Int J Clin Exp Pathol 2017;10(3):2870-2878
www.ijcep.com /ISSN:1936-2625/IJCEP0039626

Original Article

miR-320 inhibits human coronary artery smooth muscle cell proliferation and migration by targeting Krüppel-like factor 5

Yuhua Sun, Zhenghai Zhang, Haibin Zhang, Lixin Zhang, Shunjin Gan

Department of Cardiovascular Medicine, Beijing Luhe Hospital, Capital Medical University, Beijing, China

Received September 7, 2016; Accepted October 24, 2016; Epub March 1, 2017; Published March 15, 2017

Abstract: Krüppel-like factor 5 (KLF5) plays an essential role in the regulation of vascular smooth muscle cell proliferation and migration. In the present study, a post-translational mechanism of miR-320 in TNF-α-induced proliferation and migration of HCASMCs by targeting KLF5 was investigated. The results indicated that TNF-α could accelerate the proliferation and migration of HCASMCs. Overexpressed miR-320 could suppress the proliferation and migration of HCASMCs in the absence and presence of TNF-α. Moreover, bioinformatic analysis showed the potential miR-320 binding sites within the 3'-UTR of KLF5. KLF5 as a direct target of miR-320 was involved in TNFα-induced proliferation and migration of HCASMCs. KLF5 knockout could reverse the increased proliferation and migration in HCASMCs induced by miR-320 loss-of-function and TNF-α treatment. In conclusion, overexpressed miR-320 was verified to protect against TNF-α-induced proliferation and migration of HCASMCs, and the underlying mechanism was mediated, at least partially, by inhibiting KLF5 expression.

Keywords: miR-320, carotid artery injuries, KLF5, TNF-α

Introduction

Vascular smooth muscle cells (VSMCs) are the major constituents of the vasculature and are crucial to maintain an appropriate blood pressure and flow distribution [1]. However, VSMCs exhibit marked differences in migration and proliferation rate suffering from vascular injury [2]. Abnormal proliferation of coronary artery smooth muscle cells (CASMCs) has been linked to coronary artery disease (CAD) [3]. Human CASMCs (HCASMCs) phenotypic changes ranging from differentiated (contractile) to a dedifferentiated (synthetic, noncontractile) are involved in vascular remodeling, restenosis and atherosclerosis [4]. In the vascular media of healthy arteries, HCASMCs exhibit a non-proliferative contractile phenotype; however, growth factors promote transdifferentiation of HCASMCs, which is thought to cause carotid artery injuries [5]. However, the underlying mechanisms of HCASMCs proliferation and migration in carotid artery diseases have not been clearly delineated.

Krüppel-like factor 5 (KLF5) is characterized by a highly conserved interfinger space sequence and has been identified in mammalian [6]. It is generally known that KLF5 regulates a wide variety of cellular functions such as growth, angiogenesis and proliferation [7, 8]. A growing body of evidence suggests that KLF5 is associated with vascular remodeling [9, 10]. In human pulmonary arterial hypertension, KLF5 contributes to pulmonary artery smooth muscle cell proliferation and resistance to apoptosis [11]. Moreover, KLF5 expression is up-regulated in angiotensin II (Ang II)-induced cell proliferation in rat vascular smooth muscle cells [12]. Silencing of KLF5 suppresses TNFα-induced phenotypic conversion of VSMCs; conversely, overexpressed KLF5 stimulates phenotypic conversion of VSMCs [13]. Interestingly, KLF5 can be regulated by oxidative stress stimuli, such as endothelin-1 (ET-1), H_2O_2 and inflammatory cytokines, including TNFα and IL-1β [14].

Recently, post-translational mechanisms of microRNAs (miRs) by regulating target genes

are involved in phenotypic changes of VSMCs within the vascular lesions [13, 15]. Recent study indicates that miR-328 targeting PIM-1 inhibits proliferation and migration of pulmonary arterial smooth muscle cells [16]. Xie et al have been found that miR-599 suppresses VSMCs proliferation and migration by targeting TGFB2 [17]. miR-145, as a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation [18]. In the present study, we had found that miR-320 was the most abundant miR in HCASMCs. However, the post-translational mechanisms of miR-320 in TNFα-treated HVSMCs were completely unknown. Therefore, we hypothesized that UA could improve TNFα-induced proliferation and migration in HCASMCs by targeting KLF5.

Materials and methods

Cell culture

Primary human coronary artery smooth muscle cells (HCASMCs) were commercially obtained (Frederick, MD, USA). HCASMCs were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco Life Technologies) that contained 10% fetal calf serum (Gibco Life Technologies), 10% L-glutamine, 0.5% penicillin/streptomycin, 10% nonessential amino acids and 10% pyruvate, in a 5% CO_2 atmosphere and incubated at 37°C.

Cell viability detection by MTT

HCASMCs proliferation were monitored by a 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) Cell Proliferation/Viability Assay kit (R&D SYSTEMS) in according to the guidelines.

Wound healing assay

HCASMCs were trypsinized and counted. 1×10^5 cells were reseeded in each well of a new 6-well plate. With incubation overnight, the confluent cells monolayers were scratched with a 10 μL sterile pipette tip. Then the non-adherent cells were washed off with sterilized PBS and serum-free medium was added into the wells. The gap area caused by the scratch was monitored by the inverted microscope (Olympus, Japan).

Luciferase reporter gene activity assay

The 3'-UTR of KLF5 containing the predicated target sites for miR-320 was obtained by PCR amplification. The fragment was inserted into the multiple cloning sites in the pMIR-REPORT luciferase microRNA expression reporter vector (Ambion, Austin, USA). Cells were co-transfected with 0.1 μg of luciferase reporters containing KLF5 3'-UTR and miR-320 mimics by Lipofectamine2000 (Invitrogen, CA, USA). The cells were harvested after 48 h transfection, and luciferase activity was performed according to manufacturer's instruction.

Mimics and inhibitor of miR-320

The FAM modified 2'-OMe-oligonucleotides were chemically synthesized and purified by high-performance liquid chromatography (GenePharma, Shanghai, China). The 2'-OMe-miR-320 mimics were composed of RNA duplexes with the following sequence: 5'-AAAAGCUGGGUUGAGAGGGCGA-3'. The sequences of 2'-OMe-miR-320 inhibitor as follows: 5'-UCGCCCUCUCAACCCAGCUUUU-3'. The 2'-Ome-scramble oligonucleotides of miR-320 as follows: 5'-CUGGCAUUGCGGCUAAGGUGCA-3'. Cells were transfected using Lipofectamine2000 (Invitrogen, CA, USA) at a final concentration of 100 nM.

Lentiviral knockdown of KLF5

Control shRNA and specific sh-RNAs targeting KLF5 were purchased from Invitrogen, and the corresponding sequences were cloned into the pSIREN-RetroQ plasmid (Addgene) for retrovirus production with 293T cells. The targeting sequences as following: 5'-CCGGTAAGCTCACCTGAGGACTCATACTCGAGTATGAGT CCTCAGGTGAGCTTTTTTTG-3'. After 48 hours, infected cells were selected for with puromycin (2 mg/ml). Then the clones were picked and cultured for further experiment.

Real time-polymerase chain reaction

RNA extraction was performed according to the TRIzol manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). Synthesis of cDNAs was performed by reverse transcription reactions with 4 μg of total RNA using Maloney murine leukemia virus reverse transcriptase (Invitrogen) with oligo dT (15) primers (Fermentas) as described by the manufacturer. MiR-320 level was quantified by the mirVana qRT-PCR miRNA detection kit (Ambion, Austin, USA) in conjunction with real-time PCR with SYBR Green. After circle reaction, the threshold cycle (Ct) was determined and relative miR-320 level was calculat-

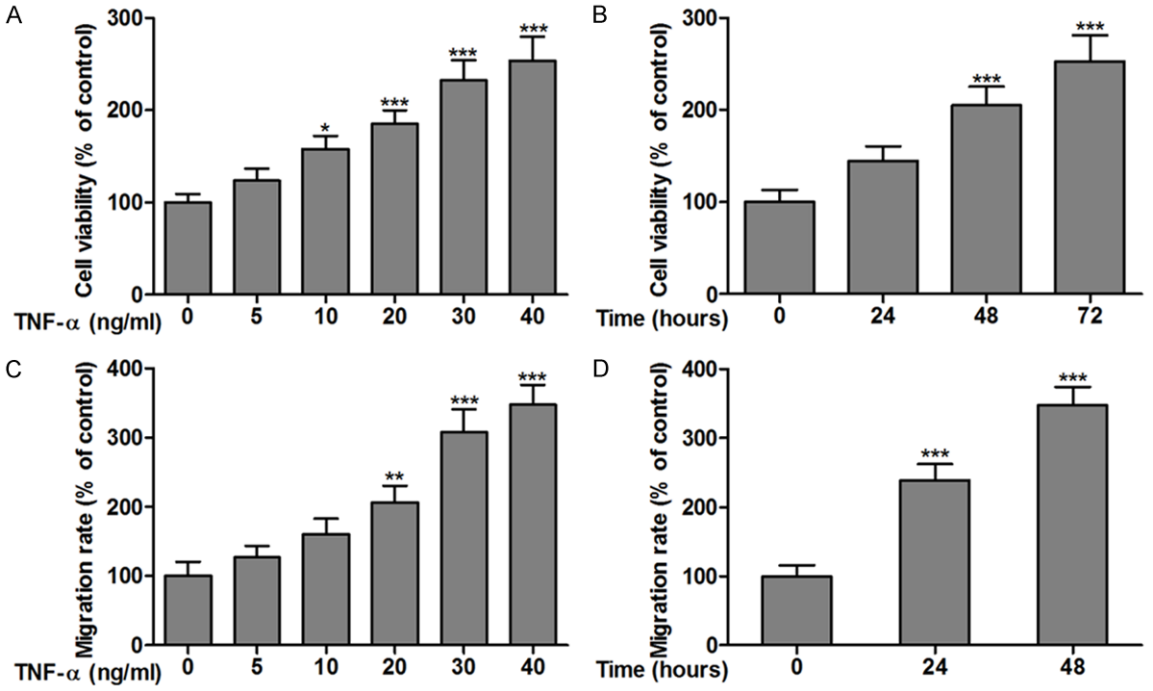

Figure 1. TNF-α promoted the proliferation and migration of HCASMCs. HCASMCs proliferation assessed by MTT assay when HCASMCs were incubated with TNF-α in different concentration for 24 h (A). The cell viability was measured by MTT assay when HCASMCs were incubated with TNF-α (20 ng/ml) in different time points (B). HCASMCs migration was determined after 24 h of PDGFBB treatment in different concentration by wound-healing assay (C). HCASMCs migration was determined in different time points in the present of TNF-α (20 ng/ml) (D). Values were expressed as mean ± SD, n = 3 in each group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ versus control group.

ed based on the Ct values and normalized to U6 level in each sample.

Western blotting

Cells were lysed in NP-40 buffer (Beyotime Institute of Biotechnology, Haimen, China). Following 5-10 min boiling, homogenate were centrifuged at ×10000 g, 4°C for 10 min to obtain the supernatant. Protein samples (50 μg) were separated by 10% sodium dodecyl sulfate-polyacrylimide gel electrophoresis and transferred to polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). Membranes were blocked with 5% (w/v) non-fat milk powder in Tris-buffered saline and 0.1% (w/v) Tween 20 (TBST), and incubated with the following primary antibodies: KLF5 and β-actin (Santa Cruz Biotechnology, Inc.; 1:1000, Dallas, TX, USA), at 4°C overnight. After being washed, the membranes were incubated with HRP-conjugated anti IgG at room temperature for 2 hour. Signal detection was carried out with an ECL system (Amersham Pharmacia, Piscataway, NJ, USA).

Statistical analysis

The data from these experiments were reported as mean ± standard deviation (SD) for each group. All s tatistical analyses were performed by using PRISM version 7.0 (GraphPad). Intergroup differences were analyzed by one-way ANOVA, and followed by Tukey's multiple comparison test as a post test to compare the group means if overall $P < 0.05$. Differences with P value of < 0.05 were considered statistically significant.

Results

Effect of TNF-α on HCASMCs proliferation and migration

Previous studies suggest that TNF-α has been associated with proliferation, migration and phenotypic modulation in VSMCs [19-21]. In line with these reports, our findings demonstrated that TNF-α at 10, 20, 30 and 40 ng/ml significantly accelerated HCASMCs proliferation by 58%, 85%, 132% and 153%, respective-

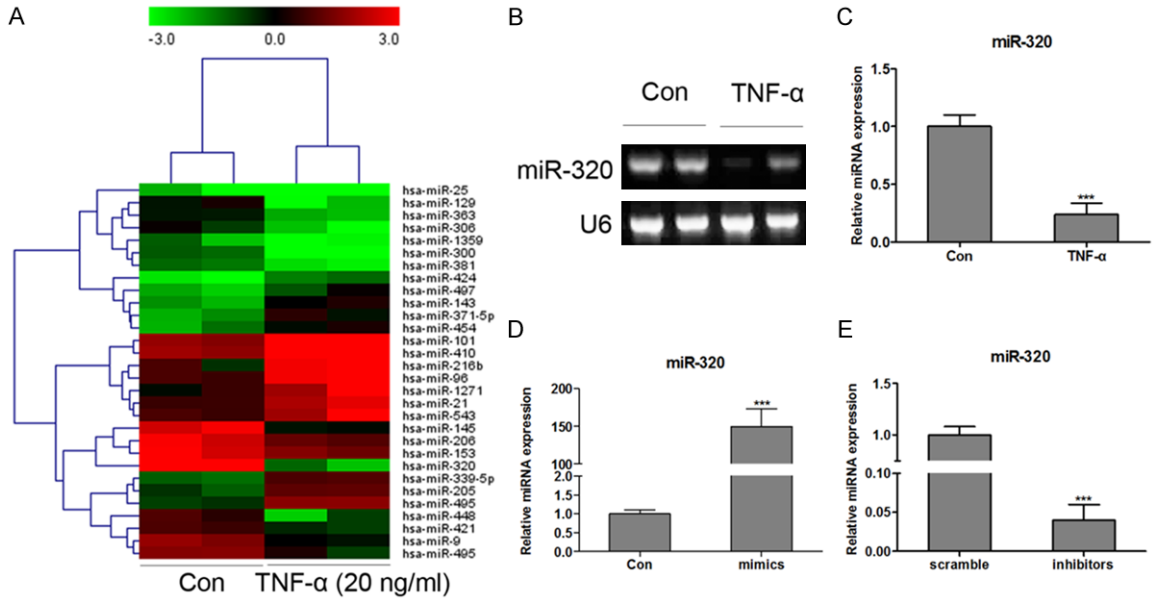

Figure 2. TNF-α inhibited miR-320 in HCASMCs. Unsupervised hierarchical clustering of differentially expressed miRs in HCASMCs treated with TNF-α (20 ng/ml) for 24 h were measured by microRNA microarray analysis. The figure is drawn by MeV software (version 4.2.6) (A). Real-time PCR analysis was performed to measure miR-320 levels in HCASMCs with or without TNF-α (20 ng/ml) treatment (B and C). Real-time PCR analysis was performed to measure miR-320 levels in HCASMCs transfected with the miR-320 mimics (D) or inhibitors (E). Values were expressed as mean ± SD, n = 3 in each group. $^{***}P < 0.001$ versus control group.

ly (**Figure 1A**). Similar results of TNF-α-induced HCASMCs proliferation were observed in increasing the duration of time (**Figure 1B**). We also evaluated HCASMCs migration by TNF-α treatment. TNF-α at 20, 30 and 40 ng/ml significantly increased HCASMCs migration by 106%, 208% and 247%, respectively (**Figure 1C**). Moreover, we found that TNF-α at 20 ng/ml could induce HCASMCs migration in a time-dependent manner (**Figure 1D**).

TNF-α inhibited miR-320 in HCASMCs

To identify aberrantly miRs expression in HCASMCs exposure to TNF-α (20 ng/ml), we performed microarray assay with small RNA libraries generated using total RNA extracted from HCASMCs with TNF-α treatment for 24 h. We found that 30 miRs were differentially expressed in HCASMCs with TNF-α treatment compared with control group. 15 miRs were increased and 15 were decreased, and the expression of miR-320 showed the lowest level of all the miRs (**Figure 2A**). Moreover, the quantitative real-time PCR results showed that the levels of miR-320 were significantly decreased in HCASMCs with TNF-α treatment as compared to control group (**Figure 2B** and **2C**). Therefore, we further investigated the functional roles of miR-320 in HCASMCs in the present of TNF-α. To further examine the roles of miR-320 in TNF-α-incubated HCASMCs, the mimics and inhibitors of miR-320 were transfected in HCASMCs. miR-320 mimics were found to be sufficient to increase the expression levels of miR-320 in HCASMCs, while miR-320 inhibitors had the opposite result; these results suggested that the mimics and inhibitors of miR-320 took effects in HCASMCs (**Figure 2D** and **2E**).

miR-320 inhibited TNF-α-induced HCASMCs proliferation and migration

miR-320 mimics and inhibitors were transfected into HCASMCs to investigate whether miR-320 mediates cell proliferation and migration in the present of TNF-α. As compared to control group, transfection of miR-320 mimics significantly inhibited HCASMCs proliferation both with and without TNF-α treatment (**Figure 3A**). In contrast to that transfection of miR-320 inhibitors markedly promoted the proliferation of HCASMCs with and without TNF-α treatment (**Figure 3B**). In addition, the effects of miR-320 on HCASMCs migration were measured using wound healing assays. The findings indicated

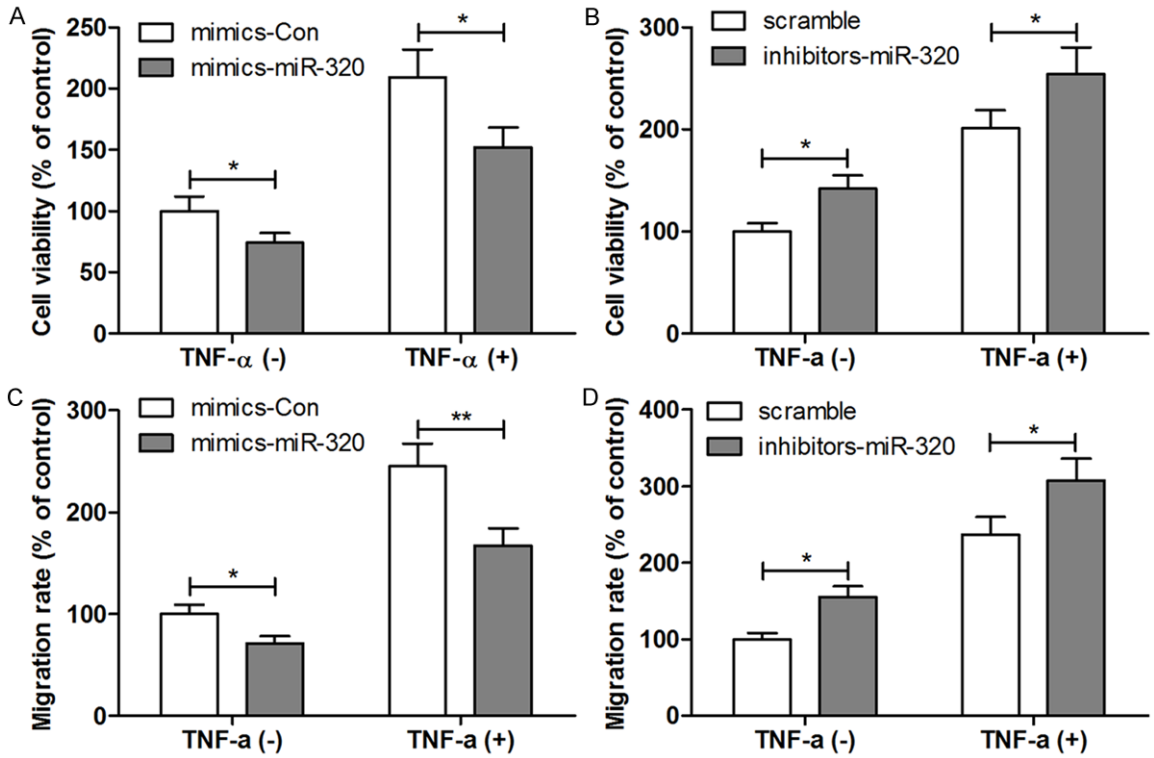

Figure 3. miR-320 inhibited TNF-α-induced HCASMCs proliferation and migration. miR-320 mimics (A) and inhibitors (B) were transfected into HCASMCs; the cell viability was measured by MTT assay with or without TNF-α treatment. miR-320 mimics (C) and inhibitors (D) were transfected into HCASMCs; the cell migration was measured by wound-healing assay with or without TNF-α treatment. Values were expressed as mean ± SD, n = 3 in each group. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group.

that transfected miR-320 mimics significantly inhibited HCASMCs migration both in the absence and presence of TNF-α (**Figure 3C**). However, miR-320 inhibitors resulted in accelerating migration of HCASMCs with and without TNF-α treatment (**Figure 3D**). These results suggested that miR-320 could suppress the proliferation and migration of HCASMCs in the absence and presence of TNF-α.

KLF5 was a direct target of miR-320

Bioinformatics (miRanda Target database, http://www.microRNA.org) revealed KLF5 RNA contains one conserved target site of miR-320 (**Figure 4A**). The wild type sequence of KLF5 (KLF5-Wild) or mutant sequence (KLF5-Mut) as shown in **Figure 4A** were subcloned into the pMIR luciferase reporter and then co-transfected with the mimics of miR-320 or mimics-Con into HCASMCs, and luciferase assays were performed 24 hours post-transfection. As shown in **Figure 4B**, the luciferase activity in KLF5 Wild-type HCASMCs transfected with miR-320 mimics was significantly decreased compared with control group. However, transfection of miR-320 mimics into KLF5 Mut-type HCASMCs, the luciferase activity did not show significant difference compared with control group. In addition, inhibition of miR-320 in HCASMCs resulted in up-regulation of KLF5 protein expression (**Figure 4C**). On the contrary, overexpressed miR-320 markedly decreased the protein expression of KLF5 in HCASMCs (**Figure 4D**). Collectively, these findings demonstrated that KLF5 is a direct target of miR-320 in HCASMCs.

KLF5 knockdown rescued HCASMCs proliferation and migration

Next, we examined whether KLF5 knockdown could prevent the increased HCASMCs proliferation by TNF-α treatment and miR-320 loss-of-function. First, we found that infection of sh-KLF5 lentivirus resulted in a remarkable reduction of KLF5 protein expression (**Figure 5A**). As shown in **Figure 5B** and **5C**, KLF5 loss-of-function could significantly prevent the

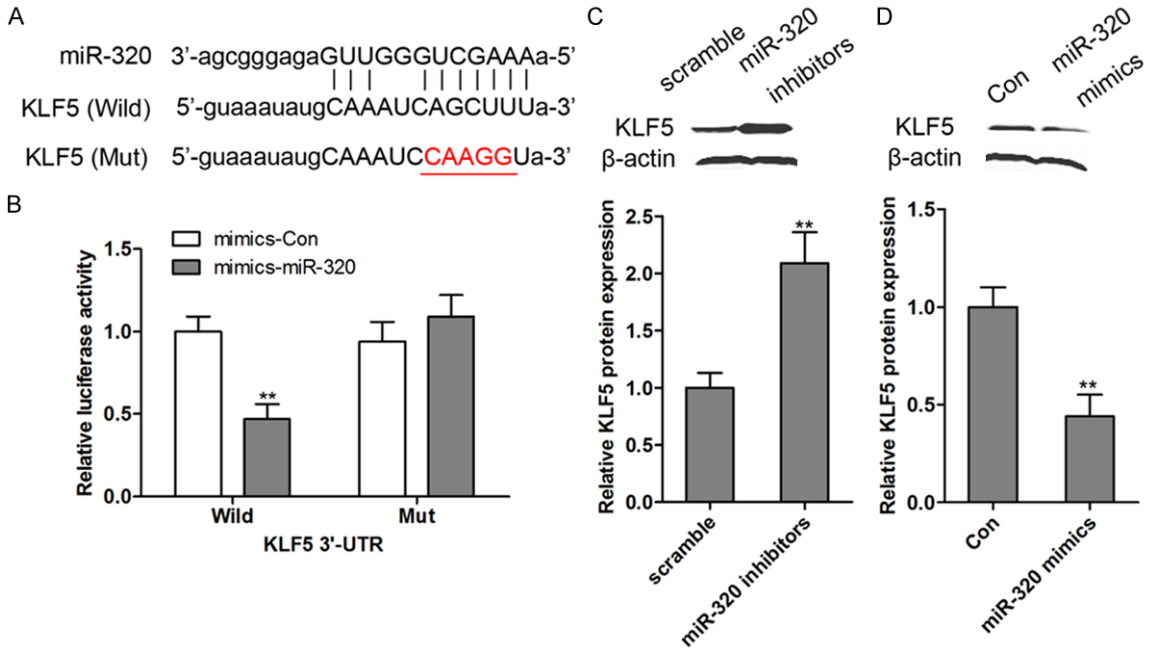

Figure 4. KLF5 was a direct target of miR-320. The putative binding sites of miR-320 on KLF5 3'-UTR was highlighted (A). In a dual-luciferase reporter assay, HCASMCs were transfected with firefly luciferase reporter inserted with wild KLF5 3'-UTR or the reporter inserted with Mut KLF5 3'-UTR, the relative luciferase activities were evaluated at 24 hours after transfection (B). The protein expression of KLF5 was measured by western blotting transfected with miR-320 inhibitors (C) or mimics (D). Values were expressed as mean ± SD, n = 3 in each group. $^{**}P < 0.01$ versus control group.

increased HCASMCs proliferation and migration in the absence and presence of TNF-α. Moreover, KLF5 loss-of-function could also reverse the increased HCASMCs proliferation and migration with or without miR-320 inhibitors (**Figure 5D** and **5E**). Taken together, these data suggested that miR-320 could suppress TNF-α-induced HCASMCs proliferation and migration by targeting KLF5.

Discussion

Our experiments demonstrated that miR-320 could inhibit TNF-α-induced proliferation and migration of HCASMCs. To further research of the molecular mechanisms of TNF-α-induced proliferation and migration in HCASMCs, bioinformatic analysis showed that KLF5-encoded mRNA contains a 3'-UTR element that was partially complementary to miR-320, indicating that miR-320 might directly target the corresponding sites to regulate the expression of KLF5. In fact, inhibition of miR-320 resulted in up-regulating, and overexpressed miR-320 markedly decreased the protein expression of KLF5 in HCASMCs. Furthermore, our findings indicated that KLF5 knockdown rescued TNF-α- or miR-320 inhibitors-induced proliferation and migration in HCASMCs.

Previous study has been found that miR-320 expression is down-regulated in several malignancies, and enhanced miR-320 inhibits cancer cells proliferation and migration [22]. miR-320 is involved in platelet-derived growth factor-BB enhancing MSC-mediated cardioprotection [23]. Moreover, miR-320 as an anti-angiogenic exosomes from cardiomyocyes can inhibit diabetes mellitus-induced myocardial vascular deficiency [24]. miR-320 loss-of-function suppresses cardiomyocyte apoptosis and protects against myocardial ischemia and reperfusion injury [25]. However, there has been no relevant report about miR-320 regulating HCASMCs functions. Our current study replenished previous studies that miR-320 possessed an inhibitory effect of TNF-α-induced proliferation and migration in HCASMCs. Moreover, our findings had identified a new target gene of miR-320. KLF5 as a direct target of miR-320 was confirmed in HCASMCs, which directly bound to the 3'-UTR region of KLF5 to

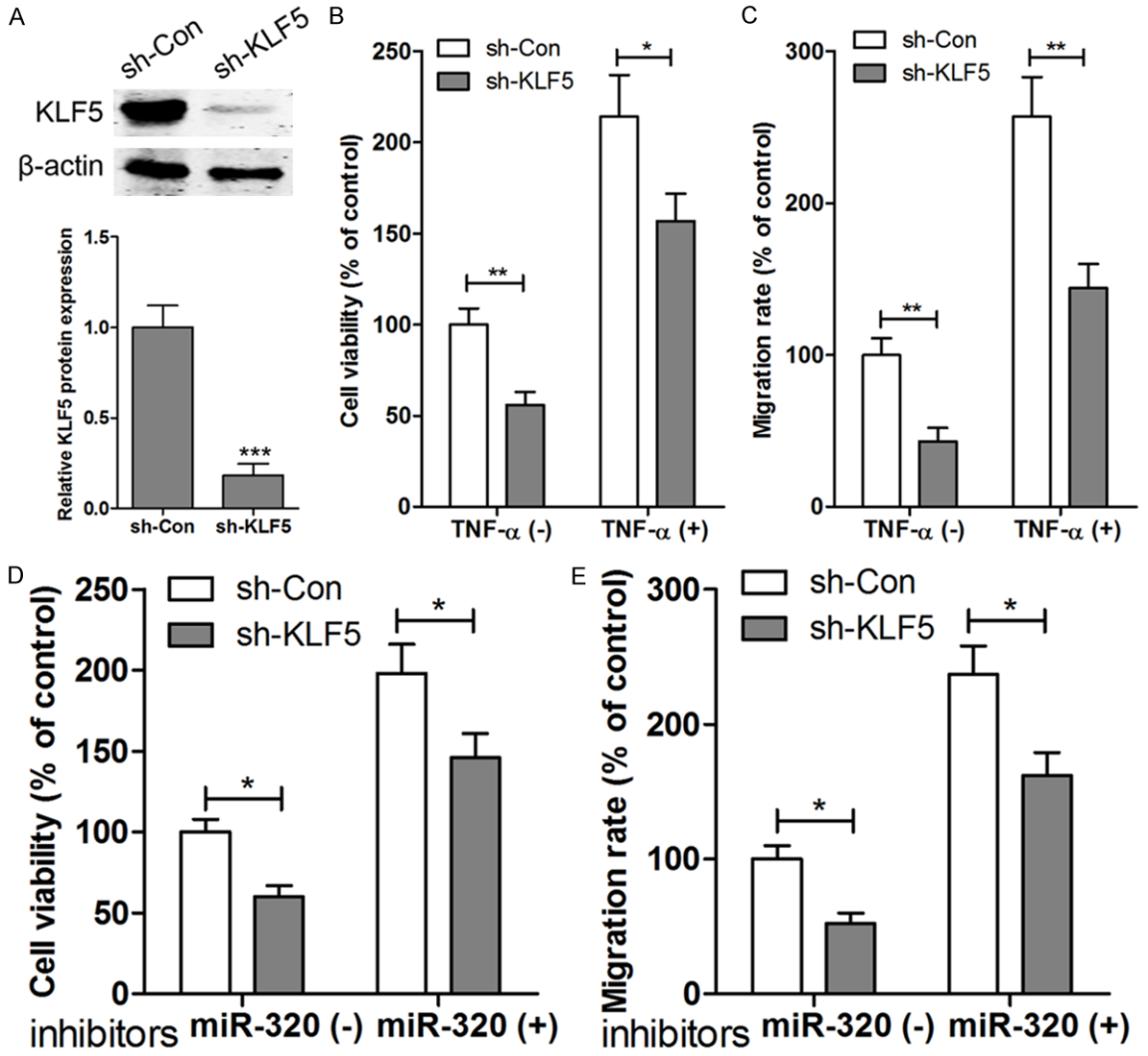

Figure 5. KLF5 knockdown rescued HCASMCs proliferation and migration. KLF5 protein expression was measured by western blotting in HCASMCs infected with sh-KLF5 lentivirus (A). HCASMCs infected with sh-KLF5, the cell viability was measured by MTT assay with or without TNF-α treatment (B), and cell migration was measured by wound-healing assay with or without TNF-α treatment (C). HCASMCs infected with sh-KLF5, the cell viability was measured by MTT assay with or without miR-320 inhibitors (D), and cell migration was measured by wound-healing assay with or without miR-320 inhibitors (E). Values were expressed as mean ± SD, n = 3 in each group. $^{*}P < 0.05$, $^{**}P < 0.01$ versus control group.

negatively regulate KLF5 expression expression. These results suggested that overexpressed miR-320 in HCASMCs might suppress proliferation and migration by dysregulating expression of KLF5. miR-320 might act as a new effective target for the treatment of carotid artery injuries.

Previous study indicates that KLF5 is an essential transcription factor in cardiovascular remodeling and a potential therapeutic target for cardiovascular disease [26]. KLF5 has been implicated in the regulation of phenotypic modulation of VSMCs and mediates TNFα-dependent phenotypic change of VSMCs [14]. Specifically, overexpressed KLF5 results in markedly increased cell proliferation and cell cycle progression of VSMCs, which directly binds to the promoter and up-regulates gene expression of cyclin D1 [27]. Our results showed that KLF5 knockout could significantly inhibit HCASMCs proliferation and migration in the absence and presence of TNF-α. Moreover, KLF5 knockout could also reverse the increased

HCASMCs proliferation and migration induced by miR-320 inhibitors. These results suggest that KLF5 plays an essential role in vascular remodeling. Cytokines stimuli, such as AngII, can induce vascular remodeling and enhance the expression of KLF5 [12]. Moreover, inflammatory cytokine, including TNF-α, can stimulate KLF5 expression [14]. KLF5 is enrichment in fetal blood vessels, but the expression of KLF5 is dramatically decreased in adult vessels [28]. In general, KLF5 is activated in VSMCs in abnormal conditions, such as restenosis and atherosclerosis [29]. Our results demonstrated that enhancement of miR-320 blocked TNFα-induced proliferation and migration in HCASMCs through increase of promoter activity of KLF5.

Taken together, TNF-α, as an important inflammatory cytokine, could regulate the proliferation and migration of HCASMCs. Overexpressed miR-320 could suppress the proliferation and migration of HCASMCs in the absence and presence of TNF-α. KLF5 as a direct target of miR-320 was involved in TNFα-induced proliferation and migration of HCASMCs. KLF5 knockout could reverse the increased proliferation and migration in HCASMCs induced by miR-320 loss-of-function and TNF-α treatment. Therefore, miR-320/KLF5 signaling pathway could be a therapeutic target for carotid artery injuries-related vascular remodeling.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Yuhua Sun, Department of Cardiovascular, Beijing Luhe Hospital, Capital Medical University, Beijing 101149, China. Tel: (+86)10-69543901; E-mail: sunyuhua5568@163.com

References

[1] Li F, Yu X, Szynkarski CK, Meng C, Zhou B, Barhoumi R, White RE, Heaps CL, Stallone JN and Han G. Activation of GPER induces differentiation and inhibition of coronary artery smooth muscle cell proliferation. PLoS One 2013; 8: e64771.

[2] Maddaluno M, Grassia G, Di Lauro MV, Parisi A, Maione F, Cicala C, De Filippis D, Iuvone T, Guglielmotti A, Maffia P, Mascolo N and Ialenti A. Bindarit inhibits human coronary artery smooth muscle cell proliferation, migration and phenotypic switching. PLoS One 2012; 7: e47464.

[3] Srivastava R, Zhang J, Go GW, Narayanan A, Nottoli TP and Mani A. Impaired LRP6-TCF7L2 activity enhances smooth muscle cell plasticity and causes coronary artery disease. Cell Rep 2015; 13: 746-759.

[4] Pena E, Arderiu G and Badimon L. Tissue factor induces human coronary artery smooth muscle cell motility through Wnt-signalling. J Thromb Haemost 2013; 11: 1880-1891.

[5] Terao Y, Satomi-Kobayashi S, Hirata K and Rikitake Y. Involvement of Rho-associated protein kinase (ROCK) and bone morphogenetic protein-binding endothelial cell precursor-derived regulator (BMPER) in high glucose-increased alkaline phosphatase expression and activity in human coronary artery smooth muscle cells. Cardiovasc Diabetol 2015; 14: 104.

[6] Qin J, Zhou Z, Chen W, Wang C, Zhang H, Ge G, Shao M, You D, Fan Z, Xia H, Liu R and Chen C. BAP1 promotes breast cancer cell proliferation and metastasis by deubiquitinating KLF5. Nat Commun 2015; 6: 8471.

[7] Shi W, Yang J, Li S, Shan X, Liu X, Hua H, Zhao C, Feng Z, Cai Z, Zhang L and Zhou D. Potential involvement of miR-375 in the premalignant progression of oral squamous cell carcinoma mediated via transcription factor KLF5. Oncotarget 2015; 6: 40172-40185.

[8] Drosatos K, Pollak NM, Pol CJ, Ntziachristos P, Willecke F, Valenti MC, Trent CM, Hu Y, Guo S, Aifantis I and Goldberg IJ. Cardiac myocyte KLF5 regulates ppara expression and cardiac function. Circ Res 2016; 118: 241-253.

[9] Li X, He Y, Xu Y, Huang X, Liu J, Xie M and Liu X. KLF5 mediates vascular remodeling via HIF-1alpha in hypoxic pulmonary hypertension. Am J Physiol Lung Cell Mol Physiol 2016; 310: L299-310.

[10] Zempo H, Suzuki JI, Ogawa M, Watanabe R, Fujiu K, Manabe I, Conway SJ, Taniyama Y, Morishita R, Hirata Y, Isobe M and Nagai R. Influence of periostin-positive cell-specific Klf5 deletion on aortic thickening in DOCA-salt hypertensive mice. Hypertens Res 2016; 39: 764-768.

[11] Courboulin A, Tremblay VL, Barrier M, Meloche J, Jacob MH, Chapolard M, Bisserier M, Paulin R, Lambert C, Provencher S and Bonnet S. Kruppel-like factor 5 contributes to pulmonary artery smooth muscle proliferation and resistance to apoptosis in human pulmonary arterial hypertension. Respir Res 2011; 12: 128.

[12] Gao D, Hao G, Meng Z, Ning N, Yang G, Liu Z, Dong X and Niu X. Rosiglitzone suppresses angiotensin II-induced production of KLF5 and cell proliferation in rat vascular smooth muscle cells. PLoS One 2015; 10: e0123724.

[13] Kim SH, Yun SJ, Kim YH, Ha JM, Jin SY, Lee HS, Kim SJ, Shin HK, Chung SW and Bae SS. Essential role of kruppel-like factor 5 during tumor necrosis factor alpha-induced phenotypic conversion of vascular smooth muscle cells. Biochem Biophys Res Commun 2015; 463: 1323-1327.

[14] Cullingford TE, Butler MJ, Marshall AK, Tham el L, Sugden PH and Clerk A. Differential regulation of Kruppel-like factor family transcription factor expression in neonatal rat cardiac myocytes: effects of endothelin-1, oxidative stress and cytokines. Biochim Biophys Acta 2008; 1783: 1229-1236.

[15] Zhao Y, Li Y, Luo P, Gao Y, Yang J, Lao KH, Wang G, Cockerill G, Hu Y, Xu Q, Li T and Zeng L. XBP1 splicing triggers miR-150 transfer from smooth muscle cells to endothelial cells via extracellular vesicles. Sci Rep 2016; 6: 28627.

[16] Qian Z, Zhang L, Chen J, Li Y, Kang K, Qu J, Wang Z, Zhai Y, Li L and Gou D. MiR-328 targeting PIM-1 inhibits proliferation and migration of pulmonary arterial smooth muscle cells in PDGFBB signaling pathway. Oncotarget 2016; [Epub ahead of print].

[17] Xie B, Zhang C, Kang K and Jiang S. miR-599 inhibits vascular smooth muscle cells proliferation and migration by targeting TGFB2. PLoS One 2015; 10: e0141512.

[18] Cheng Y, Liu X, Yang J, Lin Y, Xu DZ, Lu Q, Deitch EA, Huo Y, Delphin ES and Zhang C. MicroRNA-145, a novel smooth muscle cell phenotypic marker and modulator, controls vascular neointimal lesion formation. Circ Res 2009; 105: 158-166.

[19] Ali MS, Starke RM, Jabbour PM, Tjoumakaris SI, Gonzalez LF, Rosenwasser RH, Owens GK, Koch WJ, Greig NH and Dumont AS. TNF-alpha induces phenotypic modulation in cerebral vascular smooth muscle cells: implications for cerebral aneurysm pathology. J Cereb Blood Flow Metab 2013; 33: 1564-1573.

[20] Davis R, Pillai S, Lawrence N, Sebti S and Chellappan SP. TNF-alpha-mediated proliferation of vascular smooth muscle cells involves Raf-1-mediated inactivation of Rb and transcription of E2F1-regulated genes. Cell Cycle 2012; 11: 109-118.

[21] Li H, Cheng Y, Simoncini T and Xu S. 17beta-Estradiol inhibits TNF-alpha-induced proliferation and migration of vascular smooth muscle cells via suppression of TRAIL. Gynecol Endocrinol 2016; 32: 581-586.

[22] Vishnubalaji R, Hamam R, Yue S, Al-Obeed O, Kassem M, Liu FF, Aldahmash A and Alajez NM. MicroRNA-320 suppresses colorectal cancer by targeting SOX4, FOXM1, and FOXQ1. Oncotarget 2016; 7: 35789-35802.

[23] Xu B, Luo Y, Liu Y, Li BY and Wang Y. Platelet-derived growth factor-BB enhances MSC-mediated cardioprotection via suppression of miR-320 expression. Am J Physiol Heart Circ Physiol 2015; 308: H980-989.

[24] Wang X, Huang W, Liu G, Cai W, Millard RW, Wang Y, Chang J, Peng T and Fan GC. Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of miR-320 into endothelial cells. J Mol Cell Cardiol 2014; 74: 139-150.

[25] Song CL, Liu B, Diao HY, Shi YF, Zhang JC, Li YX, Liu N, Yu YP, Wang G, Wang JP and Li Q. Down-regulation of microRNA-320 suppresses cardiomyocyte apoptosis and protects against myocardial ischemia and reperfusion injury by targeting IGF-1. Oncotarget 2016; 7: 39740-39757.

[26] Nagai R, Suzuki T, Aizawa K, Shindo T and Manabe I. Significance of the transcription factor KLF5 in cardiovascular remodeling. J Thromb Haemost 2005; 3: 1569-1576.

[27] Suzuki T, Sawaki D, Aizawa K, Munemasa Y, Matsumura T, Ishida J and Nagai R. Kruppel-like factor 5 shows proliferation-specific roles in vascular remodeling, direct stimulation of cell growth, and inhibition of apoptosis. J Biol Chem 2009; 284: 9549-9557.

[28] Hoshino Y, Kurabayashi M, Kanda T, Hasegawa A, Sakamoto H, Okamoto E, Kowase K, Watanabe N, Manabe I, Suzuki T, Nakano A, Takase S, Wilcox JN and Nagai R. Regulated expression of the BTEB2 transcription factor in vascular smooth muscle cells: analysis of developmental and pathological expression profiles shows implications as a predictive factor for restenosis. Circulation 2000; 102: 2528-2534.

[29] Shindo T, Manabe I, Fukushima Y, Tobe K, Aizawa K, Miyamoto S, Kawai-Kowase K, Moriyama N, Imai Y, Kawakami H, Nishimatsu H, Ishikawa T, Suzuki T, Morita H, Maemura K, Sata M, Hirata Y, Komukai M, Kagechika H, Kadowaki T, Kurabayashi M and Nagai R. Kruppel-like zinc-finger transcription factor KLF5/BTEB2 is a target for angiotensin II signaling and an essential regulator of cardiovascular remodeling. Nat Med 2002; 8: 856-863.