Article

Discovery Medicine 2024; 36(182): 571–580
https://doi.org/10.24976/Discov.Med.202436182.53

Knockdown of LINC01128 Downregulates FUT8 to Inhibit OxLDL-Induced Vascular Smooth Muscle Cell Apoptosis in Atherosclerosis

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Published: 20 March 2024

Background: The apoptosis of vascular smooth muscle cells (VSMCs) contributes to the progression of atherosclerosis (AS). Long intergenic non-protein coding RNA 1128 (LINC01128) has been implicated in AS, and this study aims to uncover the role and mechanism of LINC01128 in regulating oxidized low-density lipoprotein (oxLDL)-induced VSMCs.
Methods: The position of LINC01128 in cells and its target genes were predicted using bioinformatics. The localization of LINC01128 in human VSMCs was determined through fluorescence *in situ* hybridization. VSMCs were transfected, and the interaction between LINC01128 and fucosyltransferase 8 (FUT8) was validated by chromatin immunoprecipitation assay. The apoptotic VSMC model was established using oxLDL. LINC01128 expression in VSMCs was analyzed by quantitative real-time polymerase chain reaction (qRT-PCR), and FUT8 expression was detected by qRT-PCR and western blot. VSMC viability, migration, invasion abilities, and apoptosis were assessed using cell counting kit-8, transwell assay, and flow cytometry, respectively.
Results: OxLDL (200 μg/mL) upregulated the expression of LINC01128 and FUT8 mRNA, as well as FUT8 protein, in VSMCs. LINC01128 was expressed in the nucleus of VSMCs and bound to FUT8. Knockdown of LINC01128 alleviated the inhibitory effects of oxLDL (200 μg/mL) on viability, migration, and invasion, and mitigated the promotion of apoptosis and FUT8 expression in VSMCs. On the other hand, FUT8 overexpression enhanced the suppressive effects of oxLDL (200 μg/mL) on viability, migration, and invasion activities, and amplified the facilitating effect of oxLDL on apoptosis in VSMCs. Moreover, FUT8 overexpression reversed the impact of LINC01128 silencing on viability, migration, invasion, and apoptosis in oxLDL-stimulated VSMCs.
Conclusion: The knockdown of LINC01128 downregulates FUT8, inhibiting the progression of VSMCs in AS.

Keywords: LINC01128; fucosyltransferase 8; vascular smooth muscle cells; apoptosis; atherosclerosis

Introduction

Atherosclerosis (AS) stands as the primary cause of global morbidity and mortality, often resulting in severe conditions like ischemic heart disease, myocardial infarction, and stroke [1]. This condition is characterized as a chronic inflammatory disorder marked by the accumulation of lipids, leading to artery narrowing and thrombosis. Multiple pathological mechanisms contribute to the initiation and progression of AS plaques, encompassing inflammation, lipid metabolism disorders, hemodynamic changes, and oxidative stress [2,3].

AS lesions, known as plaques, are characterized by the accumulation and transformation of lipids, smooth muscle cells (SMCs) and necrotic cell debris in the intima of blood vessels [4]. Vascular smooth muscle cells (VSMCs) play a crucial role in maintaining vascular homeostasis by mediating vessel contraction and relaxation, as well as producing extracellular matrix (ECM) [5]. They actively participate in regulating the formation and development of AS by influencing vessel and plaque biomechanics [6].

In the advanced stages of AS plaques, the apoptosis of VSMCs increases the vulnerability and risk of plaque rupture. This occurs by thining the fibrous cap of plaques and augmenting the lipid burden in the plaques [7]. Additionally, remnants of apoptotic VSMCs are retained in plaques as matrix vehicles (MVs) and serve as nucleating structures for bone formation. This process results in plaque microcalcification, further contributing to the development and rupture of plaques [8].

Long non-coding RNAs (lncRNAs) have been implicated in the regulation of cellular apoptosis by directly or indirectly modulating protein complexes and microRNAs (miRNAs) [9]. As transcripts with more than 200 nucleotides, lncRNAs can modulate gene expression and molecular functions through versatile mechanisms [10]. Cytoplasmic lncRNAs act as competitive endogenous RNAs (ceRNAs), binding to microRNAs and targeting downstream genes [11]. Additionally, lncRNAs interact with proteins to modulate gene expression through transcriptional regulation [12]. Growing evidence indicated

that lncRNAs play a role in the occurrence and progression of AS and can serve as diagnostic biomarkers and therapeutic targets for AS [13]. Long intergenic non-protein coding RNA 1128 (LINC01128) has been implicated in AS progression, and its expression is upregulated in the blood of patients with unstable AS plaques [10,14]. However, its working mechanism in AS remains unclear. Analysis from the LncATLAS database indicated that LINC01128 expression is localized in the nucleus, excluding ceRNA modulation from the mechanisms of LINC01128-mediated AS development. In this case, we further investigated the target genes of LINC01128 using the LncECell database, among which fucosyltransferase 8 (FUT8) is associated with AS.

FUT8 belongs to the fucosyltransferase family and plays a crucial role in N-glycan core fucosylation, a mechanism involved in the process of vascular calcification (VC) [15,16]. In the context of AS, VC is associated with increased mortality and AS plaque rupture [17]. A previous study has demonstrated that FUT8 is upregulated in a phosphate-stimulated VSMC calcification model, and targeting FUT8-mediated core fucosylation helps alleviate VC [18].

Based on these findings, we hypothesized that LINC01128 may promote AS progression by regulating FUT8-mediated VSMC apoptotic death. To investigate the effect of the LINC01128/FUT8 signaling pathway on VSMC apoptosis, oxidized low-density lipoprotein (oxLDL) was used in this study to establish an *in vitro* apoptosis model [19].

Materials and Methods

Bioinformatic Analysis

The lncATLAS database (http://lncatlas.crg.eu/) was employed to predict the cellular expression pattern of LINC01128. In summary, the localization of the selected gene was displayed on the website for various cell types and cellular compartments upon entering the gene name. Subsequently, the analysis of LINC01128 target genes was conducted using LnceCell (http://bio-bigdata.hrbmu.edu.cn/LnCeCell) through the "Cell Network" function.

Cell Culture and Transfection

Human aortic VSMCs (BFN60810800, BLUEFBIO, Shanghai, China) were cultured in Dulbecco's Modified Eagle's Medium (DMEM, PM150270, Procell, Wuhan, China) supplemented with 10% fetal bovine serum (FBS, 164210-50, Procell, Wuhan, China) and 1% Penicillin-Streptomycin-Amphotericin B Solution (PB180121, Procell, Wuhan, China). The cells were maintained at 37 °C in a MyTemp™ incubator (Z742534, Merck, Darmstadt, Germany) with 5% CO_2. All cells were routinely tested for STR identification and mycoplasma contamination and were mycoplasma-free.

The FUT8 overexpression plasmid (RC223075) and the negative control of overexpression plasmid pCMVA-Entry vector (NC, PS100001) were obtained from OriGene Technologies (Rockville, MD, USA). The LINC01128 overexpression plasmid, LINC01128-specific short hairpin RNA (shLINC01128, GGGATTACAGGTTACATTAGTTA), and shRNA negative control pRS vector (shNC, TR20003, TTAAAATTATGTTTTAAAATGGA) were designed and synthesized by the same company. VSMCs were seeded at 80% confluency and transfected with shNC, shLINC01128, NC, LINC01128 overexpression plasmid, or FUT8 overexpression plasmid alone. Additionally, co-transfections were performed with shLINC01128 plus FUT8 overexpression plasmid, shLINC01128 plus NC, shNC plus NC, or plasmid overexpressing FUT8 plus shNC using Lipofectamine™ 3000 Transfection Reagent (L3000001, Invitrogen, Carlsbad, CA, USA) at 37 °C for 48 h.

Fluorescence in Situ Hybridization (FISH)

The localization of LINC01128 in VSMCs was detected using a FISH kit (Bes1002, BersinBio, Guangzhou, China). VSMCs were immobilized with a 4% paraformaldehyde solution (P0099, Beyotime, Shanghai, China) for 20 min at room temperature (RT) and washed twice with DEPC reagent. Cells were permeabilized with 0.5% Triton X-100 in phosphate-buffered saline (PBS) and treated with proteinase K working solution for 15 min at 37 °C. Subsequently, cells were fixed in paraformaldehyde for 10 min and dehydrated in serial ethanol before prehybridization with 20 µL hybridization solution for 30 min at 37 °C.

LINC01128-specific FISH probe, designed and provided by Ribobio (Guangzhou, China), was labeled with digoxigenin (DIG, DIUTPS-RO, Roche, Basel, Switzerland). DIG-labeled probes (1 µL) were mixed with a hybridization solution (39 µL) on ice. The mixture (20 µL) was then subjected to denaturation with cells at 75 °C for 8 min and incubated overnight at 42 °C, shielding from light. Afterward, the hybridized mixtures were washed with 50% formamide in 2 × saline sodium citrate (SSC), reacted with Alexa Fluor 488-conjugated anti-DIG (IC7520G, 1:10, R&D systems, Minneapolis, MN, USA) overnight at 4 °C, and mounted with 4′,6-diamidino-2-phenylindole (DAPI, ab104139, Abcam, Cambridge, UK). Fluorescence images were captured using a confocal microscope (LSM700, ZEISS, Oberkochen, Germany) at a magnification of ×250, and the scale bar was set at 100 µm.

Chromatin Immunoprecipitation (ChIP)

VSMCs were transfected with either the negative control (NC) or LINC01128 overexpression plasmid. The interaction between LINC01128 and FUT8 within cells was determined using a ChIP kit (Bes5001, BersinBio, China). A total of 1×10^7 VSMCs were crosslinked in 10 mL PBS

(C0221A, Beyotime, Shanghai, China) with 1% formaldehyde (252549, Sigma-Aldrich, Darmstadt, Germany) at RT for 10 min, followed by a 5-min mixture with 1.375 M Glycine at RT. After washing with ice-cold PBS, cells were centrifuged at 1000 ×g for 5 min at 4 °C and lysed in Lysis Buffer on ice to obtain the supernatant (10% was used as Input). Cell lysates were sonicated and subjected to a 1-h incubation with 75 µL Agarose beads at 4 °C.

Subsequently, samples (0.8 mL) were probed with anti-FUT8 (3 µg, sc-271244, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or control mouse immunoglobulin G (IgG, 1 µg/µL, sc-2025, Santa Cruz Biotechnology, CA, USA) overnight at 4 °C, and then incubated with 20 µL protein A/G-beads at RT for 30 min. After being washed and eluted, samples were heated at 65 °C for 6 h to reverse crosslinks. DNA was purified and subjected to quantitative real-time polymerase chain reaction (qRT-PCR) to detect the relative LINC01128 enrichment.

OxLDL Preparation and Apoptosis Induction

LDL (L3486) from human plasma was procured from Invitrogen (Carlsbad, CA, USA). Oxidized LDL (oxLDL) was prepared by dialyzing against 5 µM $CuSO_4$ (PHR1477, Merck, Darmstadt, Germany) in PBS for 6 h at 37 °C. The oxidation reaction was halted by adding 100 µM ethylene diamine tetraacetic acid (EDTA, ST1303, Beyotime, Shanghai, China) in PBS, and the degree of oxidation was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) [20].

To induce VSMC apoptosis, both normal and transfected VSMCs were subjected to oxLDL stimulation. For normal cells, oxLDL was used at concentrations of 100 µg/mL or 200 µg/mL, and for transfected cells, the concentration was 200 µg/mL. The stimulation was carried out for the appropriate durations at 37 °C [19].

Western Blot

VSMCs were exposed to oxLDL for 24 hours. Total proteins were extracted from cells using M-PER™ Mammalian Protein Extraction Reagent (78503, Thermofisher, Waltham, MA, USA) and quantified using a Pierce™ BCA Protein Assay Kit (23225, Thermofisher, USA). The proteins were then separated by SDS-PAGE Buffer (20315ES20, YEASEN, Shanghai, China) and loaded onto PVDF membranes (ISEQ00010, univ, Shanghai, China).

After a 1-h blocking with 5% skim milk at RT, the membranes were probed with anti-FUT8 antibody (1:1000, 66 kDa, ab198741, Abcam, UK) or the loading control GAPDH (1:2500, 36 kDa, ab9485, Abcam, UK) overnight at 4 °C. Subsequently, the membranes were incubated with goat anti-rabbit IgG (1:2000, ab6721, Abcam, UK) at RT for 1 h. Bands were visualized using the ECL Substrate Kit (ab133406, Abcam, UK) and detected by ChemiDoc Touch Imager (BIO-RAD, Hercules, CA, USA). The intensity of protein bands was quantified using ImageJ software (3.0 version, National Institutes of Health, Bethesda, MA, USA), with GAPDH serving as the loading control.

qRT-PCR

VSMCs were either transfected or exposed to oxLDL induction for 24 h. Total RNAs were isolated from the cells using TriPure™ Isolation Reagent (11667157001, Roche, Switzerland). The synthesis of cDNA and qRT-PCR was carried out using the TaqMan One-Step RT-qPCR Kit (T2210, Solarbio, Beijing, China) on the Thermal Cycler Dice™ Real-Time System III (TP950, TaKaRa, Shiga, Japan) with the following parameters: 95 °C for 3 min, followed by 35 cycles of 10 s at 95 °C, 20 s at 62 °C, and 1 min at 72 °C.

Data were analyzed using the $2^{-\Delta\Delta CT}$ method, and mRNA levels were normalized to GAPDH. The primers used in this assay are listed as follows: LINC01128: 5′-CAGCAGTCAGTTCGTTACCTTGG-3′ (F), 5′-CTATGTCCTGGGTCGTCCAAGA-3′ (R); FUT8: 5′-GACAGAACTGGTTCAGCGGAGA-3′ (F), 5′-GCAGTAGACCACATGATGGAGC-3′ (R); GAPDH: 5′-CCAGCCGAGCCACATCGCTC-3′ (F), 5′-ATGAGCCCCAGCCTTCTCCAT-3′ (R).

Cell Counting Kit (CCK)-8

Cell viability was assessed using a CCK-8 kit (C0037, Beyotime, Shanghai, China). VSMCs were seeded into 96-well plates at a density of 2×10^3 cells/well and either transfected or treated with oxLDL for 0, 24, 48, or 72 h. Subsequently, 10 µL of CCK-8 solution was added to each well and incubated for 4 h at 37 °C. The absorbance was measured at 450 nm using a microplate reader (Infinite 200 PRO, Tecan, Mannedorf, Switzerland).

Transwell Assay

VSMCs were transfected or stimulated by oxLDL for 24 h. We used an HTS 24-well insert plate (354144, Corning Life Sciences, Corning, NY, USA) to detect cell migratory activity, while 24-well BioCoat™ cell culture inserts pre-coated with matrigel (354480, Corning, NY, USA) were adopted for the analysis of cell invasiveness.

VSMCs (1×10^4) were trypsinized and plated in 200 µL serum-free culture medium on apical chambers with noncoated or matrigel-coated membranes, while the lower chambers were filled with 600 µL complete culture medium containing 10% FBS. After a 24-h incubation at 37 °C, VSMCs passing through the membrane filter underside were fixed in 4% formaldehyde and stained with 0.1% crystal violet (C0121, Beyotime, Shanghai, China). Migrated/invaded VSMCs were counted and imaged under a BX51 light microscope (OLYMPUS, Tokyo, Japan) (magnification ×250, scale bar = 50 µm).

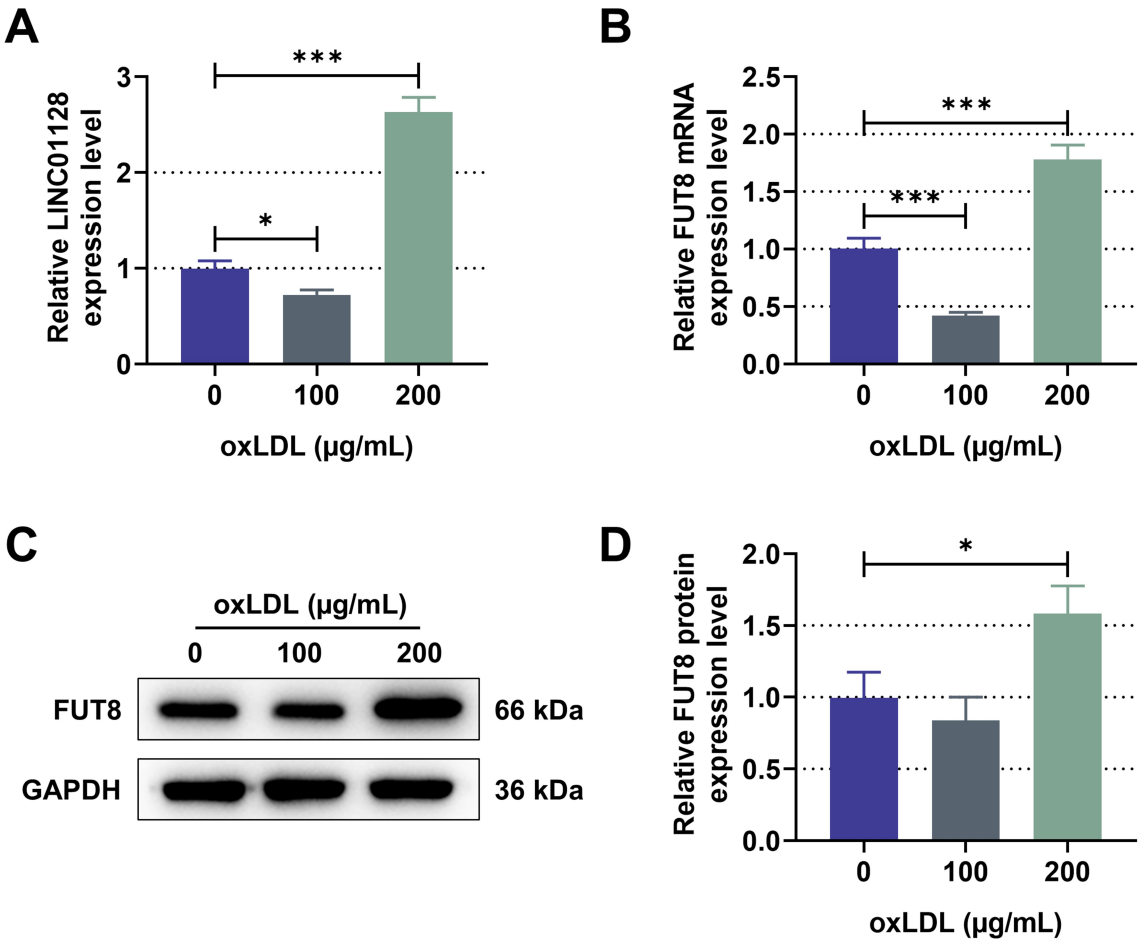

Fig. 1. OxLDL at 200 μg/mL upregulated expression of LINC01128 and FUT8 in VSMCs. (A,B) LINC01128 (A) and FUT8 (B) mRNA expression levels in VSMCs were analyzed by qRT-PCR and normalized to GAPDH. (C,D) Western blot assay was carried out to detect FUT8 protein expression in VSMCs and GAPDH served as an inner control. 0, 100, 200 oxLDL (μg/mL): human VSMCs exposed to 0, 100 or 200 μg/mL of oxLDL for 24 h at 37 °C. Abbreviations: oxLDL, oxidized low-density lipoprotein; LINC01128, long intergenic non-protein coding RNA 1128; FUT8, fucosyltransferase 8; VSMCs, vascular smooth muscle cells; qRT-PCR, quantitative real time-PCR. Linked groups were compared: $^{*}p < 0.05$, $^{***}p < 0.001$.

Flow Cytometry

VSMCs were transfected or exposed to oxLDL for 24 h. Cellular apoptosis was examined using an eBioscience™ Annexin V Apoptosis Detection Kit (BMS500FI-300, Invitrogen, Carlsbad, CA, USA). VSMCs were washed with PBS and resuspended in 200 μL Binding Buffer (2×10^5 cells/mL). Then, 195 μL of the cell suspension was mixed with 5 μL Annexin V-FITC for 10 min at RT. After being washed and suspended in Binding Buffer (190 μL), the mixture was treated with 10 μL Propidium Iodide, and Annexin V-FITC signal was detected by a CytExpert flow cytometer (Beckman Coulter, Brea, CA, USA).

Statistical Analysis

Statistically significant difference analysis ($p < 0.05$) was conducted using GraphPad Prism 8.0 (GraphPad, San Diego, CA, USA). All data were expressed as mean ± standard deviation (SD), collected from experiments repeated three times. For comparisons among multiple groups, one-way Analysis of Variance (ANOVA) was employed and repeated-measures ANOVA was used as the statistical method for repeated measures. The Bonferroni test was applied for post hoc analysis.

Results

OxLDL at 200 μg/mL Upregulated Expression of LINC01128 and FUT8 in VSMCs

The expressions of LINC01128 and FUT8 mRNA in VSMCs were reduced following the treatment with 100 μg/mL oxLDL (Fig. 1A,B, $p < 0.05$) and increased by 200 μg/mL oxLDL (Fig. 1A,B, $p < 0.001$). Furthermore, oxLDL (200 μg/mL) also elevated the FUT8 protein level in VSMCs (Fig. 1C,D, $p < 0.05$). OxLDL at the concentration

of 200 μg/mL exhibited promoting effects on the expressions of LINC01128 and FUT8, and was therefore utilized in the subsequent assays.

LINC01128 Knockdown Reduced oxLDL-Induced Inhibition of Viability, Invasion and Migration and Promotion of Apoptosis in VSMCs

LINC01128 knockdown significantly decreased LINC01128 mRNA expression in VSMCs compared with shNC (Fig. 2A, $p < 0.001$). Incubation with oxLDL for 24, 48, and 72 h impaired the viability of VSMCs (Fig. 2B, $p < 0.001$), while LINC01128 silencing reversed the inhibitory effect of oxLDL on cell viability (Fig. 2B, $p < 0.001$).

The migration rate and invasion rate in VSMCs that received oxLDL stimulation were reduced (Fig. 2C–E, $p < 0.01$). Inhibition of LINC01128 was demonstrated to offset oxLDL-mediated suppression of VSMC migration and invasion capabilities (Fig. 2C–E, $p < 0.01$). Flow cytometry results showed that oxLDL promoted the apoptosis rate of VSMCs (Fig. 2F,G, $p < 0.001$), which, however, was counteracted by shLINC01128 (Fig. 2F,G, $p < 0.001$).

FUT8 mRNA expression was elevated by oxLDL in VSMCs (Fig. 2H, $p < 0.001$). Compared with shNC-transfected VSMCs, the inhibition of LINC01128 diminished the oxLDL-induced increase of FUT8 expression in VSMCs (Fig. 2H, $p < 0.001$).

LINC01128 was Mainly Distributed in the Nucleus of VSMCs and Bound to FUT8 in VSMCs

According to the analysis of the lncATLAS database, LINC01128 was expressed in the nucleus of many cells (Fig. 3A). The targets of LINC01128 were obtained from the LnceCell database, and FUT8 was identified as a target gene of LINC01128 (Fig. 3B). FISH assay verified that LINC01128 was mainly localized in the nucleus of VSMCs (Fig. 3C). ChIP assay revealed that FUT8 antibody-induced LINC01128 enrichment in VSMCs overexpressing LINC01128 was higher than that in NC-transfected VSMCs (Fig. 3D, $p < 0.001$), indicating the interaction between LINC01128 and FUT8 in VSMCs.

ShLINC01128-Mediated Increase of Viability, Migration, Invasion and Decrease of Apoptosis in oxLDL-Induced VSMCs were Reversed by FUT8 Overexpression

In comparison to NC-transfected VSMCs, the transfection of the FUT8 overexpression plasmid elevated the FUT8 expression level in VSMCs (Fig. 3E, $p < 0.001$). FUT8 overexpression weakened the viability of oxLDL-treated VSMCs at 24, 48, and 72 h (Fig. 3F, $p < 0.05$) and reduced LINC01128 knockdown-mediated promotion of viability in oxLDL-induced VSMCs (Fig. 3F, $p < 0.001$). Inhibition of LINC01128 reversed the restraining effect of FUT8 overexpression on viability in oxLDL-stimulated VSMCs (Fig. 3F, $p < 0.05$).

The migratory ability and invasiveness of oxLDL-treated VSMCs were diminished by FUT8 overexpression (Fig. 4A–C, $p < 0.05$). FUT8 overexpression and LINC01128 knockdown counteracted each other's effect on cell migration and invasion in VSMCs that received oxLDL stimulation (Fig. 4A–C, $p < 0.05$). FUT8 overexpression facilitated the apoptosis of oxLDL-induced VSMCs (Fig. 4D,E, $p < 0.001$). Additionally, FUT8 overexpression and LINC01128 silencing offset the role of each other in cellular apoptosis in oxLDL-mediated VSMCs (Fig. 4D,E, $p < 0.001$).

Discussion

AS is a progressive disorder that can lead to cardiovascular diseases (CVDs) due to the accumulation of lipids in the arterial wall [21]. Despite significant advancements in the management of dyslipidemia, the incidence rate of AS-related CVD remains high, emphasizing the need for novel therapies targeting AS progression [22]. OxLDL plays a crucial role in AS by inducing VSMC apoptosis in AS plaques [8,23]. This study revealed that inhibiting LINC01128 could reverse oxLDL-induced cytotoxicity in VSMCs.

VSMCs are known to be present at all stages of AS and play a crucial role in modulating the development of atherogenic plaques [24]. It is well-established that VSMCs contribute to plaque stability, and a decrease in VSMC plaque content is indicative of increased plaque vulnerability [25]. The balance between VSMC migration, proliferation, death, and senescence determines the content of VSMCs within AS lesions [25]. The apoptosis of VSMCs leads to the loss of collagen and matrix, accumulation of cell debris, and intimal inflammation, promoting plaque destabilization, rupture, and ultimately driving AS development [26].

The role of lncRNAs in AS has gained significant attention, with lncRNAs being recognized as potential therapeutic targets against CVDs [27]. Targeting lncRNAs has been implicated in AS treatment by inhibiting apoptosis and promoting proliferation, migration, and invasion in VSMCs [28]. Previous studies have shown that individuals with diabetes are prone to AS plaques with calcification, suggesting that diabetes may facilitate the progression of severe AS lesions [29]. LINC01128 has been identified as a biomarker for the diagnosis and prognosis of diabetes and is considered a key lncRNA in AS [14,30]. It is enriched in the p53 signaling pathway, whose activation induces the apoptosis of plaque VSMCs [8,31]. Consistent with previous findings, our study demonstrated that LINC01128 was elevated in oxLDL-induced VSMCs. Moreover, knockdown of LINC01128 was found to alleviate oxLDL-mediated progression of VSMCs.

While previous research on the pro-proliferative and anti-apoptotic effects of LINC01128 mainly focused on the miRNA sponge mechanism [32], our study revealed

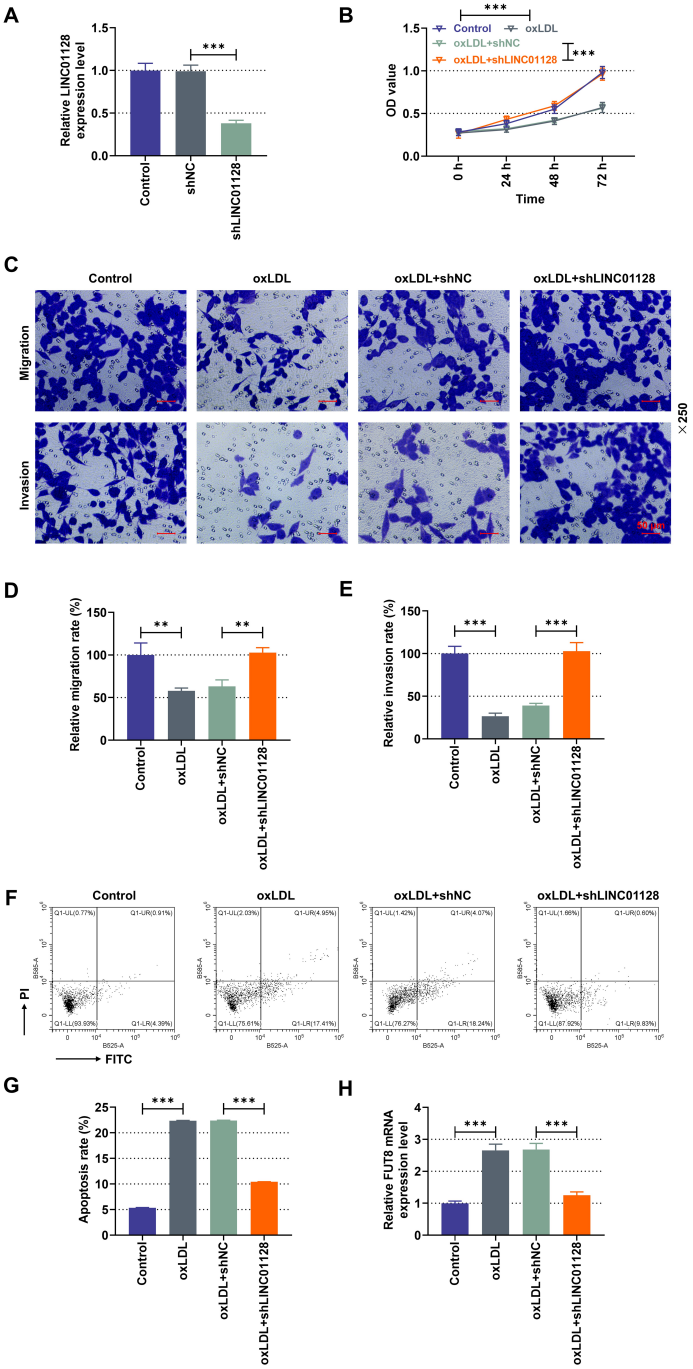

Fig. 2. LINC01128 knockdown reduced oxLDL-induced inhibition of viability, invasion, migration and promotion of apoptosis and FUT8 expression in VSMCs. (A) LINC01128 mRNA expression was measured by qRT-PCR and normalized to GAPDH. (B) CCK-8 assay was performed to assess cell viability in VSMCs at 0, 24, 48 and 72 h. (C–E) The migration (C,D) and invasion (C,E) of VSMCs were determined by transwell assay and images were captured under a light microscope (magnification ×250, scale bar = 50 μm). (F,G) VSMCs apoptosis was examined via flow cytometry. (H) FUT8 mRNA expression was measured by qRT-PCR and normalized to GAPDH. Control: normal VSMCs; shNC: shNC-transfected VSMCs; shLINC01128: shLINC01128-transfected VSMCs; oxLDL: VSMCs stimulated with 200 μg/mL oxLDL for proper times at 37 °C; oxLDL+shNC: shNC-transfected VSMCs exposed to 200 μg/mL oxLDL for proper times at 37 °C; oxLDL+shLINC01128: shLINC01128-transfected VSMCs exposed to 200 μg/mL oxLDL for proper times at 37 °C. (For CCK-8 assay, cells were stimulated with oxLDL for 0, 24, 48 and 72 h; for other experiments, cells received a 24-h incubation with oxLDL). Abbreviations: oxLDL, oxidized low-density lipoprotein; LINC01128, long intergenic non-protein coding RNA 1128; FUT8, fucosyltransferase 8; VSMCs, vascular smooth muscle cells; qRT-PCR, quantitative real time-PCR; shNC, negative control of short hairpin RNA; CCK-8, cell counting kit-8. Linked groups were compared: $^{**}p < 0.01$, $^{***}p < 0.001$.

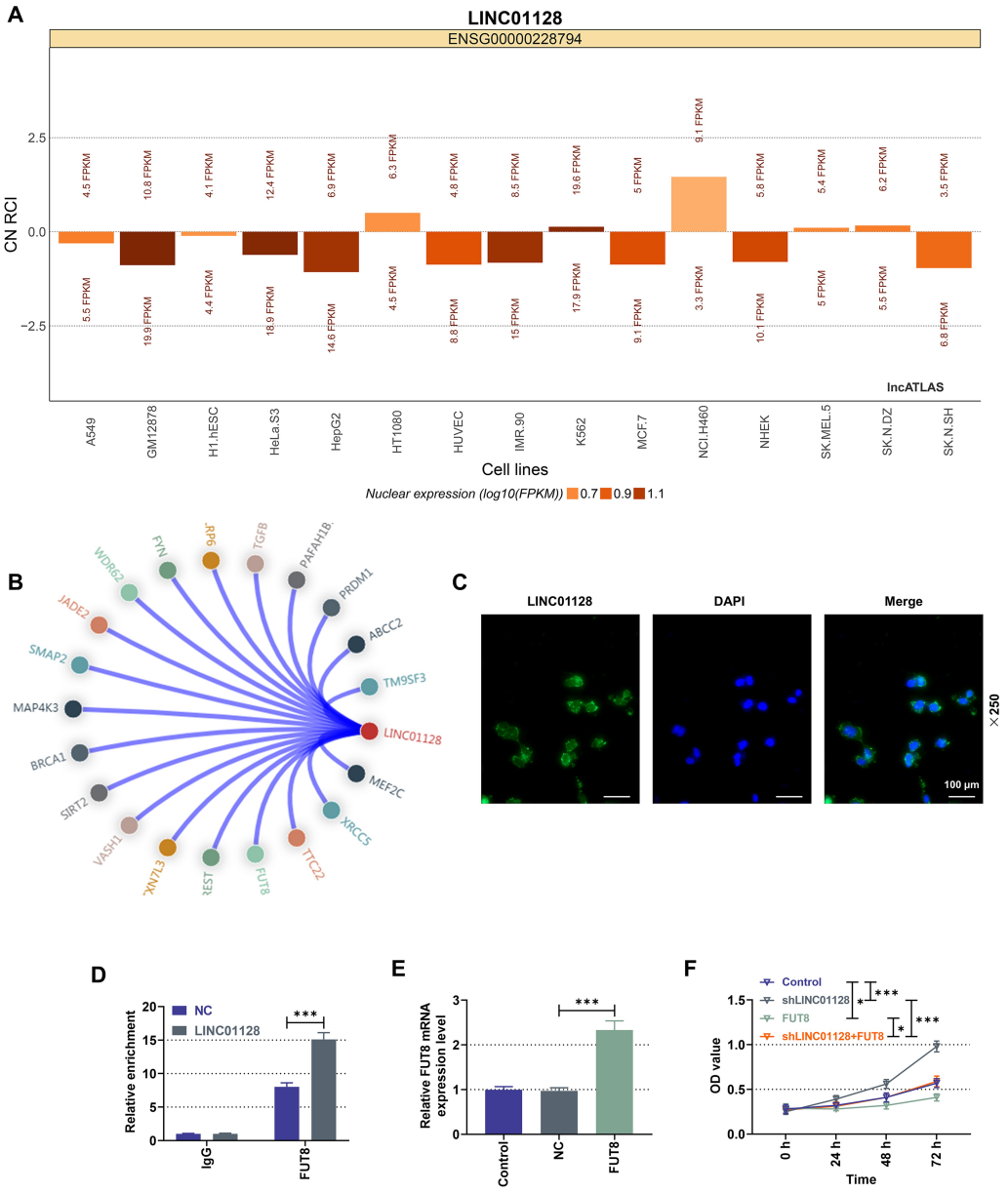

Fig. 3. LINC01128 was mainly localized in the nucleus of VSMCs and bound to FUT8 in VSMCs, and LINC01128 knockdown promoted the viability of oxLDL-stimulated VSMCs by downregulating FUT8. (A) The position of the LINC01128 expression in VSMCs was predicted by the lncATLAS database (http://lncatlas.crg.eu/). (B) LINC01128 target genes were analyzed by LnceCell (http://bio-bigdata.hrbmu.edu.cn/LnCeCell). (C) LINC01128 distribution in VSMCs was determined by FISH assay (green fluorescence, magnification ×250, scale bar = 100 μm). (D) LINC01128-FUT8 interaction in VSMCs was assessed by ChIP. Comparisons in Fig. 3D were performed using an independent sample *t*-test. (E) qRT-PCR was conducted to assess FUT8 mRNA expression in VSMCs with GAPDH as the loading control. (F) CCK-8 assay was performed to assess cell viability in VSMCs at 0, 24, 48 and 72 h. Control: normal VSMCs (D,E), VSMCs exposed to 200 μg/mL oxLDL for 0, 24, 48 and 72 h at 37 °C (F); NC: NC-transfected VSMCs; FUT8: FUT8 overexpression plasmid-transfected VSMCs (D,E), FUT8 overexpression plasmid-transfected VSMCs exposed to 200 μg/mL oxLDL for 0, 24, 48 and 72 h at 37 °C (F); LINC01128: LINC01128 overexpression plasmid-transfected VSMCs; shLINC01128: shLINC01128-transfected VSMCs exposed to 200 μg/mL oxLDL for 0, 24, 48 and 72 h at 37 °C; shLINC01128+FUT8: VSMCs co-transfected with shLINC01128 and FUT8 overexpression plasmid and then exposed to 200 μg/mL oxLDL for 0, 24, 48 and 72 h at 37 °C. Abbreviations: oxLDL, oxidized low-density lipoprotein; LINC01128, long intergenic non-protein coding RNA 1128; FUT8, fucosyltransferase 8; VSMCs, vascular smooth muscle cells; qRT-PCR, quantitative real time-PCR; NC, negative control of overexpression plasmid; FISH, fluorescence *in situ* hybridization; ChIP, chromatin immunoprecipitation; CCK-8, cell counting kit-8. Linked groups were compared: $^{*}p < 0.05$, $^{***}p < 0.001$.

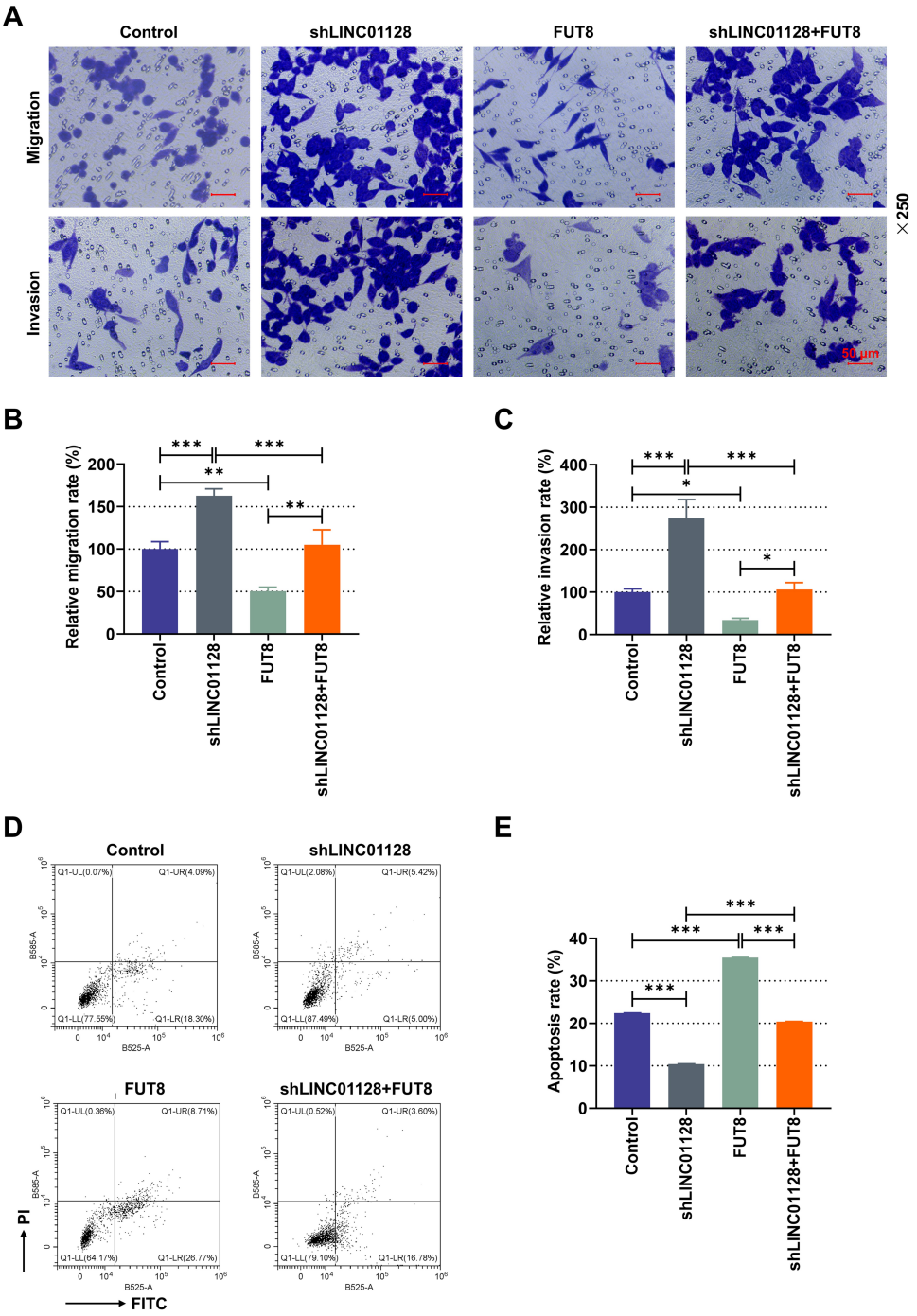

Fig. 4. LINC01128 knockdown-induced increase of cell migration and invasion activities and suppression of apoptosis in oxLDL-mediated VSMCs were offset by FUT8 overexpression. (A–C) Transwell assay was performed to assess the migratory (A,B) and invasive (A,C) activities of VSMCs, and images were observed under a light microscope (magnification ×250, scale bar = 50 μm). (D,E) VSMC apoptosis was examined by flow cytometry. Control: shNC and NC-transfected VSMCs exposed to 200 μg/mL oxLDL for 24 h at 37 °C; shLINC01128: shLINC01128 and NC-transfected VSMCs exposed to 200 μg/mL oxLDL for 24 h at 37 °C; FUT8: FUT8 overexpression plasmid and shNC-transfected VSMCs exposed to 200 μg/mL oxLDL for 24 h at 37 °C; shLINC01128+FUT8: VSMCs co-transfected with shLINC01128 and FUT8 overexpression plasmid and then exposed to 200 μg/mL oxLDL for 24 h at 37 °C. Abbreviations: oxLDL, oxidized low-density lipoprotein; LINC01128, long intergenic non-protein coding RNA 1128; FUT8, fucosyltransferase 8; VSMCs, vascular smooth muscle cells; qRT-PCR, quantitative real time-PCR; shLINC01128, LINC01128-specific short hairpin RNA; shNC, negative control of short hairpin RNA; NC, negative control of overexpression plasmid. Linked groups were compared: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

that LINC01128 is predominantly located in the nucleus of VSMCs. This suggests that it may influence VSMC progression through mechanisms other than ceRNA.

Beyond the lncRNA-miRNA network in AS, lncRNAs have also been reported to regulate the proliferation of VSMC through lncRNA-mRNA interactions with targeted genes [27]. To further investigate the working mechanism of LINC01128 in VSMC processes, we identified FUT8 as a potential target of LINC01128 through bioinformatic analysis and validated that LINC01128 could bind to FUT8 in VSMCs through ChIP. FUT8 is known to contribute to the exacerbation of AS, and oxLDL-induced upregulation of FUT8 is associated with decreased migratory activities of macrophages in AS [33]. Being the sole catalyzer of core fucosylation, FUT8 plays a crucial role in various diseases, including CVD and diabetes, where abnormal core fucosylation has been observed [34].

Evidence has shown that FUT8-mediated aberrant fucosylation is associated with weakened cell migration ability and cholesterol-enriched foam cell accumulation in the early stage of AS [35]. Additionally, FUT8 is involved in multiple biological processes such as cell proliferation, apoptosis, and metastasis via core fucosylation of epidermal growth factor receptors [15]. VC is a hallmark of AS and is commonly observed in patients with diabetes, leading to high morbidity and mortality in CVD [36]. Previous research has revealed that VC occurs after VSMC apoptosis and is positively regulated by apoptosis during AS [37]. In calcific VSMCs, silencing FUT8 leads to reduced core fucosylation, thereby alleviating VC [18].

In the current study, we found that FUT8 was upregulated by oxLDL, and its overexpression could exacerbate oxLDL-stimulated cell apoptosis and inhibition of viability, invasion, and migration in VSMCs, indicating its role in AS progression. Furthermore, LINC01128 could positively modulate FUT8, and LINC01128 knockdown and FUT8 overexpression offset each other's effects on the development of oxLDL-exposed VSMCs. These results suggest that LINC01128 may regulate FUT8 to affect the apoptosis, growth, migration, and invasion of VSMCs, thereby contributing to AS development.

Conclusion

In conclusion, this study demonstrated that the knockdown of LINC01128 can suppress VSMC apoptosis and increase cell viability, migration, and invasion by downregulating FUT8. Targeting the LINC01128/FUT8 pathway may serve as a promising method in AS prevention and management. However, the role of LINC01128 in AS models *in vivo* and the specific regulatory mechanism behind LINC01128-FUT8 signaling still need to be further studied.

Availability of Data and Materials

The analyzed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

GZN designed the research study; GZN and JX performed the research; GZN and JX collected and analyzed the data. GZN and JX have been involved in drafting the manuscript and have been involved in revising it critically for important intellectual content. GZN and JX give final approval of the version to be published. GZN and JX have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflict of Interest

The authors declare no conflict of interest.

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