige, I. & Filipowicz, W. (2010) The widespread regulation of microRNA biogenesis, function and decay. *Nat. Rev. Genet.*, **11**, 597-610.

Kucur, M., Isman, F.K., Karadag, B., Vural, V.A. & Tavsanoglu, S. (2007) Serum YKL-40 levels in patients with coronary artery disease. *Coron. Artery Dis.*, **18**, 391-396.

Luan, L., Hu, Q., Wang, Y., Lu, L. & Ling, J. (2021) Knockdown of lncRNA NEAT1 expression inhibits cell migration, invasion and EMT by regulating the miR-24-3p/LRG1 axis in retinoblastoma cells. *Exp. Ther. Med.*, **21**, 367.

Malinda, K.M., Ponce, L., Kleinman, H.K., Shackelton, L.M. & Millis, A.J. (1999) Gp38k, a protein synthesized by vascular smooth muscle cells, stimulates directional migration of human umbilical vein endothelial cells. *Exp. Cell Res.*, **250**, 168-173.

Mukherjee, S., Shelar, B. & Krishna, S. (2022) Versatile role of miR-24/24-1*/24-2* expression in cancer and other human diseases. *Am. J. Transl. Res.*, **14**, 20-54.

O'Sullivan, J.W., Raghavan, S., Marquez-Luna, C., Luzum, J.A., Damrauer, S.M., Ashley, E.A., O'Donnell, C.J., Willer, C.J., Natarajan, P., American Heart Association Council on, G., Precision, M., Council on Clinical, C., Council on Arteriosclerosis, T., Vascular, B., Council on Cardiovascular, R., et al. (2022) Polygenic Risk Scores for Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation*, **146**, e93-e118.

Ren, L., Chen, S. & Liu, W. (2024) OxLDL-Stimulated Macrophages Transmit Exosomal MicroRNA-320b to Aggravate Viability, Invasion, and Phenotype Switching via Regulating PPARGC1A-Mediated MEK/ERK Pathway in Proatherogenic Vascular Smooth Muscle Cells. *T ohoku J. Exp. Med.*, **262**, 13-22.

Schroder, J., Jakobsen, J.C., Winkel, P., Hilden, J., Jensen, G.B., Sajadieh, A., Larsson, A., Arnlov, J., Harutyunyan, M., Johansen, J.S., Kjoller, E., Gluud, C. & Kastrup, J. (2020) Prognosis and Reclassification by YKL-40 in Stable Coronary Artery Disease. *J. Am. Heart Assoc.*, **9**, e014634.

Sun, W., Xue, Q., Zhao, Y. & Zheng, J. (2021) The effects of YKL-40 on angiogenic potential of HUVECs are partly mediated by syndecan-4. *Int. J. Med. Sci.*, **18**, 3759-3767.

Tan, H., Qi, J., Fan, B.Y., Zhang, J., Su, F.F. & Wang, H.T. (2018) MicroRNA-24-3p Attenuates Myocardial Ischemia/Reperfusion Injury by Suppressing RIPK1 Expression in Mice. *Cell Physiol. Biochem.*, **51**, 46-62.

Tiwari, A., Mukherjee, B. & Dixit, M. (2018) MicroRNA Key to Angiogenesis Regulation: MiRNA Biology and Therapy. *Curr. Cancer Drug Targets*, **18**, 266-277.

Turk-Adawi, K., Sarrafzadegan, N., Fadhil, I., Taubert, K., Sadeghi, M., Wenger, N.K., Tan, N.S. & Grace, S.L. (2018) Cardiovascular disease in the Eastern Mediterranean region: epidemiology and risk factor burden. *Nat. Rev. Cardiol.*, **15**, 106-119.

Virmani, R., Kolodgie, F.D., Burke, A.P., Finn, A.V., Gold, H.K., Tulenko, T.N., Wrenn, S.P. & Narula, J. (2005) Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. *Arterioscler. Thromb. Vasc. Biol.*, **25**, 2054-2061.

Wang, R., Sun, S., Wang, Z., Xu, X., Jiang, T., Liu, H., Li, X. & Ren, Z. (2022) MCPIP1 promotes cell proliferation, migration and angiogenesis of glioma via VEGFA-mediated ERK pathway. *Exp. Cell Res.*, **418**, 113267.

Wang, X., He, Y., Mackowiak, B. & Gao, B. (2021) MicroRNAs as regulators, biomarkers and therapeutic targets in liver diseases. *Gut*, **70**, 784-795.

Xu, W., Chao, R., Xie, X., Mao, Y., Chen, X., Chen, X. & Zhang, S. (2024) IL13Ralpha2 as a crucial receptor for Chi3l1 in osteoclast differentiation and bone resorption through the MAPK/AKT pathway. *Cell Commun. Signal.*, **22**, 81.

Xue, Q., Chen, L., Yu, J., Sun, K., Ye, L. & Zheng, J. (2021) Downregulation of Interleukin-13 Receptor alpha2 Inhibits Angiogenic Formation Mediated by Chitinase 3-Like 1 in Late Atherosclerotic Lesions of apoE(-/-) Mice. *Front. Physiol.*, **12**, 690109.

Yan, L., Ma, J., Zhu, Y., Zan, J., Wang, Z., Ling, L., Li, Q., Lv, J., Qi, S., Cao, Y., Liu, Y., Cao, L., Zhang, Y., Qi, Z. & Nie, L. (2018) miR-24-3p promotes cell migration and proliferation in lung cancer by targeting SOX7. *J. Cell Biochem.*, **119**, 3989-3998.

Yu, G., Jia, Z. & Dou, Z. (2017) miR-24-3p regulates bladder cancer cell proliferation, migration, invasion and autophagy by targeting DEDD. *Oncol. Rep.*, **37**, 1123-1131.

Zhang, Y., Wang, Z., Lan, D., Zhao, J., Wang, L., Shao, X., Wang, D., Wu, K., Sun, M., Huang, X., Yan, M., Liang, H., Rong, X., Diao, H. & Guo, J. (2022) MicroRNA-24-3p alleviates cardiac fibrosis by suppressing cardiac fibroblasts mitophagy via downregulating PHB2. *Pharmacol. Res.*, **177**, 106124.

Zheng, J.L., Lu, L., Hu, J., Zhang, R.Y., Zhang, Q., Chen, Q.J. & Shen, W.F. (2010) Increased serum YKL-40 and C-reactive protein levels are associated with angiographic lesion progression in patients with coronary artery disease. *Atherosclerosis*, **210**, 590-595.

Zhu, Y., Xian, X., Wang, Z., Bi, Y., Chen, Q., Han, X., Tang, D. & Chen, R. (2018) Research Progress on the Relationship between Atherosclerosis and Inflammation. *Biomolecules*, **8**, 80.