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Original Article

Honokiol inhibiting proliferation of vascular smooth muscle cells by targeting lncRNA (BC033150) via FTO

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Abstract: As proliferation of vascular smooth muscle cells (VSMCs) plays an important role in the pathogenesis of atherosclerosis, the present study aims to explore new molecules which can inhibit the proliferation of VSMCs. Honokiol is identified as the most effective one which can inhibit the proliferation and promote the apoptosis of VSMCs in a time-dependent manner by cell counting kit-8 test and fluorescence-activated cell sorting. This process is associated with a reduction in the levels of FTO and relative RNA expression, identified by western-blot and qRT-PCR. Furthermore, after over-expression FTO in VSMCs, the expression of lncRNA (BC033150) is obviously increased. Meanwhile, the proliferation-inhibited and apoptosis-promoted effect of Honokiol can be attenuated by siRNA. These results indicate that Honokiol can inhibit VSMCs proliferation and promote apoptosis by targeting lncRNA (BC033150) via FTO, and the Honokiol-FTO-lncRNA axis is indispensible for Honokiol's role performance, suggesting Honokiol has potential to serve as a novel agent for atherosclerosis prevention and therapy.

Keywords: VSMCs, honokiol, FTO, LncRNA, atherosclerosis, proliferation

Introduction

Atherosclerosis leading to myocardial infarction and stroke is the major cause of death in the world. It is a chronic inflammatory condition that results from complex interactions of modified lipoproteins and various cell types including vascular smooth muscle cells (VSMCs) and monocyte-derived macrophages and so on [1]. VSMCs proliferation is thought to play an important role in the pathogenesis of both atherosclerosis and restenosis [2]. During their migration and intimal proliferation, VSMCs partially loose their contractile phenotype and acquire a synthetic one, involving the appearance of abundant endoplasmic reticulum and the Golgi apparatus and the secretion of proteoglycans and collagen [3]. Exploring new molecules, which can inhibit the proliferation and migration of VSMCs, bring great significance not only for better understanding of mechanism but also for strategy of pertinent therapeutic remedies.

Honokiol is a bioactive compound and obtained from several species of genus Magnolia (officinalis, obovata, and grandiflora) of Magnoliaceae family [4], which has been shown to exhibit pleiotropic anticancer effects in many cancer types. Studies have shown that honokiol is able to target many pathologically relevant pathways, including nuclear factor kappa B (NF-κB), signal transducers and activator of transcription 3 (STAT3), epidermal growth factor receptor (EGFR) and mammalian target of rapamycin (m-TOR), which have great relevance during cancer initiation and progression [5]. Honokiol exerts anti-proliferative activity with the cell cycle arrest at the G0/G1 phase and sequential induction of apoptotic cell death in a concentration-dependent manner [6]. However, little is known as to whether Honokiol targets proliferation and migration of VSMCs, if so, what is the mechanism of Honokiol performing its function. In this study, we investigate the effect of Honokiol on the proliferation and apoptosis of VSMCs, identified fat mass and obesity-associated (FTO) gene as the most suppressed gene by Honokiol.

FTO gene locates on chromosome 16. As one homolog in the AlkB family proteins, it is the

first mRNA demethylase that has been identified [7]. The discovery of the FTO-mediated oxidative demethylation of m6A in nuclear RNA initiate further investigations on biological regulation based on reversible chemical modification of RNA. Long non-coding RNAs (LncRNA), non-protein coding transcripts longer than 200 nucleotides, generally lack strong conservation, which is often cited as evidence of non-functionality [8]. However, accumulating evidence suggest that the majority of these are likely to be functional [9]. LncRNAs modulate the function of transcription factors by several different mechanisms, including functioning themselves as co-regulators, modifying transcription factor activity, or regulating the association and activity of co-regulators [10]. Local LncRNAs can also recruit transcriptional programs to regulate adjacent protein-coding gene expression [11]. The presence of appreciable amounts of m6A in lncRNAs suggests that m6A can also be important in cellular processes other than protein synthesis.

Considering the aforementioned roles of LncRNAs in the regulation of gene transcription, we hypothesize that Honokiol can regulate the proliferation and migration of VSMCs through regulation of gene transcription via targeting LncRNA. However, whether LncRNA involved the function of Honokiol is unknown. Here, we demonstrate the role of Honokiol in regulation of gene transcription and the expression of lncRNA by microarray, and also confirm LncRNA (BC033150) as the target lncRNA of Honokiol.

Materials and methods

Reagents and antibodies

Purified Honokiol was purchased from Quality Phytochemicals, LLC (Edison, NJ). Boyden Chambers and polycarbonate membranes (8 mm pore size) for cell migration assays were obtained from Neuroprobe (Gaithersburg, MD). The antibodies specific for FTO and GAPDH were purchased from Cell Signaling Technology (Beverly, MA). PI/Annexin V antibody kit (C1062) for FACS was from Beyotime biotech company (Shanghai, China).

Cell lines and cell culture

Human coronary artery smooth muscle cells (CASMCs) were purchased from Lonza (Walkersville, MD) which characterized by positive immunostaining for alpha-smooth muscle actin (α-SMA) and negative immunostaining for factor VIII. Cells were grown in smooth muscle basal medium (SmBM) supplemented with hEGF, insulin, hFGF-B and gentamin/amphotericin-B (SmGM-2 SingleQuots; Lonza), 5% (v/v) heat inactivated fetal bovine serum (Hyclone Defined FBS; Thermo Ficher Scientific, Waltman, MA), 100 U/mL penicillin, 100 μg/mL streptomycin and 2 mmol/L glutamine (Sigma-Aldrich, St Louis, MO, USA), in a humidified atmosphere with 5% CO_2 in air. Culture medium was changed every 2 days. Experiments were performed using cells between the 5th and 10th passage.

Immunofluorescence

After various experimental treatments, the adherent cultured cells were washed twice with cold phosphate buffered saline (PBS) and fixed in a paraformaldehyde solution (4% in PBS, pH 7.4) for 30 min at 4°C. The fixed cultures were then washed twice with PBS, treated with permeabilization solution (0.1% Triton X-100 in 0.1% sodium citrate) on ice for 2 min, and incubated in blocking buffer (3% BSA in TBST) for 1 h. The cells were then incubated with the indicated antibody in TBST containing 3% BSA overnight at 4°C. After incubation, the cells were washed and incubated with the indicated fluorescence-labeled secondary antibody in the dark at 37°C for 1 h. After washing, the cells were observed under a fluorescence microscope. Confocal images were captured under a Leica, TCS SP5 confocal microscope.

Cell viability assay

The effect of honokiol on the proliferative capacity or cell viability of the VSMCs was determined using CCK8 assay. A total of 10000 cells were plated into each well of a 96-well plate and cultured for 24 hours. After this the culture medium was removed, different concentration of honokiol media were added, and the VSMCs were cultured for 1, 2, or 3 days. Subsequently, 10 μL of CCK-8 was added to each well and incubated for 3 hours, and a microplate reader was used to detect absorbance at 450 nm.

Apoptosis assay

Apoptosis was measured using an Annexin V/fluorescein isothiocyanate (FITC) apoptosis

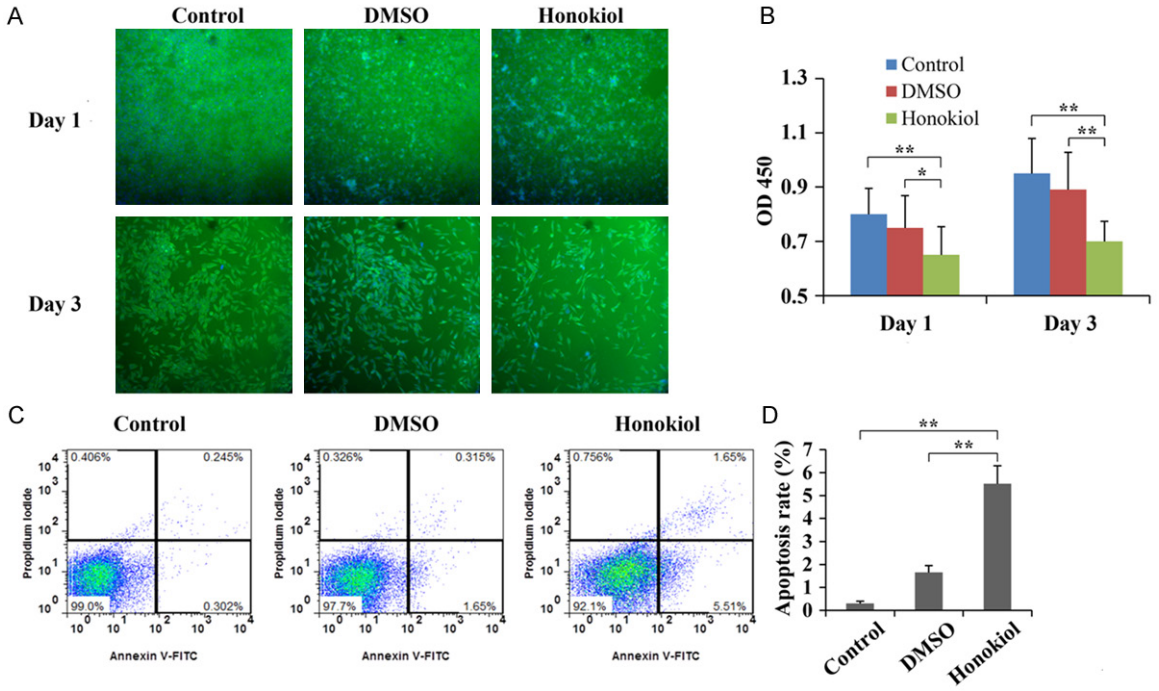

Figure 1. Honokiol inhibit the proliferation of VSMCs (A) with a time-independent manner (B), and promote the apoptosis of VSMCs (C, D). There was a significant difference in OD450 value between control group and Honokiol treated group ($P<0.01$). Moreover, the OD450 value was obviously lower at 72 h in Honokiol treated group than control group ($P<0.01$) (B). The apoptosis rate was obviously higher at 72 h in Honokiol treated group than control group or DMSO treated group ($P<0.01$) (C, D).

detection kit (Beyotime biotech company, China). Briefly, 2×10^5 cells cultured in 6-cm dishes were trypsinized, washed, stained with FITC-conjugated anti-Annexin V antibody under darkness for 15 min at room temperature, and then analyzed by flow cytometry (FAC-SCalibur; Becton-Dickinson).

Western blotting

VSMCs were treated with or without honokiol for desired time period, thereafter the cells were harvested, and lysed with ice-cold lysis buffer supplemented with protease inhibitors. Proteins were electrophoretically resolved on 8-10% Tris-Glycine gels and transferred onto a nitrocellulose membrane. After blocking the non-specific binding sites, the membrane was incubated with the primary antibody at 4°C overnight. Membranes were washed and then incubated with the peroxidase-conjugated secondary antibody and the specific protein bands were detected using the enhanced chemiluminescence reagents. To verify equal loading of proteins on the gels, the membrane was stripped and reported with either anti-GAPDH antibody.

Quantitative RT-PCR analysis

Osteosarcoma cell lines were used to isolate total RNA by using RNeasy kit according to the manufacturer's protocol (Qiagen, Valencia, CA). Briefly, first-strand cDNA was reverse-transcribed from 1 μg total RNA using the SuperScript First-Strand cDNA System (Invitrogen), and was amplified by Platinum SYBR Green qRT-PCR SuperMix-UDG (Invitrogen). A master mix was prepared for each PCR reaction, which included Platinum SYBR Green qPCR SuperMix-UDG, forward primer, reverse primer, and 10 ng of template cDNA. PCR conditions were 5 min at 95°C, followed by 40 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s.

PCR-assay

VSMCs' RNA was converted to cDNA using RT^2 first strand kit (SA Bioscience). Real-time PCR was done using Super Array RT^2 SYBR Green qPCR Master Mix (SA Bioscience) and RT^2 Profiler™ PCR Array contains gene-specific PCR arrays for a set of LncRNA genes relevant to FTO and three RNA quality control elements. PCR cycling conditions were as follows: initial

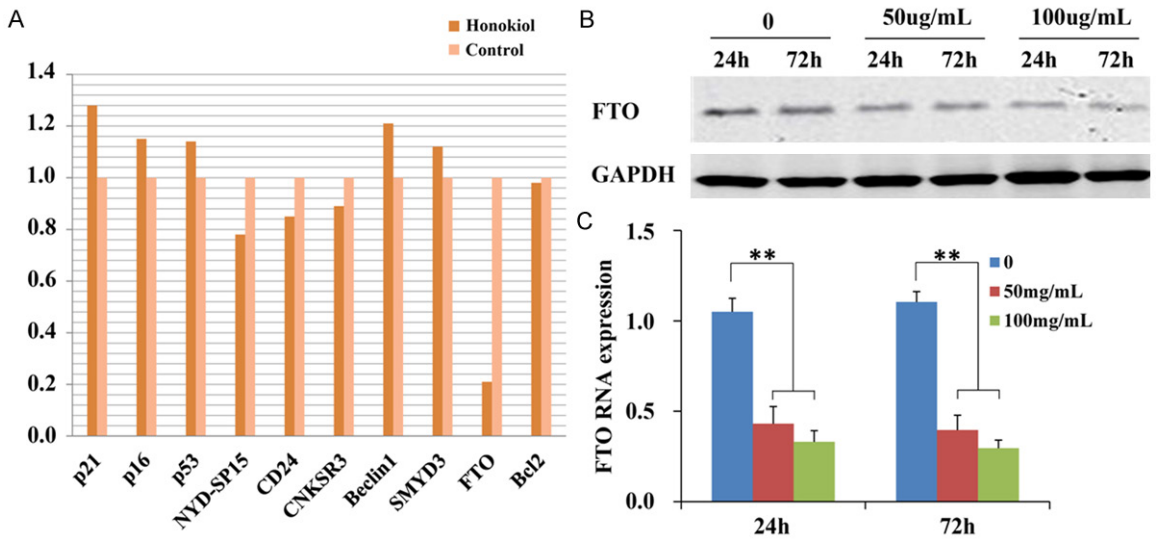

Figure 2. Gene expression of FTO is obviously inhibited by Honokiol (A), and also the RNA expression and protein level of FTO are down regulated by Honokiol (B, C).

denaturation at 95°C for 10 min, followed by 40 cycles of amplifications at 94°C for 15 s, 60°C for 60 s. Data were analyzed by PCR Array Data Analysis Web Portal using the default set format.

Choice of differentially expressed gene list using volcano plot and heat map analysis

We obtained the microarray date from Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/), and the GEO accession number is GSE39890. The date was generated using the genechip Affymetrix Human Genome U133 plus 2.0 Array (GEO accession number is GPL570), which completely coverage Human Genome U133 Set plus 6500 additional genes for analysis of over 47,000 transcripts. Significantly different expression genes were extracted by volcano plot analysis with the filtering criteria of a 10.0-fold change and P<0.001 using GeneSpring GX, version 7.3. The heat map of related LncRNA genes relevant to FTO was created using a method of hierarchical clustering by GeneSpring GX, version 7.3.

Statistical analysis

For cell apoptosis and viability assays, the data were compared among groups separately using one-way analysis of variance (ANOVA) using GraphPad Prism version 4.00 for Windows software (GraphPad Software, San Diego, California. www.graphpad.com.). A two-tailed *P* value of less than 0.05 was considered statistically significant.

Results

The effect of honokiol on the proliferation and apoptosis of VSMCs

First, we tested the effect of Honokiol on the proliferation and apoptosis of VSMCs. As shown in **Figure 1**, incubation of VSMCs with Honokiol for 24 h and 72 h in Boyden chamber resulted in an obvious inhibition of VSMCs proliferation. Additionally, a time-dependent manner was observed (**Figure 1B**): the inhibition-ratio of VSMCs at 72 h was obviously higher than at 24 h. There was a significant difference in OD450 value between control group and Honokiol treated group (P<0.01). Moreover, the OD450 value was obviously lower at 72 h in Honokiol treated group than control group (P<0.01). Contrary to proliferation, the apoptosis of VSMCs was promoted by Honokiol. As shown in **Figure 1C**, after treated by Honokiol, apoptotic cells of VSMCs was obviously increased. The apoptosis rate was around 5 percent after treatment by Honokiol, but was still maintain almost zero in the control. The apoptosis rate was obviously higher at 72 h in Honokiol treated group than control group or DMSO treated group (P<0.01). These data showed that Honokiol could be an effecting factor of inhibit-

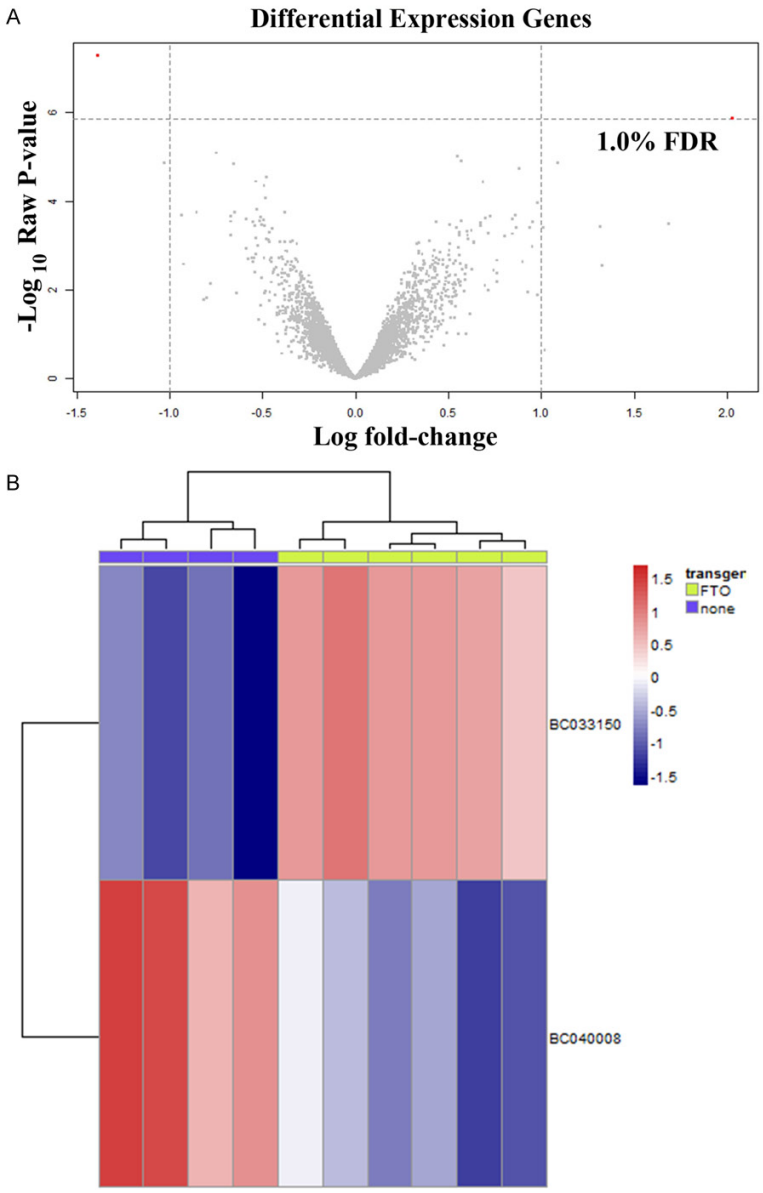

Figure 3. FTO promotes LncRNA (BC033150) expression in FTO overexpressed VSCMs. After overexpression of FTO in VSMCs, lncRNA (BC033150) in contrary to lncRNA (BC04008), in which lncRNA (BC033150) was up-regulated and lncRNA (BC04008) was down-regulated (A, B).

ing VSMCs proliferation and promoting apoptosis.

Identification of the most suppressed gene by honokiol from cell proliferation-associated genes

To further confirm the most suppressed gene by Honokiol, the PCR-array was adopted to screen from gene clusters associated with cell proliferation. As we can see from **Figure 2A**, the FTO was the greatest suppressed one, after screened from P21, P16, P53, NYD-SP15, CD24, CNKSR3, Beclin1, FTO and Bcl2. This also was confirmed by Western-Blot, level of FTO was decreased with an obvious dose-dependent manner but with an unclear time-dependent manner (**Figure 2B**). In addition, we confirmed that the FTO RNA expression in VSMCs was decreased after treated by Honokiol. There was a significant difference in FTO RNA expression between control group and Honokiol (50 or 100 mg/mL) treated group ($P<0.01$) (**Figure 2C**). From these data, we proposed that Honokiol performs its function via FTO.

Confirmation of the target lncRNA which involved in the function of FTO

As shown in **Figure 3A** and **3B**, the microarray data of pcDNA3 vector as control in the selection of differentially expressed genes related to FTO transfer. After removed the redundant and unannoated sequences, with FDR<1%, 1 gene was found to be significantly down-regulated and 1 genes to be significantly up-regulated ($P<0.0001$) in FTO-transfer group compared to pcDNA3 vector group (**Figure 3A**). We found that the expression of lncRNA (BC033150) at the highest level in FTO-transfer group (**Figure 3B**). These results suggested that both lncRNA (BC033150) and lncRNA (BC04008) were involved in the function of FTO, although an opposite expression pattern was observed. Meanwhile, FTO could regulate the expression of lncRNA (BC033150).

LncRNA (BC033150) was indispensable in honokiol-FTO-lncRNA axis

To further determine the exact role of LncRNA (BC033150) involved in Honokiol-FTO-lncRNA

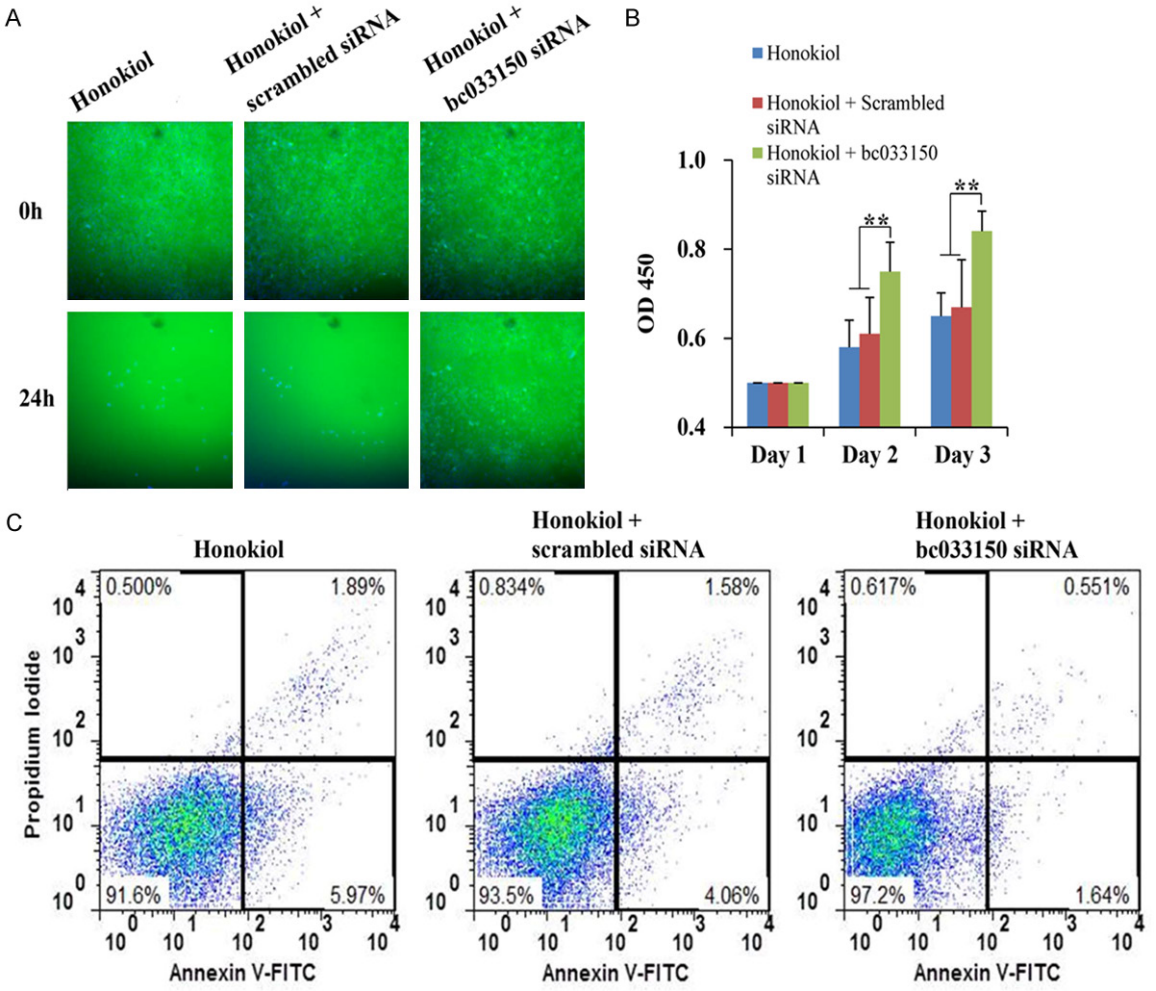

Figure 4. After lncRNA (BC033150) was silenced by target siRNA, the proliferation-inhibit (A, B) and apoptosis-promote (C) effect of Honokiol was attenuated. (A) Interference the expression of LncRNA (BC033150) inhibits VSMCs proliferation. (B) There was a significant difference in OD450 value after Honokiol treated between lncRNA (BC033150) silenced Group andcontrol group ($P<0.01$). (C) The apoptosis ratio of VSMCs was higher in lncRNA (BC033150) silenced Group than in control group ($P<0.01$).

axis, we silenced LncRNA (BC033150) using siRNA. After silenced by siRNA, the proliferation-inhibit (**Figure 4A**, **4B**) and apoptosis-promote (**Figure 4C**) effect of Honokiol was attenuated. There was a significant difference in OD450 value after Honokiol treated between lncRNA (BC033150) silenced Group andcontrol group ($P<0.01$) (**Figure 4B**). The apoptosis ratio of VSMCs was higher in lncRNA (BC033150) silenced Group than in control group ($P<0.01$) (**Figure 4C**). LncRNA (BC033150) was indispensible in Honokiol-FTO-lncRNA axis and was as a core function-exertion factor to Honokiol.

Discussion

The proliferation of VSMCs played important roles in the pathogenesis of both atherosclerosis and restenosis. Exploring new molecules inhibiting VSMCs proliferation was helpful for the strategy of atherosclerosis-therapeutic remedies. The discovery of new agents that could alter VSMCs proliferation in preclinical models would set the stage for clinical trials in humans. Honokiol had been shown to exert antitumor effects in various types of cancer [5]. However, there was little known to whether Honokiol targeted the proliferation and migration of VSMCs, and the mechanism of Honokiol performing its function was not clear.

The present study firstly explored the effects of Honokiol on VSMCs and demonstrated that Honokiol could inhibit the proliferation and promote the apoptosis of VSMCs. Furthermore, we

indentified that FTO gene was the most suppressed gene by Honokiol, and confirmed LncRNA (BC033150) as the target lncRNA which involved in the function of FTO. Honokiol reduced LncRNA (BC033150) expression via FTO suppression seems to be the mechanism of Honokiol's effects on VSMCs.

Honokiol-mediated apoptosis had been described in other cells types, consistent with these studies [12-14], the present data revealed that honokiol was able to induce apoptosis in VSMCs, as determined by CCK8 assay kit and reflected by FACS of annexin V/propidium iodide-labelled cells. Others found that Honokiol was shown to inhibit the growth in breast cancer cell lines in a dose dependent manner regardless of their HR, HER2 or p53 status [15]. Growth inhibitory effects of honokiol were shown to occur through arrest of cell cycle at the G0/G1 phase and induction of apoptosis [16], which indicated an interpretation to the mechanism of Honokiol in VSMCs.

We further screened from the cluster of proliferation-associated genes and found that FTO gene was the most suppressed one. FTO was a kind of demethylase which was first discovered to catalyze demethylation of 3-methylthymine in single-stranded DNA, and 3-methyluridine in single-stranded RNA, with low efficiency [17]. Therefore, we detected the expression of lncRNA by microarray. We found that the expression of lncRNA (BC033150) was at the highest level in FTO-transfer group, suggesting that lncRNA (BC033150) participated in this process and FTO were able to regulate the expression of lncRNA (BC033150).

LncRNAs were considered to be longer than ~200 nucleotides, on the basis of a convenient practical cut-off in RNA purification protocols that excluded small RNAs [18]. Given their unexpected abundance, lncRNAs were initially thought to be spurious transcriptional noise resulting from low RNA polymerase fidelity, but now emerging recognition that any transcript, regardless of coding potential, could have an intrinsic function was becoming the mainstream of more and more studies [9]. The functional repertoire of lncRNAs was broad and complex, which included roles in high-order chromosomal dynamics, telomere biology and subcellular structural organization. Research of lncRNAs in cardiovascular diseases was becoming a hot-area. Leung provided the first identification of Ang II-regulated lncRNAs, which suggested functional roles for these lncRNAs in mediating cellular responses to Ang II. Furthermore, they identified an Ang II-regulated lncRNA was responsible for the production of microRNAs implicated in VSMC proliferation [19]. Wu identified lincRNA-p21 as a novel regulator of cell proliferation and apoptosis and suggested that this lncRNA could serve as a therapeutic target to treat atherosclerosis and related cardiovascular disorders [20]. Consistence with these researches, we found that the expression of lncRNA (BC033150) was at the highest level in FTO-transfer group, suggesting that lncRNA was involved in the function of FTO/Honokiol. Although exact mechanism needs further exploration, we identified that LncRNA (BC033150) was indispensible in Honokiol-FTO-lncRNA axis. Based on early discoveries linking lncRNA to proliferation and apoptosis, we proposed that LncRNA (BC033-150) may affect the expression of nearby protein-coding genes. Future studies aimed at determining the mechanisms that control this context-dependency will be necessary to identify signaling nodes that could be targeted by therapeutic interventions.

Other limitations of our study include lacking observation on other apoptosis related molecules, without *in vivo* proof, and without proof by knocking-down or knocking out models of FTO/LncRNA (BC033150) pathway. Although these defects was companied with our study, we identified for the first time that honokiol promotes the apoptosis of VSMCs and proved that LncRNA (BC033150) was involved the mechanism of Honokiol's effects.

Further mechanism and intervention strategies for Honokiol targeting key signaling molecules of the FTO/LncRNA (BC033150) pathway might represent promising options to inhibit dysplasia of VSMCs, and serve as the basis for prevention or therapy of atherosclerosis and restenosis. Honokiol promoted VSMCs apoptosis by targeting FTO/LncRNA pathway, and indicated that it had potential to serve as a novel agent for the prevention and therapy of atherosclerosis and restenosis.

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Disclosure of conflict of interest

None.

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References

[1] Libby P, Ridker PM and Hansson GK. Progress and challenges in translating the biology of atherosclerosis. Nature 2011; 473: 317-325.

[2] Lacolley P, Regnault V, Nicoletti A, Li Z and Michel JB. The vascular smooth muscle cell in arterial pathology: a cell that can take on multiple roles. Cardiovasc Res 2012; 95: 194-204.

[3] Owens GK, Kumar MS and Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev 2004; 84: 767-801.

[4] Esumi T, Makado G, Zhai H, Shimizu Y, Mitsumoto Y and Fukuyama Y. Efficient synthesis and structure-activity relationship of honokiol, a neurotrophic biphenyl-type neolignan. Bioorg Med Chem Lett 2004; 14: 2621-2625.

[5] Arora S, Singh S, Piazza GA, Contreras CM, Panyam J and Singh AP. Honokiol: a novel natural agent for cancer prevention and therapy. Curr Mol Med 2012; 12: 1244-1252.

[6] Park EJ, Min HY, Chung HJ, Hong JY, Kang YJ, Hung TM, Youn UJ, Kim YS, Bae K, Kang SS, Lee SK. Down-regulation of c-Src/EGFR-mediated signaling activation is involved in the honokiol-induced cell cycle arrest and apoptosis in MDA-MB-231 human breast cancer cells. Cancer Lett 2009; 277: 133-140.

[7] Jia G, Fu Y, Zhao X, Dai Q, Zheng G, Yang Y, Yi C, Lindahl T, Pan T, Yang YG, He C. N6-methyladenosine in nuclear RNA is a major substrate of the obesity-associated FTO. Nat Chem Biol 2011; 7: 885-887.

[8] Struhl K. Transcriptional noise and the fidelity of initiation by RNA polymerase II. Nat Struct Mol Biol 2007; 14: 103-105.

[9] Mercer TR, Dinger ME and Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet 2009; 10: 155-159.

[10] Nagano T, Mitchell JA, Sanz LA, Pauler FM, Ferguson-Smith AC, Feil R, Fraser P. The Air noncoding RNA epigenetically silences transcription by targeting G9a to chromatin. Science 2008; 322: 1717-1720.

[11] Wang X, Arai S, Song X, Reichart D, Du K, Pascual G, Tempst P, Rosenfeld MG, Glass CK, Kurokawa R. Induced ncRNAs allosterically modify RNA-binding proteins in cis to inhibit transcription. Nature 2008; 454: 126-130.

[12] Chae JI, Jeon YJ and Shim JH. Downregulation of Sp1 is involved in honokiol-induced cell cycle arrest and apoptosis in human malignant pleural mesothelioma cells. Oncol Rep 2013; 29: 2318-2324.

[13] Jeong JJ, Lee JH, Chang KC and Kim HJ. Honokiol exerts an anticancer effect in T98G human glioblastoma cells through the induction of apoptosis and the regulation of adhesion molecules. Int J Oncol 2012; 41: 1358-1364.

[14] Hahm ER, Arlotti JA, Marynowski SW and Singh SV. Honokiol, a constituent of oriental medicinal herb magnolia officinalis, inhibits growth of PC-3 xenografts in vivo in association with apoptosis induction. Clin Cancer Res 2008; 14: 1248-1257.

[15] Leeman-Neill RJ, Cai Q, Joyce SC, Thomas SM, Bhola NE, Neill DB, Arbiser JL, Grandis JR. Honokiol inhibits epidermal growth factor receptor signaling and enhances the antitumor effects of epidermal growth factor receptor inhibitors. Clin Cancer Res 2010; 16: 2571-2579.

[16] Hou W, Chen L, Yang G, Zhou H, Jiang Q, Zhong Z, Hu J, Chen X, Wang X, Yuan Y, Tang M, Wen J, Wei Y. Synergistic antitumor effects of liposomal honokiol combined with adriamycin in breast cancer models. Phytother Res 2008; 22: 1125-1132.

[17] Gerken T, Girard CA, Tung YC, Webby CJ, Saudek V, Hewitson KS, Yeo GS, McDonough MA, Cunliffe S, McNeill LA, Galvanovskis J, Rorsman P, Robins P, Prieur X, Coll AP, Ma M, Jovanovic Z, Farooqi IS, Sedgwick B, Barroso I, Lindahl T, Ponting CP, Ashcroft FM, O'Rahilly S, Schofield CJ. The obesity-associated FTO gene encodes a 2-oxoglutarate-dependent nucleic acid demethylase. Science 2007; 318: 1469-72.

[18] Kapranov P, Cheng J, Dike S, Nix DA, Duttagupta R, Willingham AT, Stadler PF, Hertel J, Hackermüller J, Hofacker IL, Bell I, Cheung E, Drenkow J, Dumais E, Patel S, Helt G, Ganesh M, Ghosh S, Piccolboni A, Sementchenko V, Tammana H, Gingeras TR. RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 2007; 316: 1484-1488.

[19] Leung A, Trac C and Jin W. Novel long noncoding RNAs are regulated by angiotensin II in vascular smooth muscle cells. Circ Res 2013; 113: 266-278.

[20] Wu G, Cai J, Han Y, Chen J, Huang ZP, Chen C, Cai Y, Huang H, Yang Y, Liu Y, Xu Z, He D, Zhang X, Hu X, Pinello L, Zhong D, He F, Yuan GC, Wang DZ, Zeng C. LincRNA-p21 regulates neointima formation, vascular smooth muscle cell proliferation, apoptosis and atherosclerosis by enhancing p53 activity. Circulation 2014; 130: 1452-1465.