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Original Article

Cholesterol induces dedifferentiation of vascular smooth muscle cells by regulating monocyte chemotactic protein-1-induced protein 1

Bo Shu[1], Ming Zhuo[1], Zhijiang Liu[2], Zhe Lu[1], Minzhang Qian[1]

[1]*Department of Biochemistry, Zunyi Medical University, Zunyi, Guizhou, P. R. China;* [2]*Department of Cardiology, The First Affiliated Hospital, Zunyi Medical University, Zunyi, Guizhou, P. R. China*

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Abstract: Objective: To discover the effect of cholesterol on dedifferentiation of VSMCs *in vitro* and the underlying mechanisms. Methods: Vascular smooth muscle cells (VSMC) were employed to evaluate the role of cholesterol in regulating the dedifferentiation of VSMCs *in vitro*. Immunofluorescent staining, western blot, and RT-PCR were applied to uncover the inducing effect of cholesterol at a molecular level. Results: We demonstrated that the cholesterol was capable of inducing the dedifferentiation of VSMCs. Mechanistic studies revealed that monocyte chemotactic protein-1-induced protein 1 (MCPIP1) composed the most influential factor in the regulation of VSMCs during the process of cholesterol induction. When MCPIP1 was overexpressed in VSMCs, the dedifferentiation, proliferation and migration of the cells was enhanced, and the expression of miR-145 was suppressed. In contrast, knocking down MCPIP1 by siRNA promoted the differentiation and prohibited the migration of VSMCs after cholesterol treatment. These results demonstrate that MCPIP1 plays an important role in regulating cholesterol-induced dedifferentiation of VSMCs *in vitro*.

Keywords: Cholesterol, dedifferentiation, vascular smooth muscle cell, SM-actin, MCPIP1

Introduction

Atherosclerosis represents one of the most common cardiovascular diseases and results in high mortality by causing serious arrhythmia, myocardial infarction, and stenocardia [1]. Cholesterol has been identified as the main factor that induces atherosclerosis through regulating the process of VSMCs [2]. The VSMCs are highly specialized cells that play important roles in the contraction and regulation of blood vessel tone, blood pressure, and blood flow [2]. Under physiologic conditions, VSMCs mainly show a differentiated phenotype, also known as a contractile phenotype, which has a low proliferation rate and low synthetic activity [3]. The differentiated VSMCs express certain contractile proteins and show significant plasticity. However, VSMCs can also exhibit a phenotype with a high rate of proliferation, migration, and extracellular matrix synthesis that is acquired in response to environmental challenges [4]. This process of phenotypic switching is called dedifferentiation. The dedifferentiated cell plays a major pathophysiologic role in the development of atherosclerosis [5, 6]. Identification of the key mechanisms regulating the dedifferentiation of VSMCs will help to clarify the cellular responses to vascular injury in atherosclerosis.

Monocyte chemotactic protein-1-induced protein 1 (MCPIP1) is a member of the CCCH zinc finger protein family. It was first found in human monocytes after treatment with monocyte chemotactic protein-1 [7]. Recent studies have shown that MCPIP1 is involved in atherosclerosis [8, 9]. In human atherosclerotic lesions, smooth muscle actin (SM actin)-positive cells showed high expression levels of MCPIP1, and MCPIP1 mRNA levels were also found to be much higher in the aorta from ApoE knockout mice, a mouse model of atherosclerosis, than in wild-type mice [10]. Our previous study also showed that the expression of MCPIP1 was increased during VSMC dedifferentiation [11]. These results suggest that MCPIP1 may play an

important role in atherosclerosis, and they raised the question of how MCPIP1 is involved in regulating the dedifferentiation of VSMCs, which has not been answered yet.

MicroRNAs (miRNAs) are effective regulators of gene expression and require complex maturation processes to become functional [12, 13]. An important role of MCPIP1 is to suppress the maturation of certain miRNAs, including miR-135b, -146a, -21, -155, -143, and -145 [14]. Among these, miR-145 is a phenotypic regulator of VSMCs and has been reported to help maintain the differentiation state of VSMCs [15, 16]. In the present study, we tested the hypothesis that MCPIP1 suppresses miR-145 levels, thus contributing to VSMC dedifferentiation after cholesterol treatment.

Materials and methods

Materials

Cholesterol and fetal calf serum (FBS) were obtained from Sigma Chemicals, St Louis, MO, USA. Dulbecco's modified Eagle's medium (DMEM) was from Invitrogen, CA, USA. Penicillin and streptomycin were both from Beyotime (Shanghai, China). The MCPIP siRNA SMART pool was purchased from Thermo Fisher Scientific. The MCPIP and GAPDH primers were synthesized by Shanghai Sangon while the miR-145 and U6 primers were synthesized by Shanghai Gene Pharma. All other reagents were from Aladdin Reagent Co., Ltd. (Shanghai, China) and were analytical grade if not mentioned elsewhere.

Cell culture and cholesterol treatment

Human VSMC was obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were maintained in DMEM supplied with 10% FBS, 80 U/ml penicillin and 100 U/ml streptomycin at 37°C in 5% CO_2. For cholesterol treatment, 1×10^6 VMSC cells were seeded in a cell culture dish. After overnight incubation, the old culture medium was replaced with fresh and FBS-free medium containing 50 μg/mL cholesterol. The co-incubation occurred for 48 h to induce cell dedifferentiation [11].

Immunofluorescent staining

For immunofluorescent staining, 1×10^6 VSMCs without treatment of cholesterol were seeded on the glass chamber slides and cultured for 48 h. Then the slides were washed with phosphate-buffered saline (PBS) followed by fixation using 4% paraformaldehyde (Beyotime) for 10 min. After washing the slides with PBS again, the samples were permeabilized with 0.1% Triton X-100 (Beyotime) and then blocked in PBS with 3% goat serum (Bioss, Beijing, China) for 2 h. SM actin was detected by co-incubating with goat anti-SM actin antibody (1:1000, Abgent, Wuxi, China) for 2 h and then exposed to fluorescein isothiocyanate (FITC)-conjugated mouse anti-goat secondary antibody (1:1000, Bioss) for 1 h. Finally, the cells were counterstained with 4', 6-diamidino-2-phenylindole (DAPI) and the qualitative analysis was visualized using an Olympus IX17 fluorescence microscope (Olympus Corporation, Tokyo, Japan).

Plasmid construction and transfection

The GFP-MCPIP1 fusion protein was generated by cloning of the human MCPIP1 cDNA encoding the full-length MCPIP1 gene (GenBank accession number: AY920403) into the green fluorescent protein (GFP)-expressing vector pEGFP/N1 (Sangon Biotech, Shanghai, China). Plasmid transfection of VSMCs was performed using the Lipofectamine PLUS Reagents (Invitrogen). For cell transfection, 1×10^6 VSMCs were seeded on a culture dish and allowed to grow to 70-90% confluent. The plasmid DNA-lipid complex was made following the manufacturer's instructions and then applied to the cells. After 6 h of incubation, the transfection medium was replaced with fresh medium and continued to culture for further experiments. Of great importance, the transfected VSMCs were divided into two groups: oe-MCPIP1 group (VSMCs transfected with the pEGFP/N1-MCPIP1 vector) and vector group (VSMCs transfected with pEGFP/N1 vector). The transfection efficiency was evaluated by EGFP fluorescence under a fluorescence microscope, and western blot was further used to confirm the increase in MCPIP1 protein levels in cells.

Small interfering RNA (siRNA) transfection

1×10^5 VSMCs were seeded in six-well plates and cultured for 24 h until they reached 70-80% confluence followed by transfection with corresponding siRNA. The transfection was performed using RNAiFect reagent (Thermo Fisher Scientific, MA, USA) according to the manufac-

turer's instructions. Subsequently, the old medium in each well of the plates was replaced by 2 ml of fresh complete DMEM medium. After the cellscontinued to culture for 48 h, the knockdown efficiency was determined by real-time PCR and western blot analysis. The MCPIP siRNA SMART pool contained 4 siRNAs with the following sense sequences: 5'-GUAAGAAGCCACUCACUUUUU-3', 5'-GCAAGCGGGUGGUGUGCUAUU-3', 5'-CCAACACGGUGCUGGGUGAUU-3', 5'-AUACUAAGCUGUGUGGUGUUU-3', and the following antisense sequences: 5'-AAAGUGAGUGGC-UUCUUACUU-3', 5'-UAGCACACCACCCGCUUGCUU-3', 5'-UCACCCAGCACCGUGUUGGUU-3', 5'-ACACCACAC-AGCUUAGUAUUU-3'. The specificity of the siRNAs was verified using the nonspecific siRNA as a reference, with a sequence of 5'-UAGCGACUAAACACAUCAA-3'.

Cell proliferation assay

The proliferation of VSMCs was determined using the Cell Counting Kit-8 (CCK-8) assay kit. VSMCs were seeded on 96-well plates at the density of 2×10^3 cells per well. Before treatment with cholesterol, the VSMCs were respectively transfected with vector, oe-MCPIP1, NS siRNA, and MCPIP1 siRNA. After co-incubation with cholesterol for 0 h, 24 h, or 48 h, 10 μL of CCK-8 solutions were added into each well of the plates and allowed to incubate with VMSCs for 4 h at 37°C. Then the medium in the plates was carefully removed and replaced by an equivalent volume of PBS, and the absorbance at 450 nm was measured using a microplate reader. The values reflecting the amount of living cells per well were represented as a percentage of the control group.

Cell migration assay

Migration of VSMCs was evaluated by Transwell chamber assay (Corning Inc., MA, USA). For experiments, VSMCs, which have been respectively transfected with vector, oe-MCPIP1, NS siRNA, and MCPIP1 siRNA, were transplanted into the upper chambers. In the meanwhile, the lower chambers were coated with 1 mL of fresh DMEM. After 24 h of incubation, the cells that migrated to the lower chamber were fixed with methyl alcohol for 10 min and then stained with DAPI for 20 min. For quantitative evaluation, the amount of migrated cells in the lower chamber was counted using a fluorescence microscope.

Quantitative real-time PCR assay

Total RNA from VSMCs was isolated using TRIzol reagent (Invitrogen), and the genomic DNA was removed by treatment with RNase-free DNase I (Invitrogen). The first-strand cDNA, which was used as the template in the real-time PCR, was synthesized from 1 μg of total RNA using a cDNA synthesized kit (Takara, Tokyo, JAPAN). Of great importance, each sample was run in triplicate Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), used as a reference gene in the present study. The sequences of primers are: MCPIP1 forward: 5'-AGTCTGACGGGATCGTGGTT-3', reverse: 5'-GGGAGACGTACGGGAGTGAG-3', GAPDH forward: 5'-GCACCGTCAAGGCTGAGAAC-3', reverse: 5'-TGGTGAAGACGCCAGTGGA-3', hsa-mir-145 forward: 5'-ATTATATTGTCCAGTTTTCCCAGG-3', reverse: 5'-AAAGGTTGTTCTCCACTCTCTCTC-3', U6 forward: 5'-ATTGGAACGATACAGAGAAGATT-3', U6 reverse: 5'-GGAACGCTTCACGAATTTG-3'. The quantitative real-time PCR was performed with an iCycler Thermal Cycler (Bio-Rad, Hercules, CA, USA) using 2X SYBR Premix Ex Taq (Takara) and conducted according to the manufacture's instruction. The PCR is consisted of 95°C for 3 min and 40 cycles of 95°C for 12 seconds and of 62°C for 40 seconds. Finally, the quantitative evaluation was performed by the delta cycle time method.

Western blotting

The obtained VSMCs pellets were lysed with RIPA lysis buffer (Beyotime) followed by separation using 10% SDS-polyacrylamide gel electrophoresis. After that, the samples were transferred to polyvinylidene difluoride membranes (Millipore, MA, USA). Subsequently, the membranes coated with samples were blocked with 0.5% bovine serum albumin (BSA) followed by incubation with various primary antibodies: goat anti-SM actin, rabbit anti-epiregulin, and rabbit anti-actin (all at 1:1000, Abgent). Then the membranes were washed by PBS and incubated with the horseradish peroxidase-linked goat anti-mouse or goat anti-rabbit (1:5000, Bioss). The target bands were visualized with a Western Lighting ECL chemiluminescent detection kit (Perkin Elmer, Waltham, MA, USA) and the images were captured and quantified using a Gel Doc 2000 video documentation system (Bio-Rad).

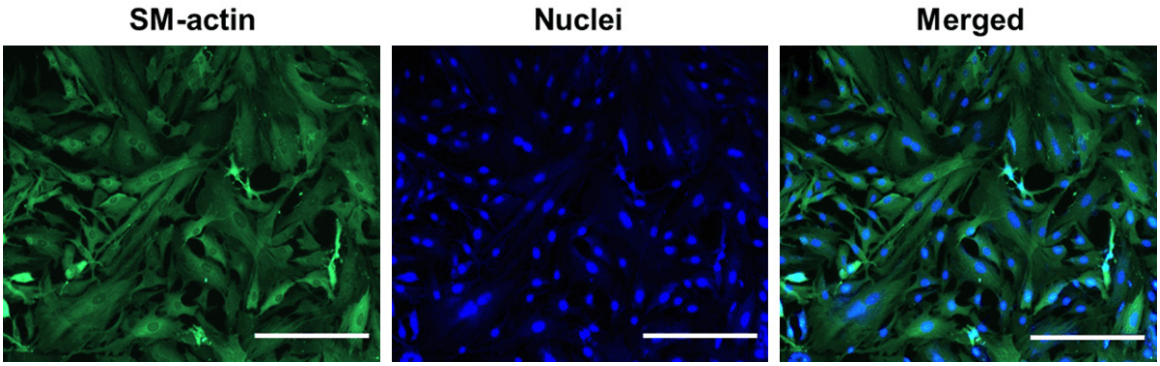

Figure 1. Characterization of the VSMCs by immunofluorescence analysis. The green fluorescent signal represents the SM-actin, with the nuclei of VSMCs visualized by the blue signal. Original magnification 200 ×. The bar represents 100 μm.

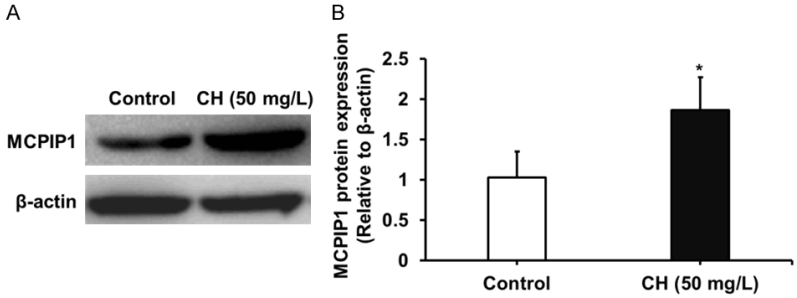

Figure 2. Expression of MCPIP1 in the VSMCs after treatment by cholesterol. A. Qualitative images of the MCPIP1 expression in VSMCs after 48 h of incubation with 50 μg/ml cholesterol. B. Semi-quantitative analysis of the level of MCPIP1 in the cholesterol-treated VSMCs. All experiments were performed 3 times, and represented as the mean ± SD. *P<0.05, significantly higher than the control group.

Statistical analysis

The obtained data were analyzed using the IBM SPSS Statistics (SPSS 18.0, SPSS Inc., USA). All data are represented as the mean ± SD. Data were analyzed using one-way analysis of variance (ANOVA) and two groups were compared using the Student's t-test. Values with P<0.05 were considered significant. All experiments were repeated at least 3 times.

Results

Characterization of VSMCs

The phenotype of VSMCs was confirmed by determination of SM actin expression. Immunofluorescent staining analysis revealed that almost all of the VSMCs highly expressed SM-actin, with obvious positive green signal observed in the in the cytoplasm (**Figure 1**). Moreover, the positive fluorescent signal displayed the typical shape of VSMCs and SM-actin. Based on this result, it could be validated that the VSMCs in our study had a satisfactory purity.

Treatment of cholesterol significantly up-regulated the MCPIP1 level in VSMCs

As demonstrated by the western blot experiments, the expression of MCPIP1 in VSMCs was dramatically increased after treatment with cholesterol (**Figure 2A**). For further confirmation, the RT-PCR assay was performed to quantitatively evaluate the levels of MCPIP1 in the cholesterol-treated VSMCs and compared with the untreated cells. As shown in **Figure 2B**, the group of cholesterol-treated cells displayed an obvious higher expression of MCPIP1 than the untreated VSMCs. Quantitative analysis revealed that the level of MCPIP1 in the cholesterol group was 1.86 fold times the controls (VSMCs without treatment by cholesterol). These results indicated that cholesterol treatment significantly increases the protein level of MCPIP1 in VSMCs compared with no treatment.

Evaluation of the transfection efficiency

The clone of MCPIP1 fragment was verified by the electrophoresis approach. As shown in **Figure 3A**, both fragments were of estimated

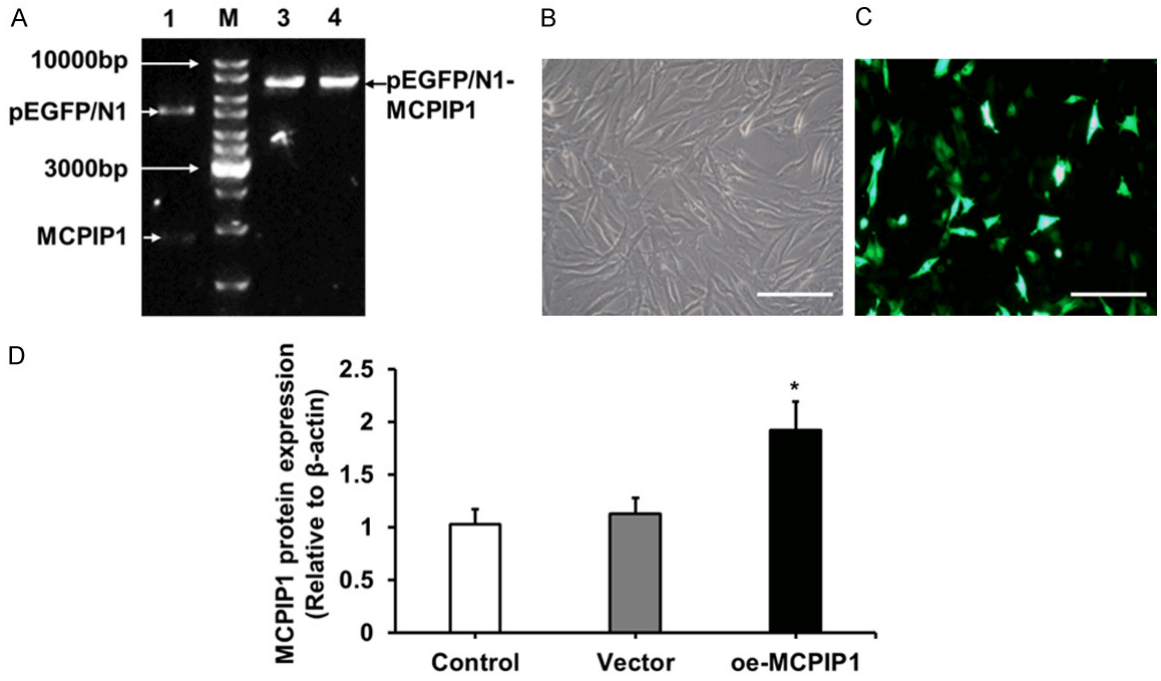

Figure 3. Evaluation of the transfection efficiency of oe-MCPIP1. A. Identification of oe-MCPIP1 plasmid by restriction enzyme digestion. Lane 1 represents the MCPIP1 recombinant plasmid digested by PstI and HindIII. Lane M represents a DNA marker, containing 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500 bp bands, respectively. Lane 3 represents the oe-MCPIP1 plasmid digested by PstI. Lane 4 represents the oe-MCPIP1 plasmid digested by HindIII. B. The morphology of the cultured VSMCs. Original magnification 100 ×. The bar represents 100 μm. C. Green fluorescent signal of MCPIP1 determined by the immunofluorescence analysis. Original magnification 100 ×. The bar represents 100 μm. D. The levels of MCPIP1 in VSMCs after the transfection of oe-MCPIP1 evaluated by western blot. All experiments were performed 3 times, and represented as the mean ± SD. *P<0.05, significantly higher than the control group.

sizes of 4600 bp and 1800 bp, respectively. Such results indicated that the MCPIP1 fragment was successfully cloned in our study.

The transfection efficiency of MCPIP1 in VSMCs was subsequently evaluated by immunofluorescence assay. As shown in **Figures 3B** and **3C**, approximately 40-50% of VMSCs displayed positive green fluorescence, indicating these cells overexpressed MCPIP1. Further quantitative analysis was performed by western blot. As shown in **Figure 3D**, a much higher level of MCPIP1 protein in the oe-MCPIP1 group was observed than that of the vector group. However, there was no significant difference in MCPIP1 protein levels between the vector group and the untransfected group.

Transfection with MCPIP1 siRNA decreased the level of MCPIP1 in VSMCs

Knockdown efficacy of the MCPIP1 siRNA was also determined by RT-PCR and western blot, respectively. As shown in **Figure 4A**, the level of MCPIP1 in VSMCs was significantly lower in the MCPIP1 siRNA group compared with the NS siRNA group or control group. Such results were further confirmed by the western blot experiments. As exhibited in **Figure 4B**, the VSMCs transfected with NS siRNA displayed an obvious higher expression of MCPIP1 than that of the MCPIP1 siRNA transfected cells. However, no significant difference was observed between the NS siRNA group and the control group. These results together indicated that the transfection of MCPIP1 siRNA was efficientto knock down MCPIP1 expression in VMSCs.

Cholesterol induced dedifferentiation of VSMCs through activation of MCPIP1

As shown in **Figure 5A**, the expression of SM-actin in VSMCs was significantly decreased after the cholesterol treatment. However, in contrast to the decrease of SM-actin expression, the level of MCPIP1 was markedly elevat-

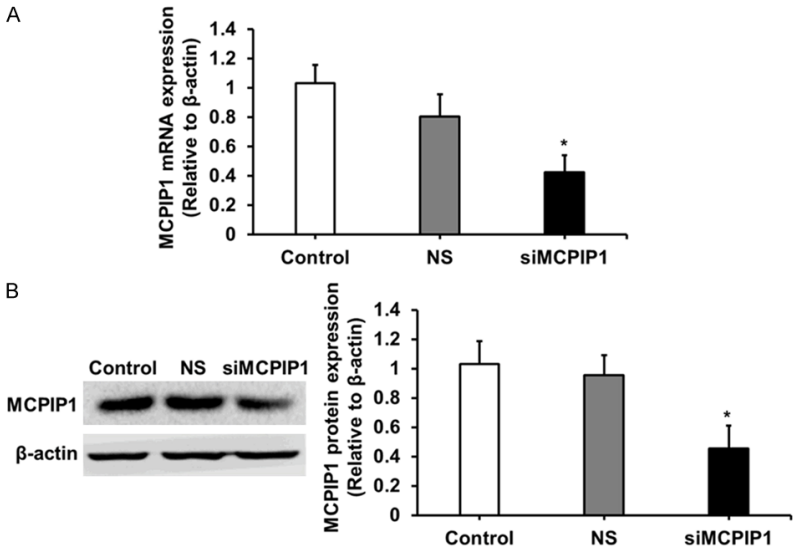

Figure 4. Investigation of the knock down efficacy of MCPIP1 siRNA. A. Real-time PCR analysis of MCPIP1 mRNA level in VSMC after siRNA transfection. B. MCPIP1 protein level in VSMC after siRNA transfection determined by western blot analysis. The left panel is a representative image and the right panel is the result of semi-quantitative analysis. All experiments were performed 3 times, and the results are represented as the mean ± SD. *P<0.05, significantly lower than the control group.

ed after treatment of cholesterol. Moreover, the positive signal of SM-actin could be further weakened by transfection with oe-MCPIP1. These results might provide evidence that cholesterol induced dedifferentiation of VSMCs through activation of MCPIP1.

For further confirmation, corresponding siRNAs were introduced to knock down the MCPIP1 expression in VSMCs before cholesterol treatment. As shown in **Figure 5B**, the decreased expression of SM-actin in the cholesterol-treated VSMCs was dramatically up-regulated after transfection with MCPIP1 siRNA. In contrast, the level of MCPIP1 in the MCPIP1 siRNA-transfected cells was significantly lower than that of the NS siRNA group.

Activation of MCPIP1 by cholesterol dramatically promoted the proliferation and migration of VSMCs

We further investigated the effect of cholesterol on proliferation of VSMCs by CCK-8 assay. As shown in **Figure 6A**, the cells incubated with cholesterol, which had overexpression of MCPIP1, exhibited an obvious faster growth rate compared with the controls. Moreover, the proliferation of VSMCs treated by cholesterol could be further enhanced after transfection of oe-MCPIP1. In contrast, the stimulatory effect of cholesterol on cell growth was counteracted by transfection of MCPIP1 siRNA to knock down the MCPIP1 expression. Based on this, the MCPIP1 was deemed as the key regulator of cholesterol-induced VSMC proliferation.

The migration of VSMCs promoted by treatment of cholesterol was evaluated by a transwell migration experiment. As demonstrated in **Figure 6B**, treatment of cholesterol led to significantly higher migration rate compared with cells without any treatment. Similar to the results of the proliferation assay, the positive effect of cholesterol could be markedly enhanced by transfection with oe-MCPIP1 while inhibited by transfection with MCPIP1 siRNA. Thus, we could draw a conclusion that the MCPIP1 played an essential role in regulating the VSMC migration in response to cholesterol treatment.

MCPIP1 regulated the cholesterol-induced dedifferentiation of VSMCs by activation of miR-145 activity

The underlying mechanisms of MCPIP1 regulating the cholesterol-induced dedifferentiation of VSMCs were further investigated. As shown in **Figure 7**, when compared with the VSMCs without any treatment, a higher expression of miR-145 was also observed in the MCPIP1 overexpressed VSMCs which were treated by cholesterol. For further confirmation, the MCPIP1 in VSMCs was knocked down before any treatment. Results displayed that knockdown of MCPIP1 in the cholesterol-treated cells led to significant down-regulation of miR-145 RNA levels. In contrast, the expression of miR-145 was dramatically increased after transfection of the VSMCs with MCPIP1 siRNA. These results together suggested that MCPIP1 had an important effect on miR-145 levels, which in turn

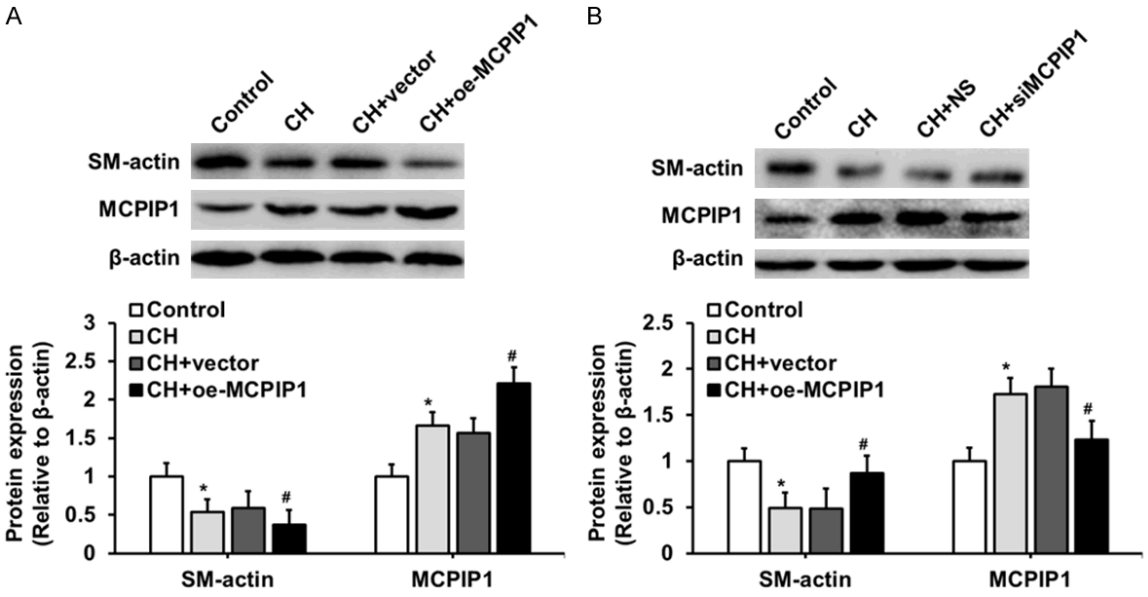

Figure 5. Cholesterol induced dedifferentiation of VSMCs through activation of MCPIP1. A. Qualitative and semi-quantitative evaluation of the levels of SM-actin and MCPIP1 in VSMCs after respectively being treated by cholesterol, cholesterol + vector, and cholesterol + oe-MCPIP1. B. Qualitative and semi-quantitative evaluation of the levels of SM-actin and MCPIP1 in VSMCs after the MCPIP1 was knocked down by transfection with MCPIP1 siRNA. All experiments were performed 3 times, and the results are represented as the mean ± SD. *P<0.05, compared with the control group, #P<0.05, compared with the cholesterol + vector group.

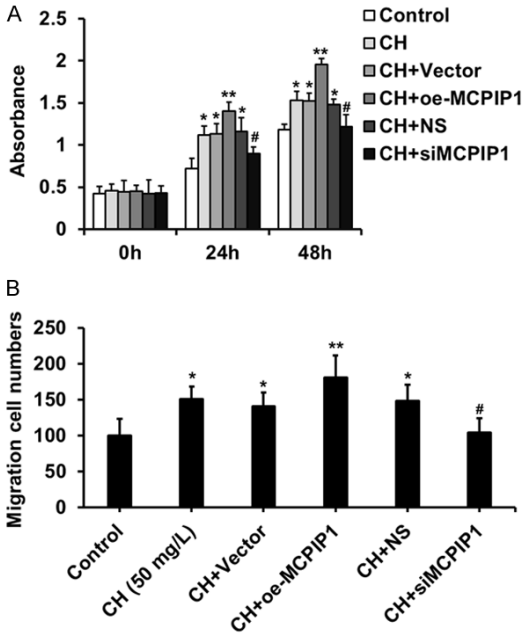

Figure 6. The effect of cholesterol on the proliferation and migration of VSMCs. A. The proliferation of VSMCs after treated by various strategies, including cholesterol, cholesterol + vector, cholesterol + oe-MCPIP1, cholesterol + NS, and cholesterol + siMCPIP1. B. Migration rate of VSMCs after the above treatments by determination of the migration cell numbers. All experiments were performed 3 times, and represented as the mean ± SD. *P<0.05, **P<0.01, compared with the control group, #P<0.05, compared with the cholesterol + NS group.

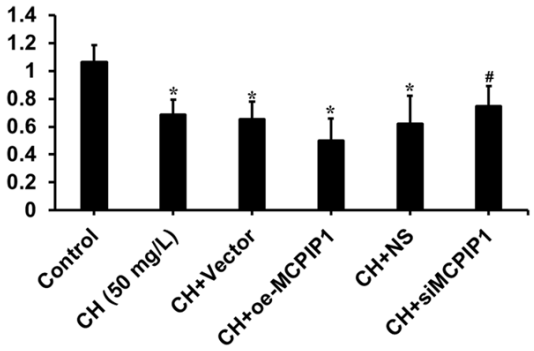

Figure 7. Effect of cholesterol on miR-145 expression in VSMCs determined by RT-PCR after treated by various strategies, including cholesterol, cholesterol + vector, cholesterol + oe-MCPIP1, cholesterol + NS, and cholesterol + siMCPIP1. All experiments were performed 3 times, and represented as the mean ± SD. *P<0.05, compared to the control group, #P<0.05, compared to the cholesterol + NS group.

regulated the cholesterol-induced dedifferentiation of VSMCs.

Discussion

High levels of plasma cholesterol have been reported to be strongly associated with atherosclerosis, and cholesterol deposits in the arterial wall are the most common characteristic of atherosclerosis in all animals [17]. Our previous

study demonstrated that cholesterol could induce a phenotypic switch of VSMCs and promote proliferation of VSMCs [11]. By determining the characteristic proteins, including the SM-actin and epiregulin, the present study showed that treatment with cholesterol significantly decreased the level of SM actin and increased the epiregulin expression in VSNCs, indicating that cholesterol was capable of stimulating VSMC dedifferentiation with a phenotypic change.

As an important regulator of cell vitality, differentiation and apoptosis, MCPIP1 has been demonstrated to play a pivotal role in the occurrence and progression of a wide array of vascular diseases [18-20]. The most likely mechanisms of MCPIP1 inducing many vascular diseases are derived from the fact that MCPIP1 is able to enhance endothelial cell proliferation, migration, and expression of angiogenesis-related genes, which finally leads to capillary-like tube formation [21, 22]. Also, anabnormal increase in the MCPIP1 level is generally correlated to endothelial dysfunction [8]. It has been demonstrated that the MCPIP1 was overexpressed in VSMCs of the atherosclerotic lesions, indicating MCPIP1 was relevant to development of atherosclerosis [23]. As proven in our previous study, MCPIP1 plays an important role in MCP1 inducing VSMC proliferation [23]. In this study, we further demonstrated that the cholesterol-induced VSMC dedifferentiation was associated with increased MCPIP1 expression. Additionally, the cholesterol-induced dedifferentiation and migration of VSMCs could be enhanced by overexpression of MCPIP1 through transfection with a plasmid. In contrast, knocking down of MCPIP1 with siRNAs significantly inhibited the dedifferentiation, proliferation, and migration of VSMCs. These results together further confirmed that MCPIP1 was a key regulator in cholesterol-induced VSMC dedifferentiation.

In recent years, miRNAs have been found to play very important roles in atherosclerosis by regulating cell proliferation and angiogenesis [24-26]. A previous study indicated that the miR-145 might play a significant role in the regulation of VSMC phenotype switch through elevating the levels of KLF4 and myocardin [27]. Additionally, MCPIP1 has also been reported to be able of suppressing the corresponding miRNAs by cleaving the terminal loops of precursor miRNAs, such as miR-145 [28, 29]. Our study revealed that the expression of miR-145 in VSMCs could be dramatically down-regulated after the treatment by cholesterol. Further experiments exhibited that overexpression of MCPIP1 leaded to a marked increase of miR-145 expression, indicating that the MCPIP1 was involved during the cholesterol inhibiting the activity of miR-145. It can be demonstrated by the fact that knocking down MCPIP1 resulted in a higher level of miR-145 in VSMCs after treatment with cholesterol.

In conclusion, the results of the present study demonstrated that MCPIP1 hasa role in the dedifferentiation of VSMCs that is induced by cholesterol. We further confirmed that suppression of the MCPIP1 level resulted in prominent inhibition of proliferation and translocation of VSMCs induced by cholesterol. These findings provided a comprehensive understanding of the underlying mechanisms of cholesterol-induced dedifferentiation of VSMCs, which finally led to serious atherosclerosis.

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Disclosure of conflict of interest

None.

Abbreviations

MCPIP1, Monocyte chemotactic protein 1 induced protein 1; VSMC, vascular smooth muscle cell; SM-actin, smooth muscle actin; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; DAPI, 4', 6-diamidino-2-phenylindole; FITC, fluorescein isothiocyanate; GFP, green fluorescent protein; CCK-8, cell counting kit-8; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Address correspondence to: Minzhang Qian, Department of Biochemistry and Molecular Biology, Zunyi Medical University, Xuefuxi Road, Zunyi, Guizhou, P. R. China. E-mail: qian_mzh@hotmail.com

References

[1] Alexander MR, Owens GK. Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease. Annu Rev Physiol 2012; 74: 13-40.

[2] Julio MM, Noemi RJ, Aranda F. MicroRNAs and atherosclerosis. Curr Atheroscler Rep 2013; 15: 322-330.

[3] Chang S, Song S, Lee J, Yoon J, Park J, Choi S, Park JK, Choi K, Choi C. Phenotypic modulat ion of primary vascular smooth muscle cells by short-term culture on micropatterned substrate. PLoS One 2014; 9: 880-89.

[4] Gan J, Li P, Wang Z, Chen J, Liang X, Liu M, Xie W, Yin R, Huang F. Rosuvastatin suppresses platelet-derived growth factor-BB-induced vascular smooth muscle cell proliferation and migration via the MAPK signaling pathway. Exp Ther Med 2013; 6: 899-903.

[5] Gomez D, Owens GK. Smooth muscle cell phenotypic switching in atherosclerosis. Circ Res 2012; 95: 156-64.

[6] Li P, Zhu N, Yi B, Wang N, Chen M, You X, Zhao X, Solomides CC, Qin Y, Sun J. MicroRNA-663 regulates human vascular smooth muscle cell phenotypic switch and vascular neointimal formation. Circ Res 2013; 113: 1117-1127.

[7] Zhou L, Azfer A, Niu J, Graham S, Choudhury M, Adamski FM, Younce C, Binkley PF, Kolattukudy PE. Monocyte chemoattractant protein-1 induces a novel transcription factor that causes cardiac myocyte apoptosis and ventricular dysfunction. Circ Res 2006; 98: 1177-118.

[8] He M, Liang X, He L, Wen W, Zhao S, Wen L, Liu Y, Shyy JY, Yuan Z. Endothelial dysfunction in rheumatoid arthritis: the role of monocyte chemotactic protein-1-induced protein. Arterioscler Thromb Vasc Biol 2013; 33: 1384-1391.

[9] Yu F, Du F, Wang Y, Huang S, Miao R, Major AS, Murphy EA, Fu M, Fan D. Bone marrow deficiency of MCPIP1 results in severe multi-organ inflammation but diminishes atherogenesisin hyperlipidemic mice. PLoS One 2013; 8: 80089-99.

[10] Qi Y, Liang J, She ZG, Cai Y, Wang J, Lei T, Stallcup WB, Fu M. MCP induced protein 1 suppresses TNFα-induced VCAM-1 expression in human endothelial cells. FEBS J 2010; 584: 3065-3072.

[11] Shu B, Yang YJ, Qian MZ. Study on VSMC phenotypic switch induced by cholesterol in vitro. CJCMI 2014; 30: 725-728.

[12] Song JL, Nigam P, Tektas SS, Selva E. microRNA regulation of Wnt signaling pathways in development and disease. Cell Signa 2015; 27: 1380-91.

[13] Hosin AA, Prasad A, Viiri LE, Davies AH, Shalhoub J. MicroRNA s in atherosclerosis. J Vasc Res 2014; 51: 338-49.

[14] Suzuki HI, Arase M, Matsuyama H, Choi YL, Ueno T, Mano H, Sugimoto K, Miyazono K. MCPIP1 ribonuclease antagonizes dicer and terminates microRNA biogenesis through precursor microRNA degradation. Mol Cell 2011; 44: 424-436.

[15] Ohnaka M, Marui A, Yamahara K, Minakata K, Yamazaki K, Kumagai M, Masumoto H, Tanaka S, Ikeda T, Sakata R. Effect of microRNA-145 to prevent vein graft disease in rabbits by regulation of smooth muscle cell phenotype. J Thorac Cardiovasc Surg 2014; 148: 676-82.

[16] Hutcheson R, Terry R, Chaplin J, Smith E, Musiyenko A, Russell JC, Lincoln T, Rocic P. MicroRNA-145 restores contractile vascular smooth muscle phenotype and coronary collateral growth in the metabolic syndrome. Arterioscler Thromb Vasc Biol 2013; 33: 727-736.

[17] Mahley RW. The role of dietary fat and cholesterol in atherosclerosis and lipoprotein metabolism. West J Med 1981; 134: 34-42.

[18] Qi D, Huang S, Miao R, She ZG, Quinn T, Chang Y, Liu J, Fan D, Chen YE, Fu M. Monocyte chemotactic protein-induced protein 1 (MCPIP1) suppresses stress granule formation and determines apoptosis under stress. J Biol Chem 2011; 286: 1692-1700.

[19] Skalniak L, Dziendziel M, Jura J. MCPIP1 contributes to the toxicity of proteasome inhibitor MG-132 in HeLa cells by the inhibition of NF-Jb. Mol Cell Biochem 2014; 395: 253-263.

[20] Yang L, Chao J, Kook YH, Gao Y, Yao H, Buch SJ. Involvement of miR-9/MCPIP1 axis in PDGF-BB-mediated neurogenesis in neuronal progenitor cell. Cell Death Dis 2013; 4: e960.

[21] Jin Z, Liang J, Wang J, Kolattukudy PE. Delayed brain ischemia induced by electroacupuncture pretreatment is mediated via tolerance MCP-induced protein 1. J Neuroinflammation 2013; 10: 63.

[22] Niu J, Azfer A, Zhelyabovska O, Fatma S, Kolattukudy PE. Monocyte chemo-tactic protein (MCP)-1 promotes angiogenesis via a novel transcription factor, MCP-1-induced protein (MCPIP). J Biol Chem 2008; 283: 14542-14551.

[23] Yang YJ, Wu LS, Shu B, Qian MZ. MCPIP1 mediates MCP-1-induced vascular smooth muscle cell proliferation. Acta Physiologica Sinica 2013; 65: 616-22.

[24] Julio MM, Noemi RJ, Aranda F. MicroRNAs and atherosclerosis. Curr Atheroscler Rep 2013; 15: 322-330.

[25] Emanueli C, Shearn AI, Angelini GD, Sahoo S. Exosomes and exosomal miRNAs in cardiovascular protection and repair. Vascul Pharmacol 2015; 71: 24-30.

[26] Song X, Shan D, Chen J, Jing Q. miRNAs and lncRNAs in vascular injury and remodeling. Sci China Life Sci 2014; 57: 826-35.

[27] Lovren F, Pan Y, Quan A, Singh KK, Shukla PC, Gupta N, Steer BM, Ingram AJ, Gupta M, Al-Omran M, Teoh H, Marsden PA, Verma S. MicroRNA-145 targeted therapy reduces atherosclerosis. Circulation 2012; 126: 81-90.

[28] Boucher J, Gridley T, Liaw L. Molecular pathways of Notch signaling in vascular smooth muscle cells. Front Physiol 2012; 3: 81.

[29] Gao H, Steffen MC, Ramos KS. Osteopontin regulates α-smooth muscle actin and calponin in vascular smooth muscle cells. Cell Biol Int 2012; 36: 155-61.