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Metformin attenuates carotid neointimal hyperplasia by modulating the vascular smooth muscle cell phenotype transformation through upregulation of TET2, Nur77 and calponin

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Abstract: Metformin is a drug used to treat type 2 diabetes based on its effectiveness as well as cardiovascular safety. Metformin has been shown to modulate proliferation and migration of vascular smooth muscle cells (VSMCs), but the underlying mechanisms of the effect of metformin on VSMC function remains unclear. We found that metformin inhibits VSMC proliferation and migration and upregulates the expression of nuclear receptor subfamily 4 group A member 1 (Nur77), ten-eleven translocation 2 (TET2), and calponin *in vitro*. In the carotid artery balloon injury model of rats, metformin effectively prevented neointimal hyperplasia in the carotid artery, including neointimal thickness, increased neointimal area, and the neointimal area/medial area ratio. It also reduced the number of proliferating cell nuclear antigen $(PCNA)^+$ cells and increased the expression of Nur77, calponin and alpha-smooth muscle actin (α-SMA). These results show that metformin attenuates neointimal hyperplasia in balloon-injured carotid arteries via increased expression of TET2, Nur77 and calponin, and reduced expression of matrix metallopeptidase 9 (MMP-9).

Keywords: metformin; VSMCs; neointima; TET2; Nur77

INTRODUCTION

Cardiovascular diseases (CVDs) are the most common causes of death globally. CVDs are disorders of the heart and blood vessels and include coronary heart disease, cerebrovascular disease, and rheumatic heart disease [1]. VSMCs play an important role in the pathogenesis of vascular diseases. Generally, VSMCs are resident in the tunica media of the vascular wall and remain in a quiescent state expressing contractile proteins such as α-SMA, smooth muscle myosin heavy chain (protein) (SMMHC) and calponin, a calcium-binding protein. VSMCs become active from the differentiated state to a dedifferentiated state in response to inflammatory stimulation. This results in hyperproliferation and migration of VSMCs accompanied by downregulation of contractile proteins. This hyperproliferative and migration state of VSMCs significantly contributes to the development of vascular diseases [1]. Metformin (N,N-dimethylbiguanide) is a first-line drug used to treat type 2 diabetes [2]. Metformin inhibits hepatic gluconeogenesis and improves glucose uptake in peripheral tissues to achieve a glucose-lowering effect. Metformin also decreases the risk of cardiovascular events such as myocardial infarction [3] and stroke [4] in patients with diabetes. In the cardiovascular system, metformin ameliorates neointimal hyperplasia through inhibition of smooth muscle cell proliferation and migration [5]; it modulates the proliferation and migration of VSMCs through different molecular targets [6-9]. Although many molecular targets have been identified, the precise molecular mechanism of metformin action is not understood.

TET2 is a demethylase enzyme that catalyzes the oxidation of DNA 5-methylcytosine to 5-hydroxymethylcytosine resulting in DNA methylation and altered gene expression [10]. Expression of TET2 is downregulated in atherosclerotic plaques [11]. TET2 alleviates endothelial cell function and inhibits

inflammation [12-14]. TET2 is also found to reduce proliferation and migration of VSMCs, and to regulate VSMC phenotypic transmission [15]. However, it is unclear if TET2 is involved in metformin-mediated VSMC function.

Nur77 (NR4A1) is an immediate-early gene induced by growth factors and cytokines, including angiotensin-II [16], platelet-derived growth factor [17] and tumor necrosis factor [18]. Nur77 plays an important role in cellular processes such as proliferation, migration and apoptosis [19], and it may also have cardiovascular protective effects [20]. Nur77 controls endothelial cell proliferation and angiogenesis and reduces atherosclerotic plaque formation [21]. In VSMCs, overexpression of Nur77 inhibited VSMC proliferation and migration, and prevented neointimal hyperplasia in injured carotid arteries of mice [22,23]. Whether metformin affects Nur77 in VSMCs is unclear.

Therefore, in the present study we investigated whether TET2 and Nur77 are involved in the vascular protective role of metformin. We found that metformin effectively prevented neointimal hyperplasia by upregulating the expression of Nur77 and TET2. The present findings provide novel insight into the mechanisms underlying proliferative arrest and migratory inhibition of VSMCs mediated by metformin.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (mean weight 250 ± 20 g) were purchased from the Shanghai Experimental Animal Research Center (Shanghai, China) and housed in a room at a temperature of 25°C±2. All experimental procedures involving rats were carried out in accordance with the Animal Management Rules of China (55, 2001, Ministry of Health, China) and the Guidelines for Care and Use of Laboratory Animals (Ethics code SCXK Shanghai 2019-0006)

Rats were randomly assigned to the following experimental groups: Control group, model group (group II), and the metformin group (group IIM). Rats were intragastrically administered metformin once daily at 300 mg/kg for 21 days. Normal phosphate buffered saline (PBS) was intragastrically administrated to the control and model group II. Rats were anesthetized with isoflurane.

The carotid artery balloon injury model in rats was established as previously described [24]. A midline neck incision was made to expose the left external, internal and common carotid arteries. A balloon was inserted from the external carotid cut into the common carotid artery and inflated with water. The common carotid artery was injured three times with rotation, and the external carotid artery was ligated. In each group, the common carotid artery was harvested 21 d after injury.

VSMC culture

VSMCs were obtained from the rat thoracic aorta by the explant method as previously described [25]. The purity of VSMCs was confirmed by immunofluorescence of α-SMA (Proteintech, Rosemont, IL, USA). VSMCs were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% FBS and 1% pen/strep (Thermo Fisher, Waltham, MA, USA) in a 37°C, 5% CO_2 incubator (Thermo Fisher, Waltham, MA, USA). All experiments were performed using cells after 3-7 passages.

Lentivirus packaging and infection

The pLent-U6-GFP-puro lentiviral vectors containing TET2- or Nur77-specific small hairpin RNA (shRNAs) were constructed by Vigene Bioscience Technology (Jinan China). The pLent-U6-GFP-puro lentiviral vector was used as a negative control. A lentiviral vector and packaging plasmid mix were transfected into HEK293T cells following the manufacturer's instructions (Genomeditech, China). The medium was replaced after 6 h. Fresh medium was collected 72 h post-transfection and passed through a 0.45-μm filter. VSMCs were transduced with filtered lentiviral supernatants supplemented with 8 μg/mL polybrene (Sigma-Aldrich, St. Louis, MO, USA). Transfected cells were selected by the addition of 2.5 μg/mL puromycin to the culture medium.

CCK-8 assay

A total of 100 μL of VSMC cell suspension (0.5 x 10^3 cells) was seeded in 96-well plates, and cells were cultured in fetal bovine serum (FBS)-free DMEM for 24 h. Cells were then treated with a vehicle control, 10%

FBS DMEM or 10% FBS DMEM containing metformin at different concentrations for indicated times. Then 10 μL CCK8 solution (MedChemExpress, USA) was added to cells for 1 h. The absorbance at 450 nm was measured with a microplate reader (BioTek, Winooski, VT, USA) to determine the proliferation rate of VSMCs.

Wound healing assay

Cells were plated in 6-well plates at a density of 1.5 x 10^5 cells/well. When confluent, the cells were cultured in serum-free medium for 24 h. A scratch was then made with a sterile 200-μL pipette tip. Cells were then treated with vehicle, 10% FBS DMEM or 10% FBS DMEM with metformin at different concentrations for the indicated times. Images of scratches were captured at 0 h and at the indicated times with an inverted microscope (BX71; Olympus Corporation, Japan).

Western blot analysis

Cells were lysed in radioimmunoprecipitation assay (RIPA) buffer (Beyotime Biotechnology, China) containing a cocktail of protease inhibitors. Proteins were quantified using the bicinchoninic acid (BCA) assay (Beyotime Biotechnology, China) and separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Proteins were transferred to a nitrocellulose membrane and blocked with 5% non-fat milk in tris-buffered saline (TBS) and Polysorbate 20 (Tween 20) (TBST) for 1 h. Then the membranes were incubated with the corresponding primary antibodies at 4°C overnight. The primary antibodies used in this study were Nur77 (1:1000, Proteintech, Rosemont, IL, USA), TET2 (1:1000, Abcam, UK), calponin (1:2000, Proteintech, Rosemont, IL, USA), MMP-9 (1:2000, Proteintech, Rosemont, IL, USA), α-SMA (1:1000, Proteintech, Rosemont, IL, USA) and α-tubulin (1:2000, Proteintech, Rosemont, IL, USA). The membranes were washed and incubated with the corresponding horseradish peroxidase (HRP)-conjugated goat anti-mouse (1:10000) or goat anti-rabbit secondary antibody (1: 10000, Proteintech, Rosemont, IL, USA) for 1 h at room temperature. Enhanced chemiluminescence reagent (Absin Bioscience, China) was added to the membranes, and chemiluminescent signals were detected using an image analyzer (Tanon Science, China). ImageJ software (NIH, Bethesda, MD, USA) was used for densitometric analysis of the resultant images.

Quantitative real-time PCR

Total RNA was extracted from cells using TRIzol Reagent (Invitrogen, Carlsbad, CA, USA), and cDNA was generated using the PrimeScript RT kit (Takara, Japan) according to manufacturer's instructions. qPCR was performed with 2xSGExcel UltraSYBR mix with ROX (Sangon Biotech, China) using a Quant Studio 6 Flex PCR machine (ThermoFisher, Waltham, MA, USA). The relative gene expression was calculated using the $2^{-\Delta\Delta Cq}$ method. The primer sequences used in this study were as follows: GAPDH, 5'-AGC TTC CCA TTC TCA GCC TTG ACT-3' (forward) and 5'-ACA AGA TGG TGA AGG TCG GTG TGA-3' (reverse); Nur77, 5'-GCG GCT TTG GTG ACT GGA T-3' (forward) and 5'-GGC CAT GTC GAT CAG TGA TGA G-3' (reverse); MMP-9, 5'-GGA TGT TTT TGA TGC CAT TGC TG-3' (forward) and 5'-CCA CGT GCG GGC AAT AAG AAA G-3' (reverse).

Histopathological and immunohistochemical analysis

Carotid arteries were excised and fixed with 4% paraformaldehyde and embedded in paraffin. Hematoxylin and eosin (H&E) staining was performed on 4-μm sections. Images of sections were taken using an inverted microscope (BX71; Olympus Corporation, Japan). The intimal area and media area were determined by the lumen, internal elastic lamina, and the external elastic lamina was measured using ImageJ software.

Immunohistochemical staining was performed to detect PCNA (Proteintech, Rosemont, IL, USA), Nur77, calponin and α-SMA. Antigen retrieval was performed using 0.1 M citric acid buffer at 100°C for 20 min. Slides were then treated with 3% hydrogen peroxide for 30 min at room temperature to block endogenous peroxidase activity. Specimens were incubated with primary antibodies overnight at 4°C. Subsequently, the specimens were washed with PBS and covered with polyperoxidase-anti-mouse/rabbit IgG for 30 min at 37°C. DAB (3,3'-diaminobenzidine) was used to visualize protein expression, and specimens were counterstained with hematoxylin. ImageJ analysis software was used to analyze the average integrated optical density (IOD).

Statistical analysis

The obtained data were statistically analyzed using GraphPad Prism 7.0 software. Data are expressed as the mean±standard deviation (mean±SD). Student's t-test was used to compare the means of at least two independent samples. P<0.05 was considered statistically significant.

RESULTS

Metformin inhibits VSMC proliferation and migration

Metformin inhibited VSMC migration as revealed by reduced healing of the VSMC monolayer wound after metformin treatment (Fig. 1A, B). The limitation of VSMC migration by metformin depended on concentration and time. The optimal condition for metformin to inhibit VSMC migration was 10 mM for 24 h (Fig. 1A, B). Metformin can also inhibit VSMC proliferation. The CCK-8 assay showed that metformin suppressed VSMC proliferation in a dose-dependent manner. The minimum dose for metformin to display the inhibitive effect was 1 mM for 24 h, and the inhibition effect of metformin on VSMC proliferation was enhanced as the metformin concentration increased (Fig. 1C).

Metformin upregulates Nur77, TET2 and calponin expression in VSMCs

We studied the expression of Nur77, TET2 and calponin. The expression of Nur77 was significantly increased in VSMCs at the levels of protein (Fig. 2A, B) and mRNA (Fig. 2C) after treatment with 10 mM metformin. The protein levels of Nur77 reached a peak at 6 h and began to decline at 12 h after metformin treatment (Fig. 3A, B). The mRNA expression level of Nur77 peaked at 2 h and then gradually decreased after metformin treatment (Fig. 3C). Metformin treatment also increased the protein levels of TET2 (Fig. 3D) and calponin (Fig. 3E). These results indicated that metformin stimulated the upregulation and expression of Nur77, TET2 and calponin in VSMCs.

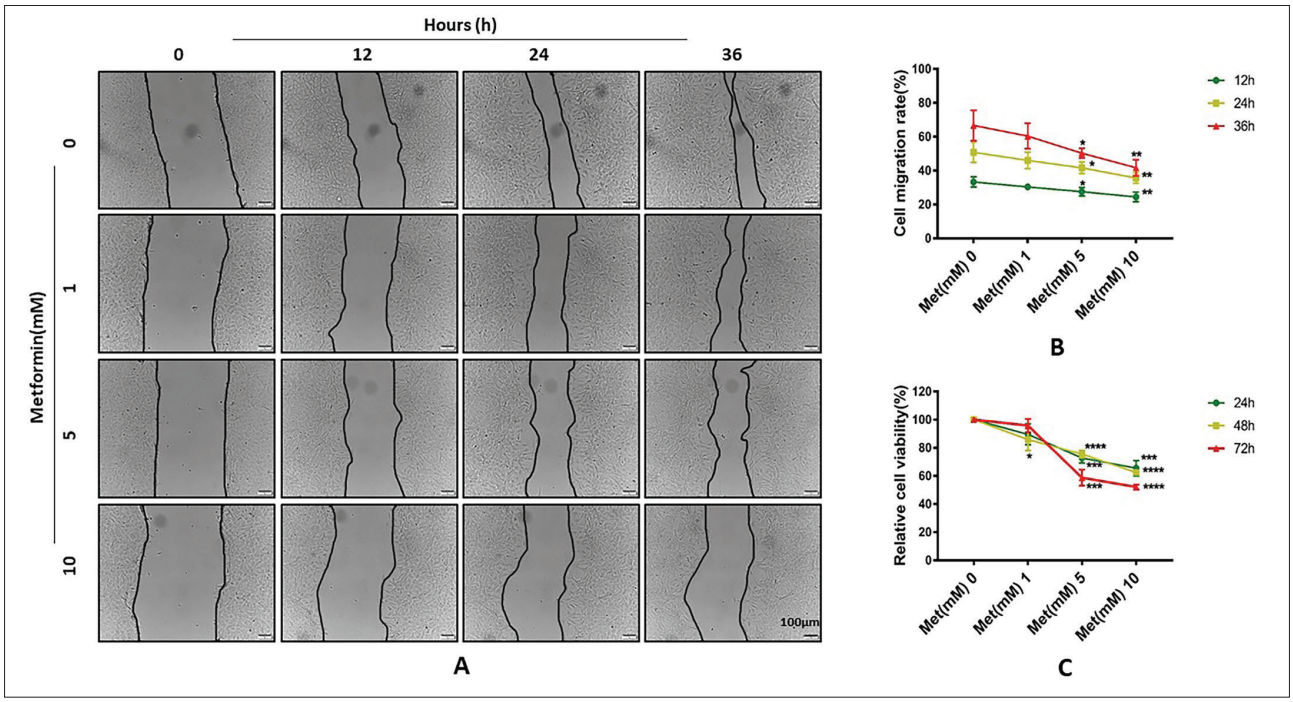

Fig. 1. Metformin inhibits vascular smooth muscle cell (VSMC) proliferation and migration. **A** – Attenuation of VSMC wound closure by metformin. VSMC wounds were treated with metformin at the indicated doses and measured at the indicated time points. **B** – Quantitation of the percent of distance of VSMC migrated toward the centerline of the monolayer wound (%). n=4; * P<0.05, ** P<0.01 vs 0 h. **C** – VSMC proliferation detected by the CCK-8 assay at the indicated time points after treatment with metformin at the indicated doses. n=3. * P<0.05, ***P<0.005, **** P<0.001 vs 0 mM metformin treatment.

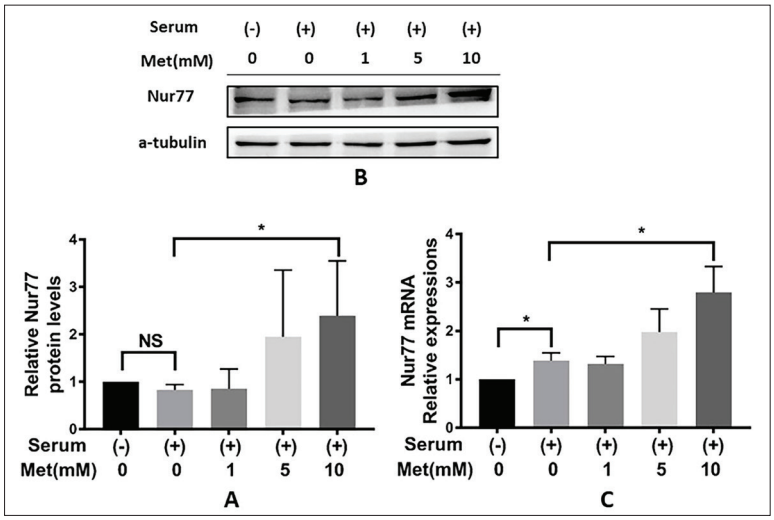

Fig. 2. Metformin upregulated Nur77 expression at the levels of mRNA and protein in VSMCs. **A** – Upregulation of Nur77 protein expression in VMSCs treated with metformin (Met); ⟨-tubulin was used as the loading control. **B** – Quantitation of the expression of Nur77 protein. n=4. * P<0.05 vs VSMCs treated with serum without metformin (Met). **C** – Quantitation of the expression of Nur77 mRNA in VMSCs treated with metformin (Met) by qRT-PCR. The results represent the fold change of Nur77 mRNA expression. n=3. * P<0.05 vs VSMCs treated with serum without metformin (Met).

Nur77 modulates the expression of MMP-9 and TET2 modulates the expression of Nur77

Metformin stimulates upregulation of Nur77, TET2 and calponin expression in VSMCs, and Nur77 and TET2 may be involved in VSMC proliferation. The level of MMP-9 mRNA expression was significantly increased after silencing Nur77 by shRNA (Fig. 4A, B). After silencing TET2 by shRNA, Nur77 protein expression was significantly reduced (Fig. 4C, D). This suggested that TET2 modulates the expression of Nur77, which subsequently regulates MMP-9 expression in VSMCs.

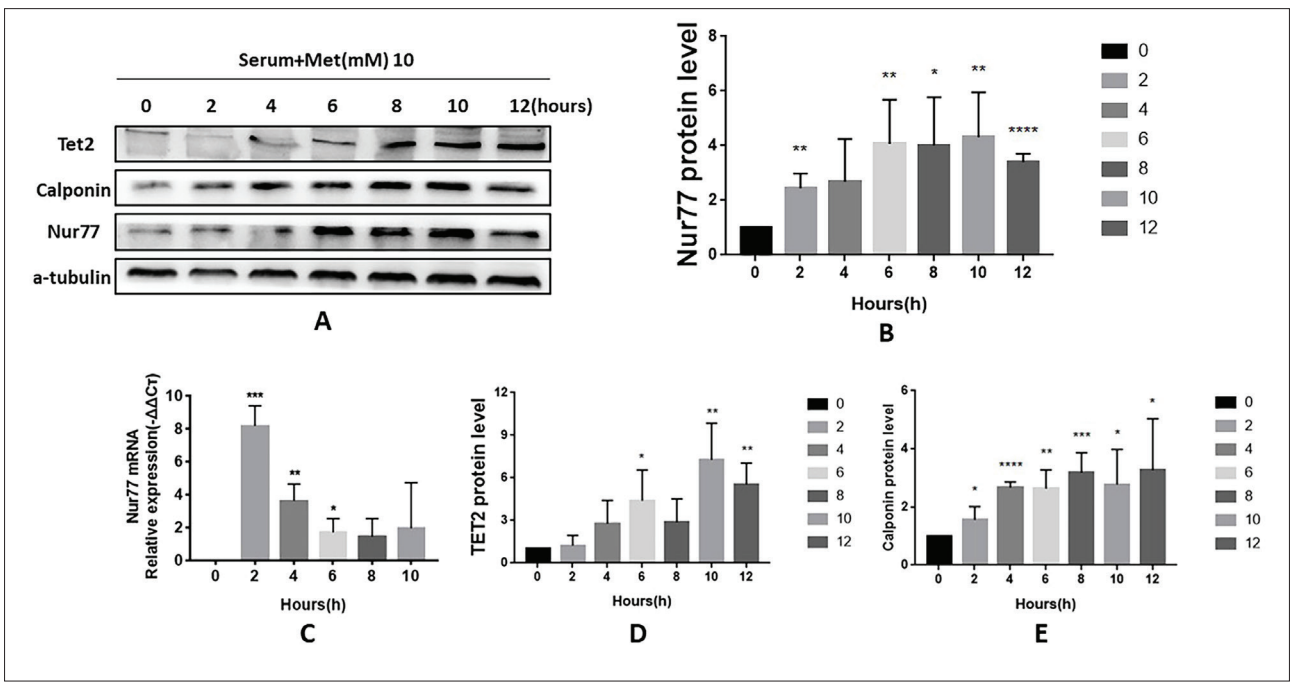

Fig. 3. Metformin upregulates the expression of Nur77, TET2, and calponin in VMSCs. **A** – Representative images showing the protein levels of Nur77, TET2 and calponin at the indicated time points in VMSCs treated with metformin (10 mM) **B** – Quantitation of the expression of Nur77 protein at the indicated time points in VMSCs treated with metformin (10 mM). n=4. * P<0.05, ** P<0.01, **** P<0.001 vs VSMCs without metformin treatment. **C** – Quantitation of Nur 77 mRNA at the indicated time points in VMSCs treated with metformin (10 mM). n=4. * P<0.05, ** P<0.01, *** P<0.005 vs VSMCs without metformin treatment. **D** – Quantitation of the expression of TET2 protein at the indicated time points in VMSCs treated with metformin (10 mM). n=4. * P<0.05, ** P<0.01 vs VSMCs without metformin treatment. **E** – Quantitation of the expression of calponin protein at the indicated time points in VMSCs treated with metformin (10 mM). * P<0.05, ** P<0.01, *** P<0.005, ****P <0.005 vs VSMCs without metformin treatment.

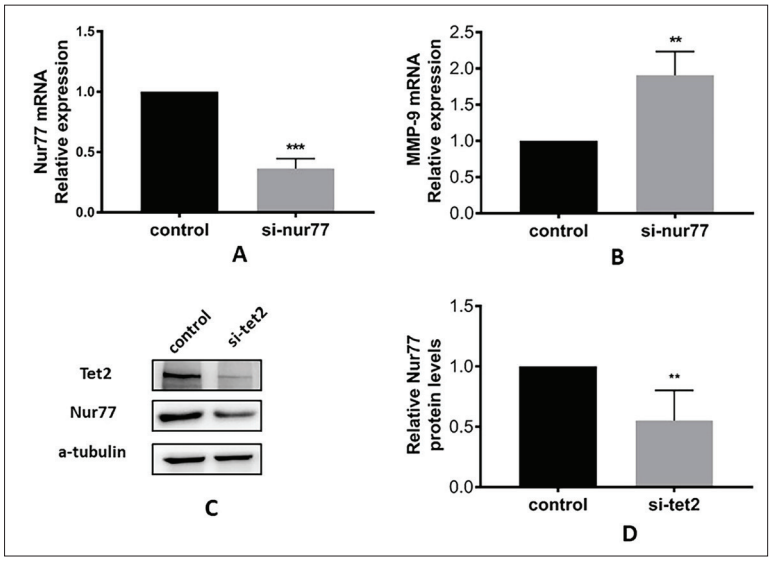

Fig. 4. Nur77 modulates the expression of MMP-9, while TET2 modulates the expression of Nur77. **A –** Reduced expression of Nur77 mRNA after Nur77-shRNA treatment in VMSCs. n=4, ** P<0.01 vs control. **B –** Reduced expression of MMP-9 mRNA in VMSCs with silenced Nur77. n=4, *** P<0.005 vs control. **C –** Representative images showing the protein levels of Nur77, TET2 and α-tubulin in VMCSs with TET2-shRNA; α-tubulin was the loading control. **D –** Reduced expression of Nur77 protein in VMSCs with TET2-shRNA. n=4, ** P<0.01 vs control.

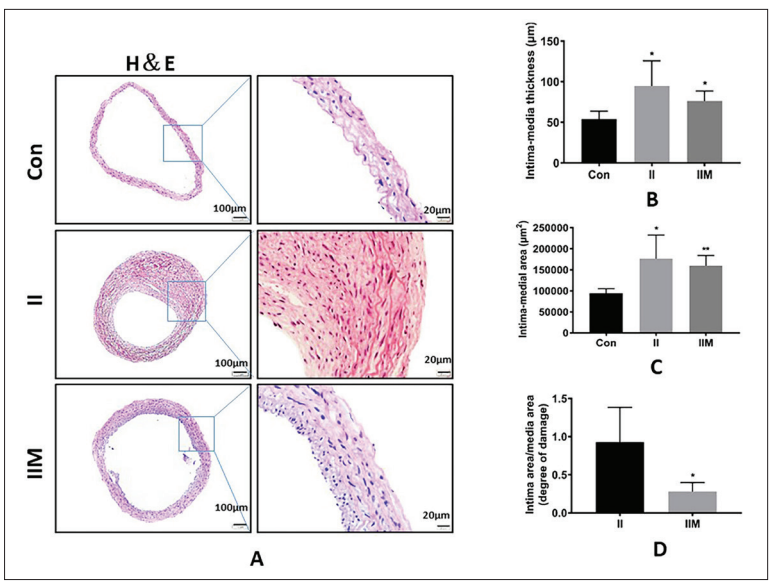

Fig. 5. Metformin reduces balloon injury-induced neointimal hyperplasia in the carotid artery.

A – Representative images showing reduced balloon injury-induced neointimal hyperplasia in the carotid artery after metformin treatment. H&E staining, Scale bar: left, 100 μm, right, 20 μm. **B –** Quantitation of reduced intima-medial thickness (μm) in the carotid artery after metformin treatment. n=4, ##P<0.01 vs control; **P<0.01 vs group II. **C –** Quantitation of reduced intima-media area (μm^2) in the carotid artery after metformin treatment. n=4. ###P<0.005 vs control; *** P<0.01 vs group II. **D –** Quantitation of the reduced degree of damage (intima area/medial area) in the carotid artery after metformin treatment. n=4. *** P<0.01 vs group II.

Metformin reduces balloon injury-induced neointimal hyperplasia

The *in vivo* experiment revealed that the balloon injury induced significant neointimal formation in the carotid artery (Fig. 5A). Administration of metformin effectively prevented neointima hyperplasia induced by balloon injury in the carotid artery (Fig. 5A), including neointimal thickness, the neointimal area and the neointimal area/medial area ratio (Fig. 5B-D)

Metformin reduces PCNA expression but enhances Nur77, calponin and α-SMA expression *in vivo*

We investigated the expression of PCNA *in vivo*. After intragastrical metformin administration, the percentage of PCNA-positive cells was significantly reduced in balloon-injured carotid arteries in rats treated with metformin (23.83±7.53%) as compared to the control group (88.71±1.42%) (Fig. 6A-C). Similar to the results from the *in vitro* experiment, the expression of Nur77 and calponin was significantly increased in balloon-injured carotid arteries of rats treated with metformin compared to control rats (Fig. 6D-I). The expression of α-SMA was also significantly increased in balloon-injured carotid arteries of rats treated with metformin compared to control rats (Fig. 6J-L).

DISCUSSION

Metformin, a long-standing and widely-used glucose-lowering drug, is also effective for prevention of cardiac and vascular diseases [26]. We demonstrated that metformin effectively prevented neointimal hyperplasia induced by balloon injury in the carotid arteries of rats by inhibiting the proliferation and migration of VSMCs. We also showed that metformin

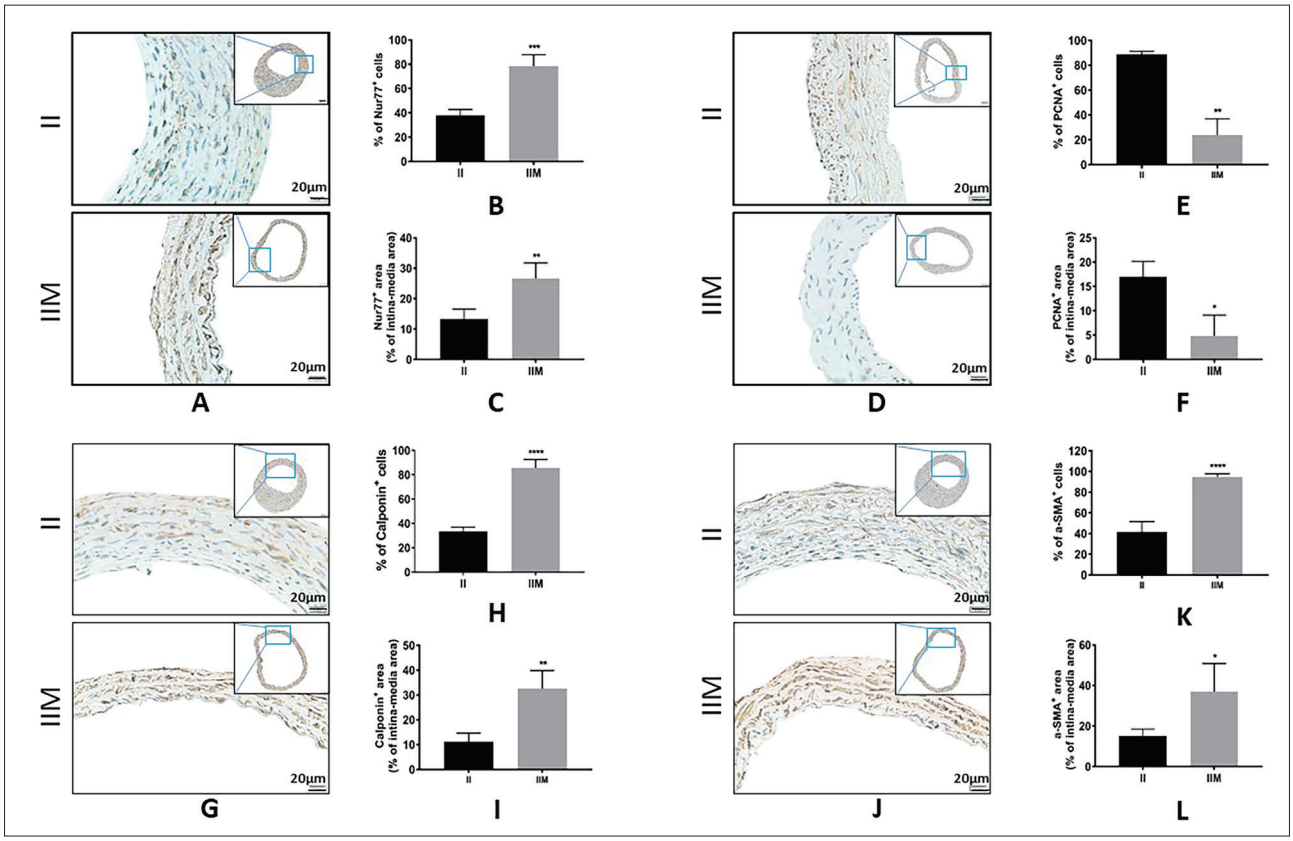

Fig. 6. Metformin reduces PCNA expression but enhances Nur77, α-SMA, and calponin expression in the carotid artery. **A –** Reduced PCNA expression in the carotid artery after metformin treatment. Immunohistochemistry (IHC) staining, scale bar=20 μm. **B –** Quantitation of the percentage of PCNA$^+$ cells (%). n=4. **P<0.01 vs group II. **C –** Quantitation of the percentage of PCNA$^+$ cell area in the intima-medial area (%). n=4. * P<0.05 vs II group. **D** – Increased Nur77 expression in the carotid artery after metformin treatment. IHC staining, scale bar=20 μm. **E –** Quantitation of the percentage of Nur77$^+$ cells (%). n=4. *** P<0.005 vs group II. **F –** Quantitation of the percentage of Nur77$^+$ cell area in the intima-medial area (%). n=4. ** P<0.01 vs group II. **G –** Increased calponin expression in the carotid artery after metformin treatment. IHC staining, scale bar=20 μm. **H –** Quantitation of the percentage of calponin$^+$ cells (%). n=4. *** P<0.005 vs group II. **I –** Quantitation of the percentage of calponin$^+$ cell area in the intima-medial area (%). n=4. ** P<0.01 vs group II. **J –** Increased α-SMA expression in the carotid artery after metformin treatment. **K –** Quantitation of the percentage of α-SMA$^+$ cells (%). n=4. **** P<0.001 vs group II. **L –** Quantitation of the percentage of the α-SMA$^+$ cell area in the intima-medial area (%). n=4. * P<0.05 vs group II.

upregulated the expression of Nur77, TET2 and calponin in VSMCs. Silenced TET2 reduced the expression of Nur77, while silenced Nur77 upregulated MMP-9 expression. These results suggested that metformin inhibited VSMC proliferation by activating TET2 and Nur77, which subsequently downregulated MMP-9.

MMP-9 belongs to the family of matrix metalloproteinases (MMPs). MMPs, including MMP-1, MMP-2, MMP-3, MMP-7, MMP-8, MMP-9, MMP-12 and MT1-MMP, are stimulated and activated by various stimuli in vascular tissues. Once activated, MMPs degrade extracellular matrix (ECM) proteins or other related signal molecules to promote the recruitment of stem/progenitor cells and facilitate migration and invasion of endothelial cells and VSMCs. Vascular cell proliferation and apoptosis can also be regulated by MMPs via proteolytical cleavage and modulation of bioactive molecules and relevant signaling pathways [27]. MMP-9 is produced by different cells, including VSMCs [28]. MMP-9 overexpression enhances vascular smooth muscle cell migration and alters remodeling in the injured rat carotid artery [29]. Targeted disruption of the MMP-9 gene impairs smooth muscle cell migration and geometrical arterial remodeling [30]. The expression of MMP-9 is regulated by TET2 [31,32], and our study revealed that metformin inhibited VMCS proliferation by upregulation of TET2, which leads to downregulation of MMP-9 expression.

TET proteins are 5-methylcytosine oxidases. There are three mammalian TET proteins, namely TET1, TET2 and TET3, which are Fe^{2+}- and 2-oxoglutarate-dependent dioxygenases [33]. TET2 regulates gene expression to control various cellular processes, including cell death [34]. TET2 is downregulated in atherosclerotic plaques and plays a role in the pathogenesis of atherosclerosis [10,11]. TET2 can also ameliorate vascular endothelial cell dysfunction, inhibit inflammation 10,11] and modulate VSMC phenotypic transmission [15]. Loss of TET2 inhibited contractile phenotypic marker expression and prevented rapamycin-induced VSMC differentiation, while overexpression of TET2 promoted a contractile phenotype [15]. TET2 mutation was associated with human pulmonary arterial hypertension [35]. Expression of TET2 can be regulated by many factors, including CD137, ox-LDL and microRNAs [36,37]. In this study, we showed that metformin stimulated VSMCs to upregulate TET2 expression, which in turn induced the expression of the critical cardioprotective transcription factor, Nur77.

Nur77 is a member of the Nur nuclear receptor family of intracellular transcription factors [38,39]. Nur77 is involved in cell cycle mediation, inflammation and apoptosis [40,41]. Nur77 is highly expressed in vascular endothelial cells and plays a role in the regulation of cell proliferation and angiogenesis [18]. Its overexpression was observed to suppress inflammation, whereas Nur77 deletion increased the development of atherosclerosis [42]. In VSMCs, Nur77 overexpression inhibited VSMC proliferation and migration *in vitro* [43]. $Nur77^{-/-}$ mice showed increased VSMC proliferation as well as elevated aortic medial areas and luminal diameters, and severe elastin disruption and collagen deposition [22]. Hyperoside inhibited VSMC proliferation and neointimal formation via upregulation of Nur77 [44]. The compound 6-mercaptopurine prevented and reversed abnormal vascular remodeling by activation of Nur77 [45]. In the present study, we observed that metformin attenuated VSMC proliferation and neointimal hyperplasia in balloon-injured carotid arteries but also upregulated Nur77 expression in VSMCs. We also demonstrated that silencing TET2 reduced the expression of Nur77, and silenced Nur77-promoted upregulation of MMP-9 expression in VSMCs. However, the mechanism and signal pathway used by metformin to inhibit VSMC proliferation need further study.

As a major component of blood vessels, VSMCs play an important role in the pathogenesis of vascular diseases [1]. Under pathological conditions, VSMCs switch from a contractile phenotype to a synthetic phenotype [1]. In the synthetic phenotype, VSMC proliferation and migration increases, and the expression of contractile phenotypic markers decreases [1]. PCNA is an essential protein, which mediates DNA replication and cell cycle regulation in eukaryotic cells. PCNA is often used as a biomarker for cell proliferation [46,47]. In animal models, the number of PCNA-positive cells was significantly decreased in response to metformin treatment in balloon-injured carotid arteries compared with that of the model group. Our study revealed that metformin treatment upregulated the expression of calponin both *in vitro* and *in vivo*. Calponin is an actin filament-bound protein that is specifically expressed in differentiated VSMCs [48]. Calponin plays a role in maintaining VSMCs in a contractile phenotype [49]. Deletion of calponin is associated with a decrease in the contractile force and increased proliferation and migration of VSMCs [50,51]. The decrease in calponin switched VSMCs from the contractile phenotype to the synthetic phenotype and contributed to pathological hypertrophic remodeling [52]. Our data indicated that upregulation of calponin by metformin may help to transform VSMCs from a synthetic to a contractile phenotype, thereby inhibiting the migration and proliferation of VSMCs and preventing vascular remodeling.

CONCLUSION

This study showed that metformin significantly inhibited VSMC proliferation, migration and neointimal hyperplasia in balloon-injured rat carotid arteries. Metformin induced the expression of calponin, Nur77, TET2 and calponin in VSMCs, while silencing TET2 repressed the expression of Nur77. Silencing of Nur77 increased the expression of MMP-9. The cardioprotective effects of metformin appear to be mechanistically linked to Nur77, TET2 and calponin upregulation as well as to MMP9 downregulation. These findings help explain the mechanism of metformin when used as a treatment for cardiovascular diseases.

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