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Long noncoding RNA Chaer mediated Polycomb Repressor Complex 2 (PRC2) activity to promote atherosclerosis through mTOR signaling

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Abstract. – OBJECTIVE: Recent studies highlighted long noncoding RNAs (lncRNAs) have been implicated in many biological processes and diseases. However, atherosclerosis is a major risk factor for cardiovascular disease, but the detailed mechanism of atherosclerosis progression remained unclear. In this study, we mainly focused on the role of lncRNA Chaer in atherosclerosis.

PATIENTS AND METHODS: RT-PCR was used to detect the expression of lncRNA Chaer in atherosclerosis patients and animal model. Moreover, the expression of Chaer in vascular smooth muscle cell dysfunction model was also measured. Proliferation ability was tested by CCK-8 and cyclin D1 assay, through loss- and gain-of-function approaches. Western-blot was used to measure the expression of H3 lysine 27 methylation, when Chaer was in different levels. RIP and ChIP assay discovered an interaction between Chaer and PRC2 through mTOR signaling.

RESULTS: Here we identified a heart-enriched long non-coding RNA, named Cardiac Hypertrophy Associated Epigenetic Regulator (Chaer). We found that the Chaer was highly expressed in serum samples from 28 patients with atherosclerosis, compared with 28 healthy volunteers. Chaer was dramatically upregulated in atherosclerotic plaques of ApoE-/- mice. We also found that the expression of Chaer was upregulated in vascular smooth muscle cell injury model. Through loss- and gain-of-function approaches, we showed that Chaer promotes cell proliferation and induces apoptosis *in vitro.* Mechanistically, Chaer interacts with Polycomb Repressor Complex 2 (PRC2) through inhibiting histone H3 lysine 27 methylation. Further, this interaction is induced upon mTOR signaling pathway.

CONCLUSIONS: According to the results, we found that lncRNA Chaer was closely related to the progression of atherosclerosis, which could be a previously uncharacterized lncRNA-dependent epigenetic checkpoint.

Key Words:
LncRNA Chaer, PRC2, Atherosclerosis, mTOR signaling.

Abbreviations

BSA= bovine serum albumin, DMEM= Dulbecco's Modified Eagle's Medium, ECL= enhanced chemiluminescence, FBS= Fetal bovine serum, GAPDH= glyceraldehyde 3-phosphate dehydrogenase, PMSF= phenylmethylsulfonyl fluoride, PVDF= polyvinylidene difluoride, RIPA= radioimmunoprecipitation assay, RT-PCR= reverse transcriptase-polymerase chain reaction, SDS-PAGE= sodium dodecyl sulphate-polyacrylamide gel electrophoresis, TBST= Tris Buffered Saline-Tween, TGF-α= Transforming Growth factor-α, PBS= phosphate buffered saline, PCR= polymerase chain reaction.

Introduction

Atherosclerosis is one of the most common chronic inflammations including stroke and coronary heart disease, and one of the leading causes of mortality worldwide[1-3]. It is considered to be a kind of vascular disorder resulting from the interaction between modified lipoproteins, monocyte-derived macrophages, T cells, and the normal cellular elements of the arterial[4-6]. As we know, long noncoding RNAs (lncRNAs) are ~200-nucleotide (nt)-long RNA molecules and stably exist in the plasma and urine, with disease and tissue specificity and without protein-coding potential. They constitute a large portion of the

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mammalian transcriptome, since only ~2% of the mammalian genome is composed of genes that encode proteins[7-9]. Recently, the role of lncRNAs in the heart has just begun to be uncovered. LncRNAs have demonstrated functional roles in the adult murine cardiac system and in human cardiovascular cells, and have strong prognostic potential as biomarkers in heart failure patients[10-12]. Of note, a number of lncRNAs are involved in epigenetic regulations, termed as Epi-lncRNAs, by directly interacting with epigenetic modifiers[13,14]. HOX antisense intergenic RNA binds to PRC2 and promotes H3K27me3 at the promoter region of target genes[15]. Genome function is highly regulated at chromatin level by epigenetic modifications, Methylation at histone H3 lysine 4 (H3K4) by trithorax group (TrxG)/mixed-lineage leukemia (MLL) complex represents a hall mark for gene activation, while methylation at H3 lys27 (K27) by polycomb repressive complex 2 (PRC2) leads to gene silence[16,17]. Changes of chromatin modifications have been demonstrated in heart diseases; however, the underlying molecular mechanism remains elusive. According to Wang et al[18], we identify a novel cardiac enriched lncRNA, named as cardiac hypertrophy associated epigenetic regulator (Chaer). However, understanding of Chaer in atherosclerosis is still unknown. In this study, we aimed at examining the functional role of lncRNA Chaer in the pathogenesis of atherosclerosis, as well as to disclose molecular mechanisms. Firstly, we measured the expression of Chaer in the serum of 28 patients with atherosclerosis. Then, proliferation ability was tested by CCK-8 and cyclin D1 assay, through loss- and gain-of function approaches. After that, RIP and ChIP assay discovered an interaction between Chaer and PRC2 through mTOR signaling. Our study, therefore, uncovered a critical function of lncRNA Chaer in atherosclerosis.

Patients and Methods

Patients

28 serum samples from patients with atherosclerosis and 28 serum samples from healthy volunteers were collected from the Third Affiliated Hospital of Chongqing Medical University Hospital in Chongqing (Chongqing, China) from June 2016 to July 2017. Enrollment included 28 patients, 38 to 76 years old, with carotid atherosclerosis as defined by B-mode ultrasonography and LDL cholesterol between the 60th and 90th percentiles[19]. All of the patients were well informed and signed informed consents. The research protocol was approved by the Institutional Review Board of the Third Affiliated Hospital of Chongqing Medical University (Chongqing, China). Information about the atherosclerosis patients and healthy volunteers were listed in **Supplementary Table I**.

Animals

The whole procedure was performed according to the National Institutes of Health (NIH) Animal Use Guidelines. 20 ApoE-/- mice were obtained from (Beijing HFK Bioscience Co., Ltd., Beijing, China). Mice received a high-fat diet, and were kept in the standard specific-pathogen-free (SPF) environment, with 12 hours light and 12 hours dark cycle at a stable temperature of 20-25°C. These mice were sacrificed after 16 weeks, and the atherosclerotic plaque and adjacent tissue were kept in the liquid nitrogen for further use.

Cell Culture

Human umbilical vein endothelial cell line HUVEC and human vascular smooth muscle cell line HA-VSMC were purchased from ATCC (Manassas, VA, USA) and cultured following the instructions of manufacturers. The cells were cultured at 37°C in a humidified incubator with 5% CO_2 and 95% air, and maintained in Dulbecco's Modified Eagle's Medium (DMEM) (HyClone, South Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (100 UI/mL and 100 μg/mL) (Beyotime, Shanghai, China).

RNA Extraction and Real-time Quantitative PCR Assays

Total RNA from clinical samples and cells was extracted by using RNAiso Plus (TaKaRa, Otsu, Shiga, Japan), following the manufacturer's protocol. Reverse transcription polymerase chain reaction (RT-PCR) was performed using PrimeScript™ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's protocol. The levels of mRNA expression were quantified by standard Real-time PCR protocol with SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan). GAPDH was used as a reference gene. The gene specific primers were listed in **Supplementary Table II**.

Construction of Plasmid, siRNA and Cell Transfection

The full-length of human Chaer cDNA was synthesized and sub-cloned into a pCDNA3.1

(Invitrogen, Carlsbad, CA, USA) vector, resulting in Chaer-pcDNA for its overexpression. For small interfering RNAs (siRNAs) analysis, three Chaer siRNAs and negative control siRNA (si-NC) were provided by Invitrogen (Carlsbad, CA, USA) and the siRNA sequences targeting the sequence of Chaer transcript were as follows: si-Chaer-1#5'– GGAAACACTTCAGCTATG-ACA-3';si-Chaer-2#5'-GAGCCAAAAACCAA-CAAGGA-3';si-Chaer-3#5'-GTGAAGGAA-CAGGGTGTGCT-3'. Cells were pre-incubated to 40-60% confluence on a six-well plate and then transfected by incubation with plasmids or siRNAs with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocols. At indicated time point post transfection, cells were harvested for further analysis.

CCK8 Assays

The target cells were seeded into 96-well plates, with the density of 2000 cells in each well. Three replicate wells were set in each group. CCK8 assay was carried out as follows: 10 ul of Cell Counting Kit 8 (Dojindo, Kumamoto, Japan) was added into 100 ul of DMEM medium in each well, which was co-cultured at the darkness for 2 hours at 37°C. The 96-well plate was placed at the absorbance of 450 nm. The data was collected for 5 days. The whole experiment was repeated three times.

Western Blotting

Target cell was harvested at 80-90% density in cell culture bottle. Phosphate-buffered saline (PBS) was used to wash the target cells for three times to remove the rest cell culture media. Protein in the cell was extracted by RIPA lysis buffer containing PMSF (Beyotime, Shanghai, China). The concentration of protein was detected according to the standard bovine serum albumin (BSA) protein quantitation assay. 50 μg of samples were added to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% denaturing gel. The protein was transferred to polyvinylidene difluoride (PVDF) membrane (Millipore, Billerica, MA, USA) after electrophoresis, which was blocked in the 5% non-fat milk for 1 hour at room temperature. TBST (Boster, China) was used to wash these membranes, which were then put into the primary antibody at 4°C over night. Respective second antibody was used to incubate these membranes. Immunoblots were visualized by ECL chemiluminescent detection system. The protein bands were quantified using densitometry analysis in Quantity One software (Bio-Rad, Hercules, CA, USA), and were analyzed by using GraphPad Prism software (GraphPad Software, La Jolla, CA, USA).

RNA Immunoprecipitation

Cells were homogenized in adequate volumes of polysomelysis buffer (10 mM HEPES-KOH [pH 7.0], 100 mM KCl, 5 mM MgCl2, 25 mM EDTA, 0.5% IGEPAL, 2 mM dithiothreitol [DTT], 0.2 mg/ml heparin, 50 U/ml RNase OUT [Life Technologies, Waltham, MA, USA], 50 U/ml Superase IN [Ambion], 1× complete protease inhibitor tablet [Roche, Basel, Switzerland]). The suspension was centrifuged at 14,000 g at 4°C for 10 min to remove debris. Lysates containing 1 mg protein were incubated with 500 ng normal IgG (Cell Signaling Technologies, Danvers, MA, USA; #2729, 1:200), anti-SUZ12 (Cell Signaling Technologies, Danvers, MA, USA; #3737, 1:200), anti-Ezh2 (Cell Signaling Technologies, Danvers, MA, USA; #5246, 1:200), anti-WDR5 (Abcam, Cambridge, MA, USA; #56919, 1:200), or anti-LSD1 (Cell Signaling Technologies, Danvers, MA, USA; #2139, 1:200) at 4°C over night on an inverse rotator. Protein A sepharose beads (Life Technologies, 50 μl each) were first blocked in NT2 buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 1 mM MgCl2, 0.05% IGEPAL) with 5% BSA, 0.02% sodium azide and 0.02 mg/ml heparin at 4°C for 1 h, and then added into the lysates followed by 3-h incubation at 4°C on an inverse rotator. The beads were subsequently washed five times in NT2 buffer. RNAs were released by incubating in proteinase K buffer (50 mM Tris [pH 8.0], 100 mM NaCl, 10 mM EDTA, 1% SDS, 1 U/ml proteinase K) for 30 min at 65°C, and pelleted by adding equal volume of isopropyl and centrifuging at 12,000 g at 4°C for 10 min. After washing once with 75% ethanol, RNAs were reverse transcribed into first strand cDNA and used for Real-time RT-PCR analysis to detect indicated lncRNAs.

ChIP-PCR Assay

HA-VSMCs were fixed with 1% formaldehyde for 10 min at room temperature, and then quenched by125 mM glycine. The samples were homogenized in lysis buffer containing 20 mM Tris-HCl (pH8.0), 150 mM NaCl, 2 mM EDTA, 1% Triton X-100, 0.1% SDS, and 1× complete protease inhibitor tablet, and sonicated to generate chromatin samples with averaged fragment sizes of 200-1000 bp. After pre-cleared with Protein A

sepharose beads, samples were incubated with anti-H3K27me3 antibody (Cell Signaling Technologies, Danvers, MA, USA), anti-Ezh2 (Cell Signaling Technologies, Danvers, MA, USA (#5246) for ChIP; Santa Cruz Biotechnology. Santa Cruz, CA, USA (#292275) for ChIP or normal control IgG at 4°C over night on an inverse rotator. The antibody-chromatin complexes were then pelleted with BSA/Salmon sperm DNA blocked Protein A sepharose beads. After standard washes, the immunoprecipitated DNA was eluted and purified with PCR purification kit.

Statistical Analysis

All the data were expressed as the mean±SD. Each assay was applied at least three independent experiments or replicates. Comparisons in multiple groups were analyzed with one-way ANOVA, followed by Student's *t*-test to calculate the *p*-value between two groups. *p*-value<0.05 was considered statistically significant. All statistical analyses were performed by Post-Hoc test using SPSS 17.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5.0 (GraphPad Software, La Jolla, CA, USA).

Results

LncRNA Chaer was Highly Expressed in Patients with Atherosclerosis

To explore the effect of Chaer in atherosclerosis, Western blot analysis for lncRNA Chaer in mouse tissues shows highly enriched expression in the heart (Figure 1A). Then, we used qRT-PCR to detect the expression of lncRNA Chaer in serum samples from 28 patients, compared with those from 28 healthy volunteers. We found that the expression of Chaer was highly expressed in patients with atherosclerosis (Figure 1B). What's more, we examined the expression of lncRNA Chaer in aortic atherosclerotic plaques of ApoE-/- mice fed a high-fat diet, a widely used animal model of atherosclerosis. Indeed, we found that the expression of Chaer was substantially higher in the aortic plaques of ApoE-/- mice when compared with that of wild type control mice (Figure 1C). These results demonstrated that lncRNA Chaer was correlated with the procession of atherosclerosis, but the effect remained unclear.

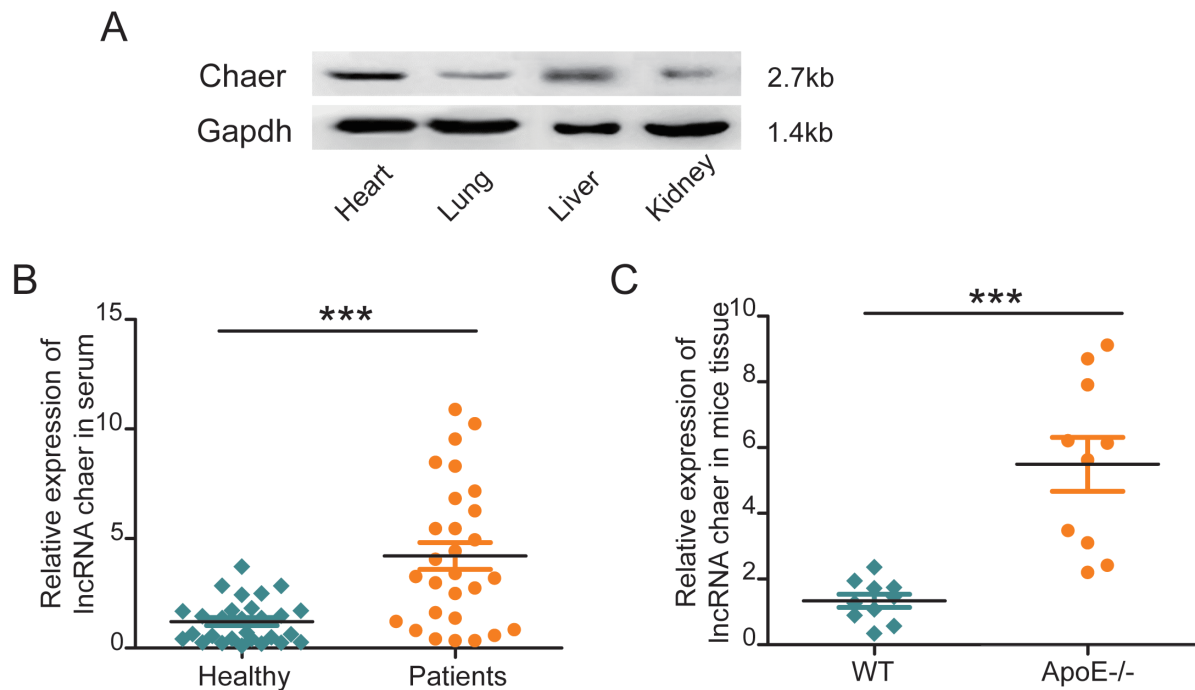

Figure 1. LncRNA Chaer was highly expressed in atherosclerosis. ***(A)*** Western blot analysis for lncRNA Chaer in mouse tissues. ***(B)*** qRT-PCR was used to detect the expression of lncRNA Chaer in serum samples from 28 patients with atherosclerosis, compared with those from 28 healthy volunteers. ***p<0.001. lncRNA Chaer expression in atherosclerotic plaques of ApoE knockout mice (ApoE-/-) and wild-type C57 control mice (WT) was measured by qRT-PCR. N=10. ***p<0.001.

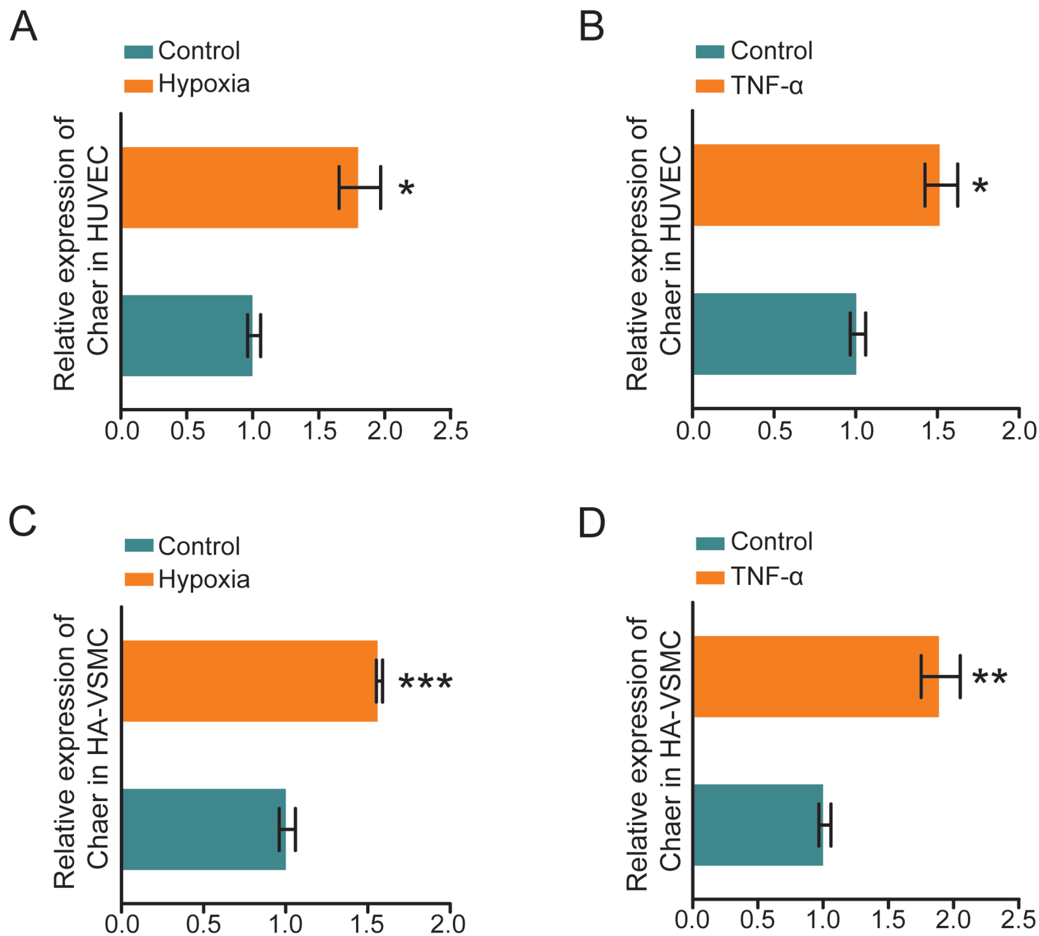

Figure 2. LncRNA Chaer was up-regulated in HUVEC cells induced with hypoxia or TNF-α. ***(A)*** The expression of Chaer was detected by qRT-PCR in hypoxia induced HUVEC cells. *$p<0.01$. ***(B)*** The expression of Chaer was detected by qRT-PCR in TNF-α (20 ng/ml) induced HUVEC cells. *$p<0.01$. ***(C)*** The expression of Chaer was detected by qRT-PCR in hypoxia induced HA-VSMC cells. ***$p<0.001$. (D) The expression of Chaer was detected by qRT-PCR in TNF-α (20 ng/ml) induced HA-VSMC cells. **$p<0.01$.

LncRNA Chaer was Upregulated in HUVECs and VSMCs Induced with Hypoxia or TNF-α

To determine the role of lncRNA Chaer, we would like to employ injury models to check it. The injury of endothelial cells plays an important role in the development of atherosclerosis and CAD. Firstly, human umbilical vein endothelial cell line HUVEC cells were induced with hypoxia condition (4% O_2) to simulate the anoxic environment. After 24 hours of treatment, the lncRNA expression profiles were detected using RT-PCR. Results revealed that the expression of lncRNA Chaer was upregulated in hypoxia induced HUVEC (Figure 2A). Then, the HUVEC was induced with TNF-α condition (20 ng/ml), lncRNA Chaer was also highly expressed (Figure 2B). Moreover, the same results come out with HA-VSMC cells, which were induced with hypoxia or TNF-α condition (Figure 2C-D). These data provide a valuable research orientation for subsequent experiments.

LncRNA Chaer Promoted the Proliferation and Suppressed Apoptosis of HUVECs and HA-VSMCs

To further demonstrate how Chaer contributes to atherosclerosis, we investigated the function of lncRNA Chaer in cell proliferation and apoptosis. We used human umbilical vein endothelial cell line HUVEC and the human vascular smooth muscle cell line HA-VSMC, both of which have

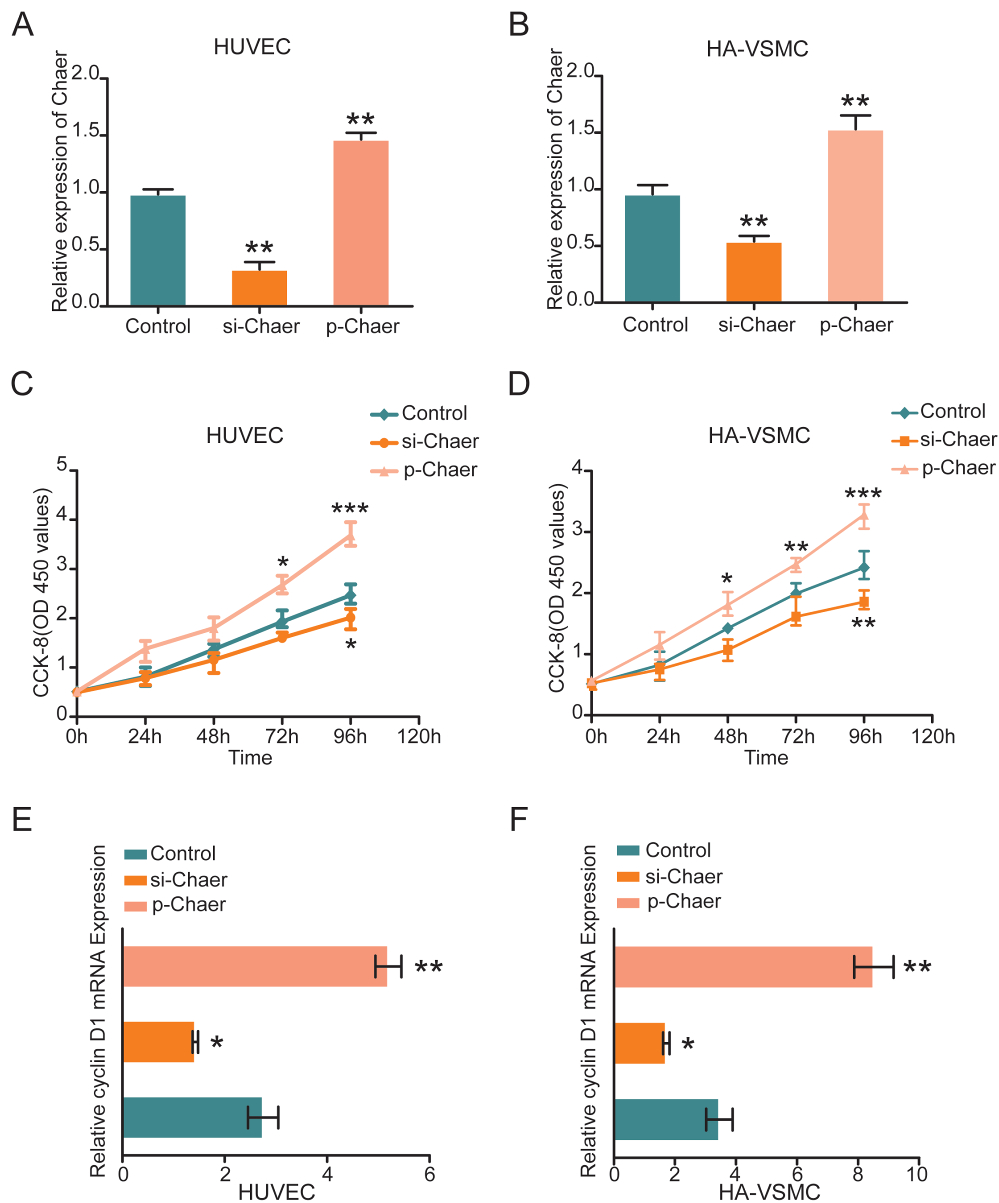

Figure 3. LncRNA Chaer regulated proliferation and apoptosis of HUVEC and HA-VSMC cells. ***(A) (B)*** Expression levels of lncRNA Chaer in HUVEC and HA-VSMC cells transfected with siRNA and plasmids. $^{**}p<0.01$. ***(C) (D)*** The proliferation and viability of HUVEC and HA-VSMC cells were measured using the Cell Counting Kit-8 (CCK-8) colorimetric assay after lncRNA Chaer knockdown or overexpressed. $^{***}p<0.001$, $^{**}p<0.01$, $^{*}p<0.05$. ***(E) (F)*** Different levels expression of lncRNA Chaer regulated the expression of cyclin D1. $^{**}p<0.01$, $^{*}p<0.05$.

been widely used to study atherosclerosis *in vitro*. Firstly, lncRNA Chaer expression levels in HUVEC and HA-VSMC transfected with siRNAs and plasmid were significantly down-regulated or upregulated compared to control group (Figure 3A-B). CCK8 and apoptosis assays were used to detect the proliferation and apoptosis ability of HUVEC and HA-VSMC. As a result, we found that lncRNA Chaer knockdown could decrease the proliferation ability, while lncRNA Chaer enforced expression could increase the proliferation ability in both cell lines (Figure 3C-D). What's more, elevated expression of lncRNA Chaer increased the expression of cyclin D1 (Figure 3E-F) by using the qRT-PCR. In conclusion, results revealed that lncRNA Chaer could improve pro-

liferation and inhibit apoptosis of HUVEC and HA-VSMC, which might accelerate the formation of atherosclerosis.

LncRNA Chaer Interacts with PRC2 through mTOR Signaling Pathway

As we have found that lncRNA Chaer could promote atherosclerosis via regulating the proliferation and apoptosis, but the molecular mechanism remained unclear. Wang et al[18] provided detailed insight into Chaer, their research implicated a potential involvement of chromatin remodeling. Histone methylation has been implicated in transcriptome reprogramming during atherosclerotic diseases[20-22]. To explore the potential mechanism, Chaer deficiency in HA-VSMC specifically increased di- and tri-methylation at H3K27 without affecting the levels of di-methylation at H3K4 or H3K9 sites (Figure 4A), while Chaer over-expression specifically reduced H3K27 tri-methylation without detectable impact on other histone methylations (Figure 4B). Di- and tri-methylation at H3K27 are catalyzed by the histone methyltransferase PRC2, which is a well-known molecular target of several regulatory lncRNAs[23,24]. Using RNA immuno-precipitation (RIP) assay, we detected a remarkable enrichment of Chaer in the interactome with PRC2 components SUZ12 and Ezh2 but not with TrxG/MLL component WDR5 or LSD1 (Figure 4C). Moreover, PRC2 chromatin immuno-precipitation (ChIP) assay showed that TNF-α treatment in HA-VSMC decreased PRC2 binding to the promoter regions of proliferation genes Anf, Myh7 and Acta1 (Figure 4D), which could be reversed by Chaer inactivation (Figure 4E). Over-expressing Chaer in HA-VSMC was also sufficient to reduce PRC2 targeting to these genes (Figure 4F). Consequently, Chaer knockdown reversed the TNF-α triggered reduction of H3K27me3 levels at the proliferation genes (Figure 4GH), while Chaer overexpression was sufficient to significantly decrease the H3K27me3 levels (Figure 4I). The interaction between Chaer and PRC2 complex upon proliferation stimulation indicates a dynamic signaling cascade involved in this process. The mTOR signaling pathway, indicated by downstream ribosomal protein S6 kinase (S6K) phosphorylation[25], was rapidly activated following TNF-α treatment in HA-VSMC (Figure 4J). Indeed, mTOR inhibition by either rapamycin[26] completely blocked the TNF-α-induced enhancement of Chaer-PRC2 interaction (Figure 4KL). These data establish an interaction between Chaer and PRC2 at the onset of proliferation stimulation; however, the Chaer-PRC2 interaction is a mTOR-dependent event.

Discussion

Long non-coding RNAs (lncRNAs) are a type of vital ncRNAs, participating in the series of epigenetic regulation. It has been verified that numerous identified lncRNAs play important regulating roles in cardiovascular diseases. In addition, lncRNAs can regulate the vessel function and growth and control phenotype of the smooth muscle cells. Li et al[27] demonstrated that higher expression of BANCR could up-regulate VSMCs proliferation and migration partly through activating the JNK pathway. Wang et al[28] showed that the overexpression of circulating exosomes and exosomal lncRNA HIF1A-AS1 could result from the activation of endothelial cells and vascular smooth muscle cells. Pan et al[29] reported that lncRNA H19 was highly expressed in atherosclerosis, which could be might regulate atherosclerosis through MAPK and NF-kB signaling pathway. These studies addressed that lncRNAs were important in regulating atherosclerosis and other cardiovascular diseases. In our study, we found that lncRNA Chaer was highly expressed in the serum of patients with atherosclerosis, as well as in the aortic plaques of ApoE-/- mice. We also found that the expression of Chaer was upregulated in VSMCs induced with hypoxia or TNF-α. Through loss- and gain-of function approaches, we showed that Chaer promotes cell proliferation and induces apoptosis *in vitro*. Further studies need to show how to identify those detailed functions and mechanisms. LncRNAs are likely powerful treatment targets because they intensively interact with their genetic environment, placing them as crucial regulators of genetic networks. Han et al[30] identified that the lncRNA Mhrt, which originates from MYH7 locus, is cardioprotective, and the restoration of Mhrt levels protects the heart from hypertrophy and failure. Zhang et al[31] reported that lncRNA CCAT1 could serve as a scaffold for two distinct epigenetic modification complexes and modulate the histone methylation of the promoter of SPRY4. Sun et al[32] found that lncRNA HOXA11-AS could interact with EZH2, LSD1 and DNA methyltransferase 1 (DNMT1) to exert oncogenic functions in GC. Ma et al[33] showed that lncRNA SNHG15 could inhibit P15 and KLF2 expression to promote pancreatic cancer proliferation through EZH2-mediated H3K27me3. These studies indicated that

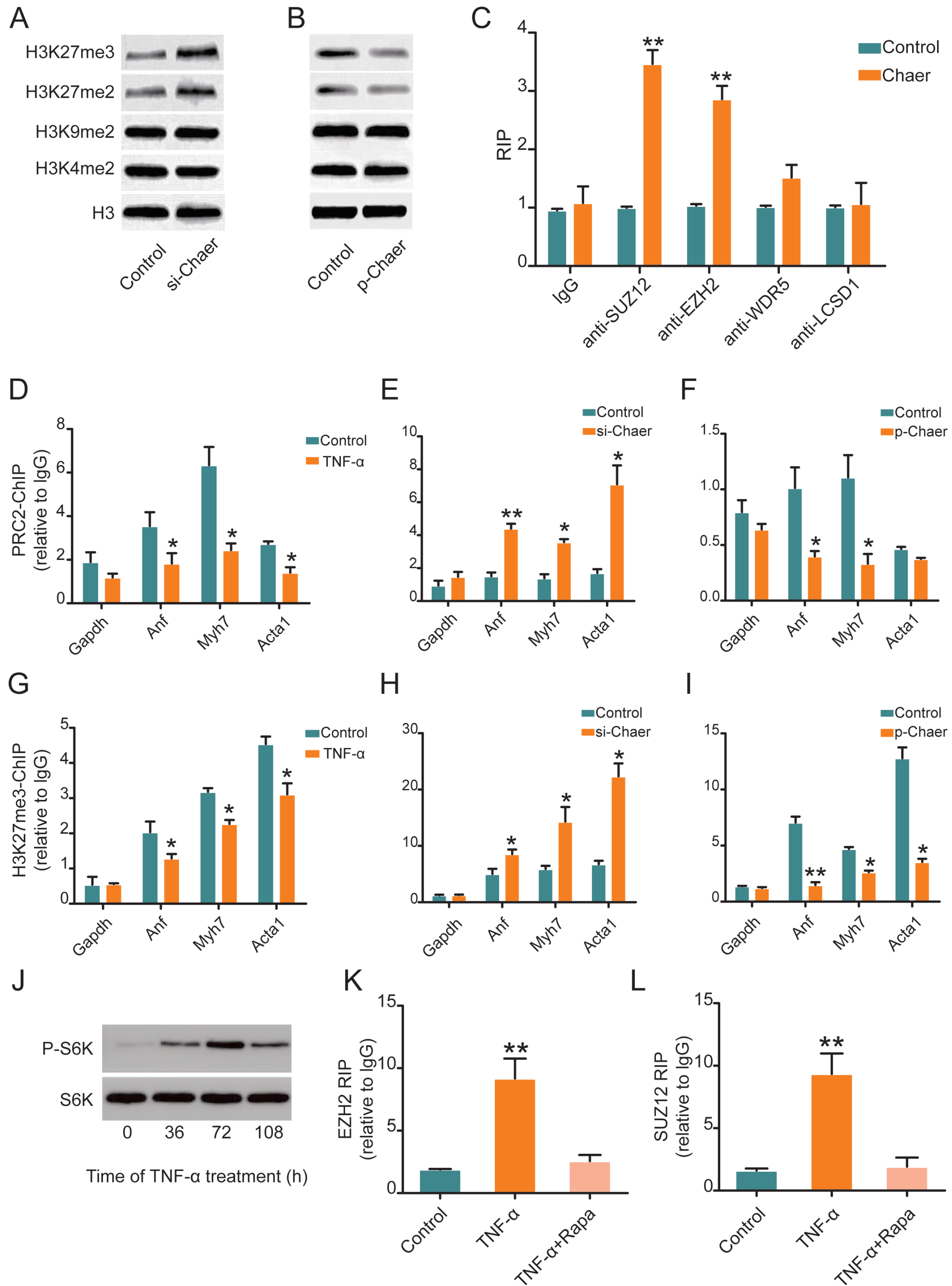

Figure 4. LncRNA Chaer interacts with PRC2 through mTOR signaling pathway. ***(A) (B)*** Effects of Chaer knock-down or over-expression on H3 methylation were measured by WB in HA-VSMC cells. ***(C)*** RNA immune-precipitation (RIP) analysis using antibodies followed by qRT-PCR for Chaer. Values were normalized to corresponding normal IgG groups. $^{**}p<0.01$. ***(D)(G)*** Chromatin immune-precipitation (ChIP) analysis with anti-Ezh2 antibody or anti-H3K27me3 antibody for the promoter regions of Anf, Myh7 and Acta1 in HA-VSMCs with and without TNF-α treatment. $^{*}p<0.05$. ***(E) (F) (H) (I)*** TNF-α-treated HA-VSMCs transfected with Chaer knockdown or overexpressed by ChIP analysis. $^{**}p<0.01$, $^{*}p<0.05$. ***(J)*** Immunoblotting analysis for ribosomal protein S6 kinase (S6K) phosphorylation in HA-VSMCs treated with TNF-α at different time points. (K) (L) RIP analyses using anti-Ezh2 and antiSUZ12 antibodies for Chaer-PRC2 interaction in non-treated and TNF-α-treated HA-VSMCs (72 h) with or without Rapa. $^{**}p<0.01$.

lncRNAs, like protein-coding genes, also contribute significantly to tissue specific responses at least in part via epigenetic regulations. Here we identified a heart-specific lncRNA Chaer as a critical regulator of atherosclerosis via interaction with PRC2. Using RIP assay, we detected a remarkable enrichment of Chaer in the interactome with PRC2 components SUZ12 and Ezh2 but not with TrxG/MLL component WDR5 or LSD1. This suggests that Chaer interaction antagonizes other PRC2 binding lncRNAs and relieves its suppressive function to target genes. Moreover, ChIP with PRC2 showed that TNF-α treatment in HA-VSMC decreased PRC2 binding to the promoter regions of proliferation genes, which could be reversed by Chaer inactivation. These data establish that interactions between Chaer and PRC2 at the onset of proliferation stimulation are sufficient to release pathological gene suppression by H3K27 tri-methylation in atherosclerosis. These findings are remarkable because they indicate that modulation of the activity of non-coding RNAs such as lincRNA Chaer may be a novel therapeutic approach to treat human cardiovascular disease.

Conclusions

LncRNA Chaer was highly expressed in patients with atherosclerosis and atherosclerotic plaque from ApoE-/- mice. Furthermore, our findings firstly uncovered that lncRNA Chaer could promote the proliferation ability of atherosclerosis by suppressing PRC2 activity through mTOR signaling pathway. Our study indicated that lncRNA Chaer might be used as a promising prognostic marker and a potential target for treating atherosclerosis.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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