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The lncRNA MALAT1 protects the endothelium against ox-LDL-induced dysfunction via upregulating the expression of the miR-22-3p target genes CXCR2 and AKT

Yong Tang [a,1], Xian Jin [b,a,1], Yin Xiang [a], Yu Chen [c], Cheng-xing Shen [a], Ya-chen Zhang [a,*], Yi-gang Li [a,*]

[a] Department of Cardiology, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China
[b] Department of Cardiology, Central Hospital of Minhang District, Shanghai, China
[c] Department of Cardiology, Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

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ABSTRACT

CXCR2 plays a key role in protecting the integrity of the endothelium. Emerging evidence has demonstrated that the long ncRNAs (lncRNA) Human metastasis associated lung adenocarcinoma transcript 1 (MALAT1) participates in the regulation of the pathophysiological processes. However, whether there is crosstalk between CXCR2 and MALAT1 remains unknown. In this study, we demonstrated that MALAT1 was upregulated in patients with unstable angina. MALAT1 silencing significantly downregulated the expression of the miR-22-3p target gene CXCR2 via reversing the effect of the miR-22-3p, resulting in the aggravation of Oxidized low-density lipoprotein (ox-LDL)-induced endothelial injury; this process was associated with the AKT pathway. Thus, MALAT1 protects the endothelium from ox-LDL-induced endothelial dysfunction partly through competing with miR-22-3p for endogenous RNA.
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1. Introduction

Oxidized low-density lipoprotein (ox-LDL) has been widely demonstrated to be involved in the development of atherosclerosis by causing an oxidative chain reaction and inducing endothelial dysfunction [1,2]. After binding to the lectin-like ox-LDL receptor-1 on the endothelial cell surface, ox-LDL and its lipid constituents induce reactive oxygen species (ROS) accumulation and nitric oxide (NO) inhibition [2]. These factors contribute to the destruction of the integrity of the endothelium and cause a series of atherosclerosis-related diseases [3]. Moreover, ox-LDL induces high expression of interleukin-8 in endothelial cells [4–6]; interleukin-8 is the ligand of the chemokine receptor CXCR2. Numerous studies have demonstrated that CXCR2 has the ability to promote cell proliferation, diminish cell apoptosis and enhance angiogenesis [7,8], indicating that CXCR2 plays a key role in protecting the endothelium from ox-LDL-induced injury. However, the regulation of the CXCR2-mediated protective effect on endothelial cells remains elusive.

Genome-sequencing projects have demonstrated that more than ninety percent of the genome is transcribed as non-coding RNAs (ncRNAs). Only 2% of the human genome contains protein-coding genes [9], indicating the need for a redefinition of the concept of ncRNAs. ncRNAs include microRNAs (miRNAs), which consist of 18–24 nucleotides, and long ncRNAs (lncRNAs), which are longer than 200 nucleotides in length. Recently, accumulating evidence has strongly implied that ncRNAs play a key role in regulating pathophysiological processes [10–12]. Human metastasis associated lung adenocarcinoma transcript 1 (MALAT1; also known as NEAT2), is an 8.7 kb lncRNA that maps to chromosome 11q13 and has been demonstrated to be overexpressed in several cancers [13]. A previous study reported that MALAT1 promoted aggressive renal cell carcinoma through Ezh2 [14]. MALAT1 silencing significantly suppresses the proliferation of esophageal squamous cell carcinoma through the cell cycle at G2/M [15]. Furthermore, the high expression levels of conserved MALAT1 are involved in the physiological progress of endothelial cells [16]

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* Corresponding authors at: Department of Cardiology, Xinhua Hospital, Shanghai Jiaotong University School of Medicine, 1665 Kong Jiang Road, Shanghai 200092, China. Fax: +86 21 6515398.

E-mail addresses: zhangyachen1965@163.com (Y.-c. Zhang), dorlyg@126.com (Y.-g. Li).

[1] Contributed equally.

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and are associated with microvascular complications [17]. In this study, we also found a marked increase in the expression of MALAT1 in endothelial cells following 24 h of ox-LDL treatment. However, little is known about the contribution of MALAT1 to endothelial cell dysfunction.

lncRNAs may function as miRNAs "sponges" by interacting through common response elements, resulting in the modulation of the miRNA activity [18]. This crosstalk between lncRNAs and miRNAs can be called the 'competitive endogenous RNA (ceRNA)' network. The ceRNA network is associated with lots of biological processes, and the intervention of the interaction between lncRNAs and miRNAs can be critical for biological development [10,14,15]. Because both the coding RNA CXCR2 and the non-coding RNA MALAT1 have an effect on the integrity of the endothelium, we hypothesed that MALAT1 post-transcriptionally regulated CXCR2 via ceRNA in response to ox-LDL-induced endothelial dysfunction. Recent examples demonstrating that MALAT1"sponges" miRNAs to regulate specific target genes support this hypothesis [14,15]. Here, we evaluated the possibility of crosstalk between CXCR2 and MALAT1 and investigating whether the regulatory mechanism was associated with the ceRNA network.

2. Materials and methods

The experiments were conducted in accordance with the Declaration of Helsinki and this study was approved by the ethics committee of the Xinhua Hospital School of Medicine, Shanghai Jiaotong University.

2.1. Patient population and blood collection

A lot of 23 patients diagnosed with unstable angina (UA) by our Division of Cardiology and 23 healthy subjects were recruited. Their diagnoses were based on a history of chest pain, coronary angiography results and characteristic ECG changes. The baseline characteristics of the two groups were compared. A 10 ml sample of peripheral blood was collected in an EDTA-containing vacutainer tube from each individual for further analysis.

2.2. Cell culture

HUVECs were isolated from fresh human umbilical cords following the methods of a previous study [19]. Briefly, the separated cord was placed in cord buffer containing NaCl (0.14 M), KCI (0.004 M), phosphate buffer (0.001 M) and glucose (0.011 M). We washed the blood out of the cord and added an appropriate amount of 0.2% collagenase type II (Sigma) into the umbilical vein for 15 min at 37 °C. After incubation, the solution containing HUVECs was rinsed into a centrifuge tube. After discarding the buffer, the cells were cultured in DMEM high glucose medium containing 20% fetal bovine serum at 37 °C in a 5% CO_2 incubator. The entire process was performed under aseptic conditions within 3 h. Cells from passages three to five were used for this study.

2.3. Cell transfection

miR-22-3p mimics and inhibitors (Dharmacon, 50 nM) were used to upregulate or downregulate the miR-22-3p expression. Cells were transfected with the CXCR2-siRNA (Ribobio), MALAT1-shRNA (Hanbio), or miR-22-3p mimics, inhibitors or controls (Dharmacon) using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Changes in RNA expression were determined by qRT-PCR 24 h after transfection, and changes in protein expression were measured by Western blotting 48 h after transfection.

2.4. RNA preparation, RT and qPCR

Total RNA was extracted using the Trizol reagent (Invitrogen) following the manufacturer's protocol. Next, the Primer-Script™ one step RT-PCR kit (TaKaRa) was used for the reverse transcription of mRNAs and lncRNAs, and the miRcute miRNA cDNA kit (Tiangen) was used for the reverse transcription of miRNAs. The SYBR kit (TaKaRa) was used for detection in the ABI7500 system (Applied Biosystems). β-Actin was used as the endogenous control for mRNAs and lncRNAs, and U6 was used as the endogenous control for miRNAs. The relative expression levels among groups were calculated by the 2^{-DDCt} method. The following primers were used:

CXCR2 F: 5′-CCGTTTTCTCCTTCCTGGGT-3′, and R: 5′-GCTGTGACCTGCTGTTATTGG-3′; MALAT1: F: 5′-ATGCGAGTTGTTCTCCGTCT-3′, and R: 5′-TATCTGCGGTTTCCTCAAGC-3′; β-actin: F: 5′-AGAGCCTCGCCTTTGCCGAT-3′, and R: 5′-TGCCAGATTTTCTCCATGTCGT-3′; miR-22-3p: 5′-AAGCTGCCAGTTGAAGAACTGT-3′; U6: 5′- CGCTTCGGCAGCACATATACTAAAATTGGAAC-3′.

2.5. Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay Kits (Elabscience) were used to detect interleukin-1or interleukin-8 in cell culture supernatants following 24 h of ox-LDL treatment.

2.6. RNA immunoprecipitation assay (RIP)

The Magna RIP RNA-Binding Protein Immunoprecipitation Kit (Millipore) was used according to the manufacturer's protocol to analyze whether MALAT1 was associated with the RISC complex. IgG (Millipore) was used as the negative control and anti-snRNP70 as the positive control (Millipore).

2.7. Western blotting (WB)

Primary antibodies targeting Ago2 (1:1000 dilution; Millipore), CXCR2 (1:1000 dilution; Cell Signaling), p-AKT, AKT (1:1000 dilution; Cell Signaling), and cleaved Casp3 (1:1000 dilution; Cell Signaling) were used. The antibody for β-actin (1:1000 dilution; Beyotime) was used as the endogenous control. The assay was performed as follows. HUVECs were lysed for 1.5 h on ice by a cell lysis solution containing 1% phenylmethanesulfonyl fluoride buffer. Then 12% SDS–PAGE gels (Millipore) were used for equal protein loading (50 μg) electrophoresis. The gels were run under the same experimental conditions.

2.8. Flow cytometric analysis

The apoptosis of HUVECs induced by ox-LDL was assessed using a FITC Annexin V Apoptosis Detection Kit I (BD) following the manufacturer's protocol. In brief, the cells were washed with cold PBS and resuspended at a density of 1×10^6 cells/ml using 1× Binding Buffer. Then, 5 μl of FITC Annexin V and 5 μl propidium iodide were added to the resuspended cells for 15 min at room temperature in the dark. The cells were analyzed with a Beckman Coulter FC500 (Beckman) within 1 h.

2.9. Capillary-like structure formation assay

An in vitro endothelial tube formation assay was performed to study the modulation of angiogenesis by miR-22-3p, CXCR2 or MALAT1 as described previously [20]. Briefly, 10^4 endothelial cells pretreated with miR-22-3p, CXCR2-siRNA or MALAT1-shRNA for 24 h were seeded into a Matrigel-coated 96-well plate. Following 6 h of incubation at 37 °C, pictures were taken using a fluorescent microscope. Each assay was repeated five times.

2.10. Migration

A total of 10^6 endothelial cells were seeded into a six-well plate and incubated at 37 °C until 60% confluent. Then, the cells were treated with miR-22-3p, CXCR2-siRNA or MALAT1-shRNA for another 24 h, and the migration distance was observed by microscope.

2.11. Luciferase activity assay

The 3′-UTR fragment of CXCR2, which contains the predicted potential miRNA binding sites, was amplified by PCR using the following primers: F: 5′-GGCGGCTCGAGACGTTCTTACTAGTTT-3′, and R: 5′-AATGCGGCCGCAGTAAAAATGGTTT-3′. The mutant was constructed by introducing point mutations into the seed binding site for miR-22-3p using the following primers: F: 5′-AAGACAG TATCCGAGCT-3′ and R: 5′-AGCTCGGATACTGTCTT-3′. The wild type and mutant fragments were subcloned into the pmiR-RB-REPORTTM vector (Ribobio). The vectors and miRNA mimics were co-transfected into HUVECs for 24 h and the luciferase activity was subsequently tested. Similarly, either the wild type or mutant 3′-UTR of AKT or the fragment of MALAT1 containing the predicted miR-22-3p targeting site (chr11:65270634–6527065

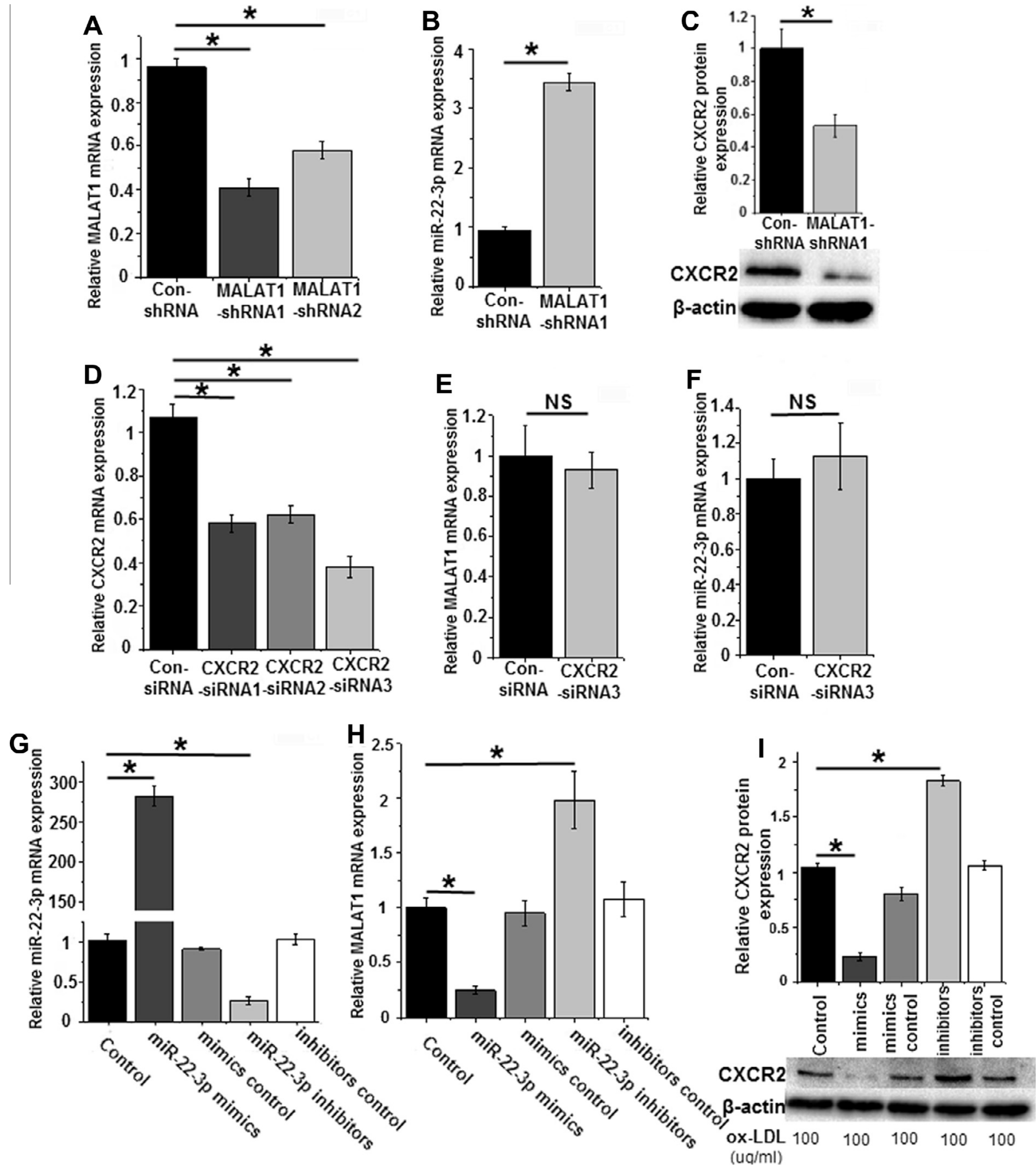

Fig. 1. The interaction among MALAT1, CXCR2 and miR-22-3p. (A) The inhibitory effect of MALAT1-shRNA on the MALAT1 mRNA level was detected by RT-PCR. After transfecting MALAT1-shRNA1 into ECs for 24 h, the expression levels of miR-22-3p (B) and CXCR2 (C) were measured by RT-PCR or WB. (D) The inhibitory effect of CXCR2-siRNA on the CXCR2 mRNA level was detected by RT-PCR. The expression of MALAT1 (E) and miR-22-3p (F) were measured following 24 h of CXCR2-siRNA3 treatment. (G) Changes in EC miR-22-3p levels 24 h after transfection with the vehicle, the miR-22-3p mimics, the mimic control, the miR-22-3p inhibitors or the inhibitor control. The expression levels of MALAT1 (H) and CXCR2 (I) were measured in response to the miR-22-3p mimics or the miR-22-3p inhibitors. *NS* not significant, $^{*}p < 0.05$.

5) were transfected and evaluated using the methods described above.

2.12. Statistics

The SPSS 18 software (SPSS Inc., USA) was used for statistical analysis. The data are presented as the mean ± standard error. The expression differences between two groups were tested using a paired samples *t*-test. A *P*-value <0.05 was considered statistically significant.

3. Results

3.1. The relationship among MALAT1, miR-22-3p and CXCR2

Several publicly available bioinformatics web sites [Starbase v2.0 (http://starbase.sysu.edu.cn/mirLncRNA.php) and TargetScan (http://www.targetscan.org/)] showed that there were six potential miRNAs with complementary base pairing with both MALAT1 and CXCR2 (Supplementary Fig. 1A): miR-1, miR-22-3p, miR-23a, miR-23b, miR-23c, and miR-376. The MALAT1-shRNA obviously decreased the expression of MALAT1 (approximately 60% reduction, Fig. 1A). Following 24 h of MALAT1 silencing treatment, the expression of miR-22-3p showed the biggest change (increased approximately 2.5-fold) among the six potential miRNAs (Fig. 1B, Supplementary Fig. 1B). By contrast, the protein expression of CXCR2 was downregulated at 48 h after transfection (approximately 50% reduction, Fig. 1C).

Although the CXCR2-siRNA3 significantly decreased the expression of CXCR2 (approximately 60% reduction, Fig. 1D), no expression changes were found in MALAT1 and miR-22-3p in response to CXCR2 silencing (Fig. 1E and F). By contrast, the expression of both MALAT1 and CXCR2 was significantly reduced in response to the miR-22-3p mimics and increased after transfection with the miR-22-3p inhibitors (Fig. 1H and I). These data suggest that a potential link may exist among the lncRNA MALAT1, miR-22-3p and CXCR2.

3.2. Changes in MALAT1 and miR-22-3p expression in UA patients and ox-LDL treated ECs

The expression levels of the lncRNA MALAT1 and miR-22-3p were measured in the plasma obtained from 23 patients with UA and 23 healthy subjects. There were no significant differences in the baseline characteristics of the two groups (Supplementary Table 1). The expression of MALAT1 was upregulated (Fig. 2A) and miR-22-3p was downregulated (Fig. 2B) in patients with UA. Following 24 h of ox-LDL (0, 30, 50, 100, and 150 μg/ml) treatment, the expression of MALAT1 and CXCR2 in the ECs was increased and the expression of miR-22-3p was decreased with the increasing ox-LDL concentration in a dose- dependent manner (Fig. 2C). Furthermore, the expression of MALAT1, CXCR2 and miR-22-3p showed a similar trend over time (Fig. 2D).

3.3. Direct binding between miR-22-3p and MALAT1

The lncRNA MALAT1 shares a miRNA response element with miR-22-3p. The luciferase assay revealed that the miR-22-3p mim-

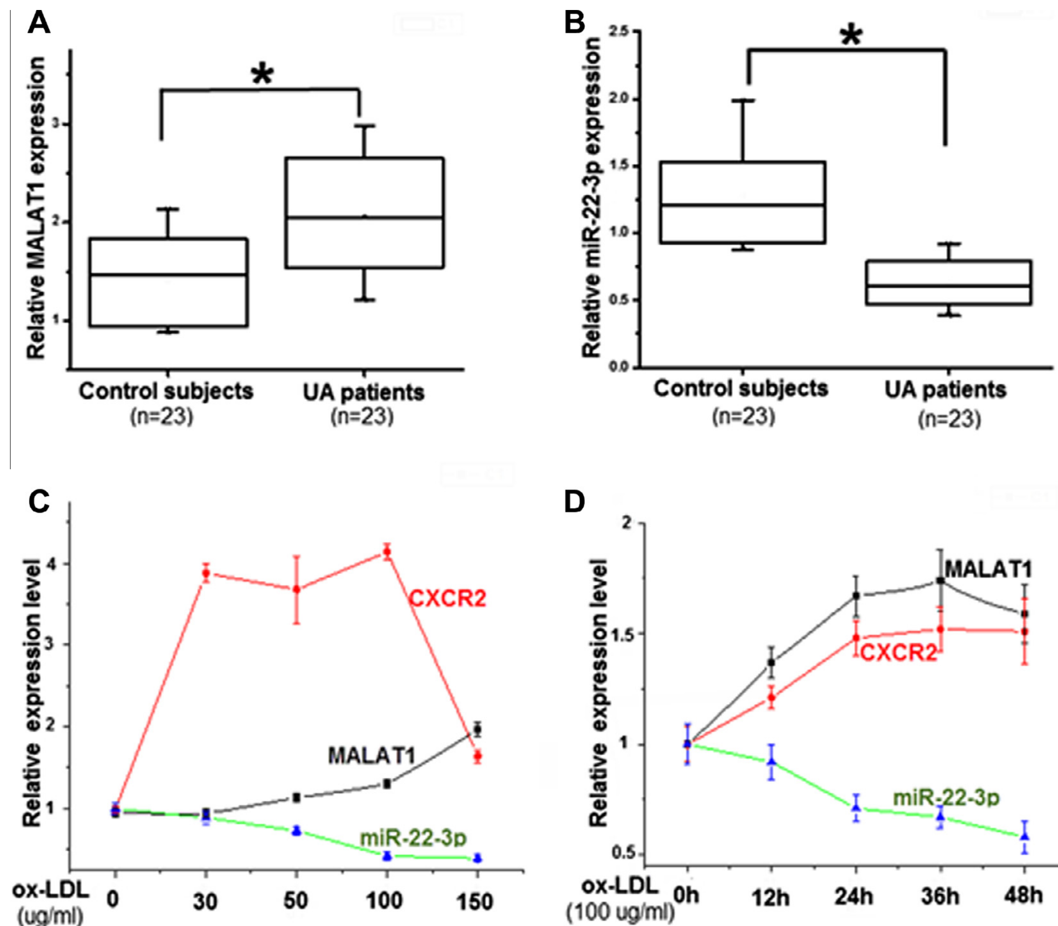

Fig. 2. MALAT1 and miR-22-3p expression in UA patients and ox-LDL treated ECs. The levels of circulating lncRNA MALAT1 (A) and miR-22-3p (B) in healthy volunteers ($n = 23$) and patients with UA ($n = 23$) were measured by RT-PCR. Following 24 h of treatment with several concentrations of ox-LDL (C), or treatment with ox-LDL (100 μg/ml) for different times (D), the mRNA expression of MALAT1, miR-22-3p and CXCR2 were measured by RT-PCR. $^{*}p < 0.05$.

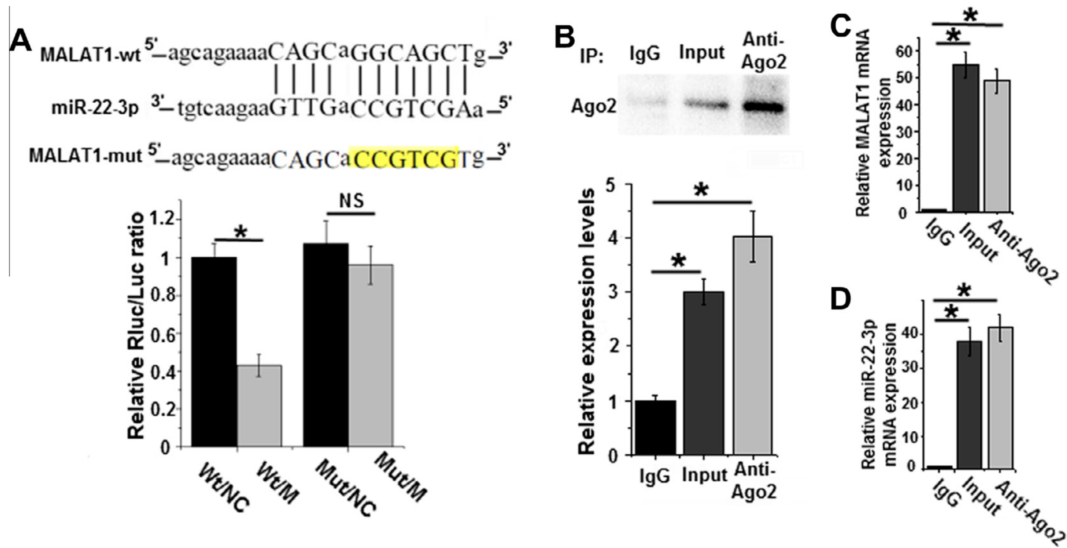

Fig. 3. The potential mechanism of the negative regulation of miR-22-3p by MALAT1. (A) The wild type and mutated miR-22-3p binding sites in the MALAT1 3′-UTR were shown in the upper panel. The miR-22-3p mimics and the luciferase constructs were co-transfected into endothelial cells. The negative control was used as a reference. Cellular lysates from ECs were used for RIP with an Ago2 antibody. The Ago2 protein level was detected by WB (B), and the mRNA expression of MALAT1 (C) and miR-22-3p (D) in the immunoprecipitate was measured by RT-PCR. *Wt* wild type, *Mut* mutant, *NC* negative control, *M* miR-22-3p mimics, $^{*}p < 0.05$.

Fig. 4. MALAT1 protects endothelial cells through the miR-22-3p/CXCR2 axis. (A) The wild type and mutated miR-22-3p binding sites in the 3′-UTR of CXCR2 were shown in the upper panel. The miR-22-3p mimics and the luciferase constructs were co-transfected into endothelial cells. The negative control was used as a reference. (B) The protein expression level of CXCR2 was measured in the absence of miR-22-3p or MALAT1 by WB. (C) The apoptotic effects of miR-22-3p, MALAT1 and CXCR2 on ECs were revealed by flow cytometry analysis. The capillary tube formation and the migration effects of miR-22-3p (D), MALAT1 and CXCR2 on ECs were also detected. $^{*}p < 0.05$.

ics decreased the luciferase activity of MALAT1; however, a reduced effect was found for the MALAT1 mutant (Fig. 3A). miRNAs function through the formation of RNA-induced silencing complex (RISC), of which the Argonaute protein 2 (Ago2) is the key component [21]. We subsequently performed an RIP experiment to investigate whether MALAT1 and miR-22-3p were in the RISC complex. The Ago2 antibody was able to precipitate the Ago2 protein from the digested cells (Fig. 3B). The mRNA expression levels of both MALAT1 and miR-22-3p were obviously enriched in the immunoprecipitates (approximately 48-fold and 42-fold, respectively Fig. 3C and D). This result suggests that direct binding exists between MALAT1 and miR-22-3p.

3.4. MALAT1 protects endothelial cells through the miR-22-3p/CXCR2 axis

miR-22-3p is a tumor-suppressive miRNA that suppresses cell proliferation and promotes apoptosis in cancers [22,23]. To investigate whether CXCR2 is a direct target of miR-22-3p, the 3′-UTR of CXCR2 was cloned into a luciferase reporter plasmid. The wild type showed significantly reduced expression in the presence of miR-22-3p, whereas no suppression of activity was observed for the mutant (Fig. 4A). We subsequently assessed CXCR2 protein levels after transfection of the miR-22-3p inhibitors or MALAT1-shRNA1 into endothelial cells and found that the decreased expression of CXCR2 in response to MALAT1-shRNA1 could be blocked by miR-22-3p knockdown (Fig. 4B).

CXCR2, which is the target gene of miR-22-3p, is involved in protecting endothelial cells from ox-LDL-induced injury [7,8]. The flow cytometric analysis revealed that the overexpression of miR-22-3p significantly increased EC apoptosis by approximately 25%. Moreover, the decreased percentage of apoptotic cells due to miR-22-3p knockdown was offset by transfection with CXCR2-siRNA3 or MALAT1-shRNA1 (Fig. 4C). Because CXCR2 is also associated with the process of angiogenesis, we subsequently measured the migration and angiogenesis effect of endothelial cells. The migration and angiogenesis inhibition effects of the CXCR2-siRNA3 and MALAT1-shRNA1 were attenuated by the miR-22-3p

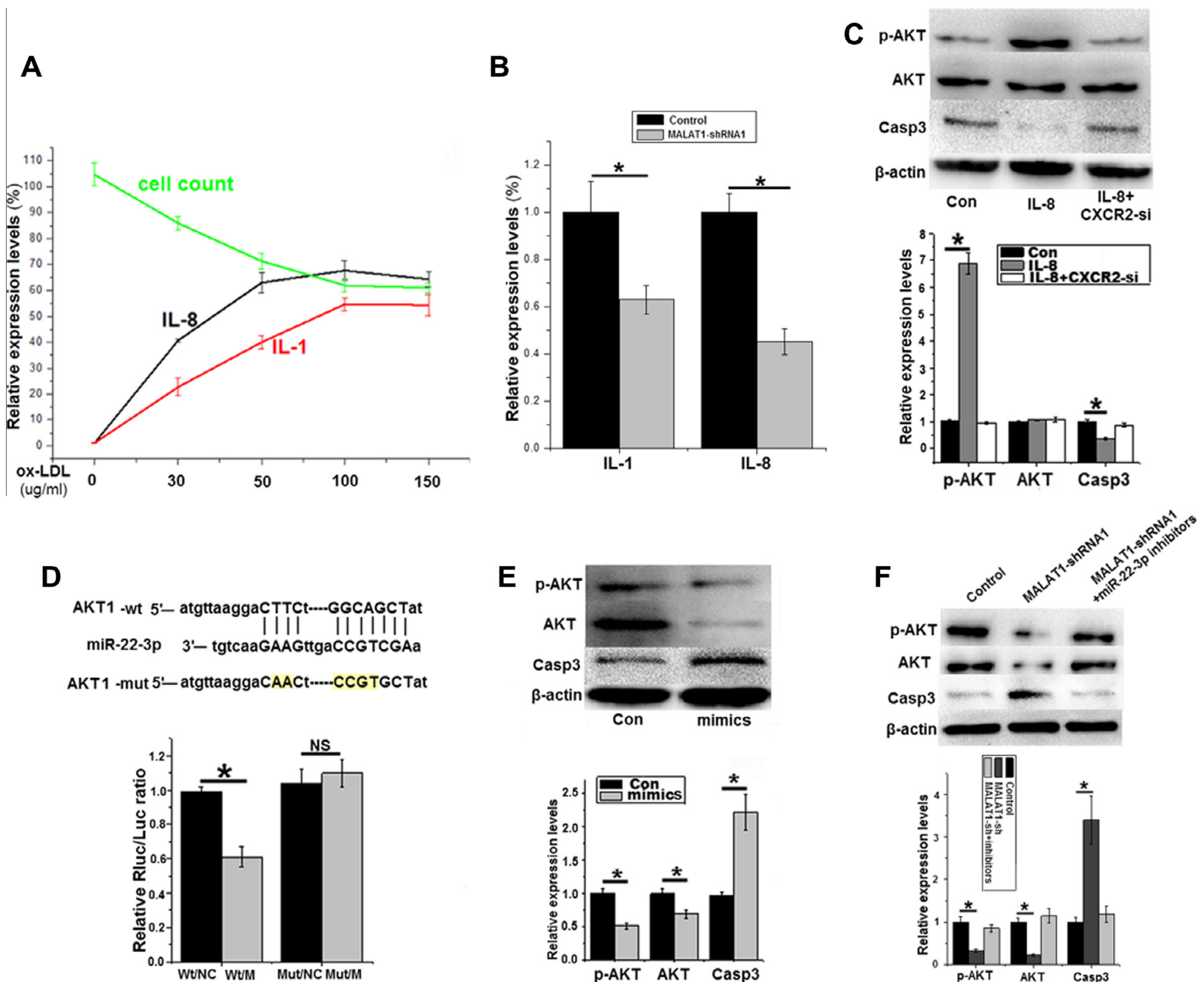

Fig. 5. MALAT1/miR-22-3p/CXCR2 signaling is associated with the AKT pathway. (A) The relationship among the cell count and the CXCR2 ligands. (B) Changes in the IL-1 and IL-8 expression in response to MALAT1 silencing. (C) After 48 h of IL-8 (50 ng/ml) or CXCR2-siRNA treatment, the protein levels of p-AKT, AKT and Casp3 were detected by WB. (D) The wild type and mutated miR-22-3p binding sites in the 3′-UTR of AKT1 were shown in the upper panel. The miR-22-3p mimics and the luciferase constructs were co-transfected into endothelial cells. The negative control was used as a reference. (E) The protein levels of p-AKT, AKT and Casp3 were modulated by the miR-22-3p mimics. (F) After 48 h of MALAT1-shRNA1 or miR-22-3p inhibitors treatment, the protein levels of p-AKT, AKT and Casp3 were detected by WB. *Wt* wild type, *Mut* mutant, *NC* negative control, *Con* control, *M* miR-22-3p mimics, $^{*}p < 0.05$.

inhibitors (Fig. 4D). These data indicate that MALAT1 protects endothelial cells partly via competing with miR-22-3p, which results in the upregulation of CXCR2.

3.5. MALAT1/miR-22-3p/CXCR2 signaling is involved in the AKT pathway

Previous results have demonstrated that IL-1 and IL-8 modulate the functions of endothelial cells through the CXCR2/AKT pathway [5,6,20,24]. There was an inverse relationship between the quantity of live cells and IL-1/IL-8 following treatment with different concentrations of ox-LDL (Fig. 5A). Furthermore, MALAT1 inhibition attenuated the expression of IL-1/IL-8 in the ox-LDL treated ECs (Fig. 5B). These results indicate that CXCR2 plays a key role in protecting the endothelium from ox-LDL-induced injury. Although the total AKT protein level was unaffected in response to IL-8 treatment, the phosphorylation of AKT was increased. This phenomenon disappeared after co-transfection with CXCR2-siRNA3 (Fig. 5C). Interestingly, AKT1 is also a target gene of miR-22-3p (Fig. 5D). The overexpression of miR-22-3p significantly decreased the AKT protein level (Fig. 5E). Furthermore, the decreased expression of AKT in response to MALAT1 silencing could be offset by the miR-22-3p inhibitors (Fig. 5F). Taken together, our results suggested that the AKT pathway might be downstream of MALAT1/miR-22-3p/CXCR2 signaling during the protection of endothelial cells from ox-LDL.

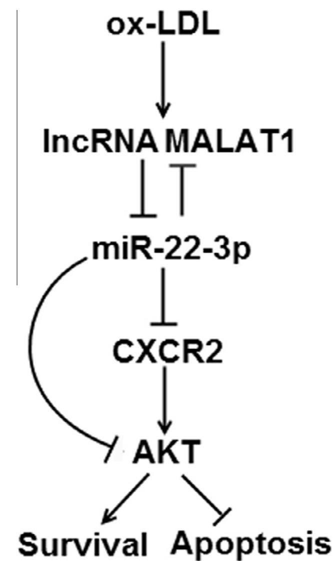

Fig. 6. The mechanism schematic. Extracellular deposition of ox-LDL induces high expression of MALAT1. Then, MALAT1 "sponges" miR-22-3p to upregulate the target genes CXCR2 and AKT. Finally, high expression of CXCR2 resists ox-LDL-induced endothelial cells dysfunction via the AKT pathway.

4. Discussion

Ox-LDL and a large number of inflammatory cytokines (i.e., the CXC chemokine family) are associated with the progression of atherosclerosis [25,26]. The deposition of ox-LDL in the endothelial barrier is an important early step in atherosclerosis [25]. Previous results have suggested that ox-LDL might promote atherosclerosis through the upregulation of CXCR2, which is a G protein-coupled receptor for chemokines [27,28]. However, other reports demonstrated that CXCR2 protected the endothelial barrier against injury by inflammatory factors during the early stage of atherosclerosis [7,8]. These reports indicate that CXCR2 may play different roles during the different stages of atherosclerosis. Therefore, it is necessary to understand the mechanism underlying the CXCR2-induced regulation.

Recently, lots of studies have advocated the important role that lncRNAs play in the regulation of gene expression. For examples, lncRNA CCAT1 levels are significantly higher in colorectal cancer [29] and promote gallbladder cancer development via negative modulation of miRNA-218-5p [10]. HOTAIR is also overexpressed in several cancers and is associated with their differentiation and aggressiveness through competition with miRNAs [30–32]. Although emerging evidence suggests that the lncRNAs participate in the progression of cancers, the roles lncRNAs play in cardiovascular diseases are poorly understood. One study noted that lincRNA-p21 might act as a novel regulator of vascular smooth muscle cell proliferation and promote neointima formation [33]. A similar report suggested that the lncRNA APF plays a key role in the regulation of autophagic cell death and myocardial infarction via targeting miR-188-3p [34]. Therefore, investigations regarding the crosstalk between lncRNAs and miRNAs could deepen our understanding of the mechanisms underlying cardiovascular diseases, which are the most prominent causes of death worldwide.

The well-conserved lncRNA MALAT1 is not only upregulated in a variety of tumors [13–15,35] but also highly expressed in endothelial cells [16]. Although MALAT1 functions in multiple pathways [14,36,37], the ceRNA hypothesis provides new insight into gene regulation. Previous results have shown that several miRNAs, such as miR-101, miR-217, miR-125b and miR-9, were negatively correlated with MALAT1 [15,35,38]. The binding of miRNAs to MALAT1 decreases miRNA levels and is accompanied by increases in the expression of miRNA target genes [39,40]. This finding indicates that MALAT1 acts as a sponge for different miRNAs in a cell-type dependent manner. However, the crosstalk between involving MALAT1, CXCR2 and miRNAs with respect to ox-LDL-induced endothelial dysfunction remains unknown. Here, we observed that the MALAT1/miR-22-3p/CXCR2 signaling pathway protected endothelial cells from ox-LDL-induced endothelial dysfunction in the setting of atherosclerosis.

In this study, the interaction among the lncRNA MALAT1, CXCR2 and miR-22-3p suggested that a potential link might exist among them. We demonstrated that the expression of MALAT1 and miR-22-3p among patients with UA was consistent with their expression changes in endothelial cells following 24 h of ox-LDL treatment. Subsequently, luciferase assay and RIP experiments showed that direct binding existed between MALAT1 and miR-22-3p. Moreover, the miR-22-3p binding site was indispensable for the attenuation of endothelial dysfunction by MALAT1. These findings suggested that MALAT1 modulated the expression of CXCR2, at least in part, by competing with miR-22-3p.

Although emerging examples of ceRNA have been reported, many questions still need to be answered. One of the key considerations concerning the crosstalk between lncRNAs and miRNAs is their abundance. The expression levels of both the lncRNAs and miRNAs would affect the ceRNA effectiveness [41]. lncRNAs with a low abundance have a limited inhibitory effect on specific miRNAs. Similarly, the regulatory effects would not be affected if the miRNAs shared a low expression level in the cells [42]. Therefore, in this study, we focused on the well-conserved lncRNA MALAT1, which is highly expressed in endothelial cells [16]. Our results suggested the possibility of a ceRNA mechanism in endothelial cells. Another important concern is the crosstalk levels. Hundreds of target genes could be regulated by one miRNA, and one lncRNA may "sponge" multiple miRNAs [43,18]. Thus, mechanism behind ceRNA is not a single-strand contact but rather a largely unknown network mode. Although more research needs to be done, the findings in our study may inspire future atherosclerosis research.

In summary, our study demonstrates the interactions among the lncRNA MALAT1, miR-22-3p and CXCR2 in ox-LDL-induced endothelial dysfunction. MALAT1 protects the endothelial barrier from injury via inhibiting the expression of miR-22-3p and upregulating the expression of CXCR2, which is associated with the AKT pathway (Fig. 6). These results may provide new insight for the prevention and cure of atherosclerosis disease.

Disclosure

The authors have no conflicts of interest to disclose.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.08.046.

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