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miR-218-5p regulates the phenotypic transformation of vascular smooth muscle cells in rat intracranial aneurysms by targeting HMGB1

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ABSTRACT: To explore the potential biological effects of miR-218-5p targeting high-mobility group protein B1 (HMGB1) mediating the phenotypic transformation of vascular smooth muscle cells (VSMCs) in intracranial aneurysm (IA) rats, an IA rat model was established, and the lentivirus vector miR-218-5p agomir or shRNA-HMBG1 was given to IA rats. The elastic fibers of the aneurysm were analyzed by Victoria Blue staining. Real-time PCR analyzed miR-218-5p, HMGB1, and phenotypic markers (α-SMA, SM-22α, MMP2, OPN). Western blot analysis of HMGB1, SM-22α, and OPN was also performed. miR-218-5p mimics and/or HMBG1 overexpression vectors were transfected into VSMCs. The effects of miR-218-5p on cell proliferation and migration were detected by EdU method and scratch method. Dual luciferase reporter gene assay verified the targeting relationship between miR-218-5p and HMGB1. The fibers of IA rats were broken, the elastic fibers were reduced, miR-218-5p was down-regulated, and HMGB1 was up-regulated. Treatment with miR-218-5p agomir and sh-HMGB1 protected tissue elastic fiber breakage induced by IA, upregulated α-SMA and SM-22α, and down-regulated HMGB1, OPN, and MMP2. Elevating miR-218-5p inhibited VSMC proliferation and migration, increased SM-22α, and decreased OPN expression. Upregulating HMGB1 had the opposite effect. The upregulation of HMGB1 expression counteracts the inhibitory impact of miR-218-5p overexpression on the proliferative, migratory, and phenotypic transformation capacities of VSMCs. By downregulating HMGB1, miR-218-5p mediates the phenotypic transformation of VSMCs in IA rats, providing a certain research basis for further research on clinical prevention and treatment of IA.

KEYWORDS: vascular smooth muscle cells, aneurysm, phenotypic transformation, high-mobility group protein

INTRODUCTION

Intracranial aneurysms (IA), a cerebrovascular disease, is associated with hypertension, cerebral arteriosclerosis, and vasculitis [1]. Rupture of IA can lead to aneurysmal subarachnoid hemorrhage, and then nerve failure or death. However, there are currently no effective interventions to prevent IA progression [2]. IA is characterized by loss of the integrity of artery wall, including endothelial dysfunction, extracellular matrix disorder, and inflammation, and long-term excessive inflammation of the blood vessel wall will promote IA growth and rupture [3]. IA is a complex pathophysiological process involving cells driving vascular wall remodeling, in which vascular smooth muscle cells (VSMCs) play a central role [4, 5].

VSMCs are located in the vascular mesoderm and make up most of the cells in the artery. The function of VSMCs is mainly to regulate blood pressure and blood flow distribution through contraction and dilation [6]. Phenotypic transformation of VSMCs is a pathophysiologic process in vascular disease development [7]. Under normal physiological conditions, VSMCs have a contractile phenotype, with characteristic markers such as smooth muscle 22α (SM-22α). When inflammation and injury trigger cell phenotype transformation into synthetics, the above contractile phenotype markers are down-regulated, matrix metalloproteinase and osteopontin (OPN) synthesis are increased, and cell proliferation and migration capacity are increased to drive vaso-related diseases [8, 9]. The phenotypic transformation of VSMCs is the key to IA, and its phenotypic transformation is regulated by miRNA transcription. Some miRNAs can regulate the function and phenotype of VSMCs [10, 11]. Sun Liqian et al found that miR-29b is down-regulated in the serum of patients with IA, and downregulating miR-29b directly activates the phenotypic transformation of VSMCs, which predicted IA rupture [12]. Huang et al pointed out that in patients with arteriosclerosis and occlusion, miR-22-3p can inhibit VSMC proliferation, migration, and intimal hyperplasia and rescue endothelial injury of IA vessels [13]. In addition, miR-218-5p has been revealed to alter the microenvironment of tumor cells and inhibit tumor cell proliferation and migration. miR-218-5p may also be involved in the regulation of VSMCs in IA, but the mechanism is not fully understood.

High-mobility group protein B1 (HMGB1) is a model molecule associated with damage and is central

to the inflammatory response [14]. When VSMCs are subject to inflammation and injury, HMGB1 mediates the phenotypic transformation of VSMCs [15]. HMGB1 can promote the formation of foam cells in VSMCs, thus promoting atherosclerosis [16]. There is evidence that HMGB1 is highly expressed in cerebral aneurysms, especially abnormal expression in ruptured aneurysms [17]. Moreover, HMGB1 in cerebrospinal fluid is a potential prognostic marker for aneurysmal subarachnoid hemorrhage [18].

This study evaluated the effect of miR-218-5p on IA VSMCs by targeting HMGB1, and the results could provide a basis for further research on the prevention and treatment strategies of IA.

MATERIALS AND METHODS

Animal and model preparation

Male Sprague Dawley (SD) rats (8 weeks of age, 180–220 g body weight, BW) were obtained. The experimental protocol was approved by the Ethics Committee of The First Hospital of Changsha, and was implemented with the Principles of Care and Use of Experimental Animals in Research (Approval No. 2020HN0233). Prior to the experiment, all rats were fed for 14 days under optimal conditions of 25 °C, 50% humidity, 12/12 diurnal cycle, and sufficient food and water.

Before the experiment began, the rats had been fasted for 24 h and allowed to drink freely. The rat model of IA was established by intraperitoneal injection of pentobarbital (40 mg/kg BW) and ligation of the left common carotid artery and posterior branch of bilateral renal arteries [19]. The same surgical incision was performed in the Sham group without ligation. After IA induction, the rats were fed a diet containing 8% sodium chloride and 0.12% 3-aminopropionitrile; and 20–30 ml normal saline was injected into the rats' abdominal cavity after surgery to prevent the rats from shock and death due to excessive fluid loss. Except for the Sham group, IA rats were divided into the following groups ($n = 5$): IA group, miR-218-5p group (10 mg/kg), miR-NC group (10 mg/kg), sh-HMGB1 group (100 μl, 1×10^9 transduction units (TU)/ml), and sh-NC group (100 μl, 1×10^9 TU/ml). The rats were injected with a micropipette through a tail vein for 3 days. After 30 days, Willis circulus arteriosus was taken from rats under general anesthesia, part of which was immersed in 4% malondialdehyde phosphate buffer, dehydrated with ethanol, and embedded with paraffin wax. The other part was frozen in liquid nitrogen and transferred to −80 °C for storage. Rats were euthanized by CO_2 asphyxiation. miR-NC agomir, miR-218-5p agomir, recombinant interfering HMGB1 lentivirus (PLVX-HMGB1-shRNA), and PLVX-MOCK-shRNA were synthesized by Sangon (Shanghai, China).

Victoria Blue staining

IA tissue specimens were dewaxed, hydrated, and then dehydrated. The samples were cut into slices of 4 μm thickness and dyed with Victoria Blue staining solution. Willis circulus arteriosus elastic fibers were analyzed with a NIKONECLIPS E100 biological microscope (Nikon, Tokyo, Japan) using a DS-U3 imaging system (Nikon).

Cell culture and transfection

Rat VSMCs (CAT#ATCCCRL-1444, ATCC, Manassas, USA) were kept in DMEM (c 319-005-CL; Wisent) with 10% FBS (Gibco, Grand Island, NE, USA), 100 U/ml penicillin, and 100 μg/ml streptomycin (Thermo Fisher Scientific, MA, USA) at 37 °C, 5% CO_2 in a humid atmosphere.

miR-218-5p mimics, miR-NC mimics, pCDNA3.1 HMGB1 overexpressed vector (HMGB1 OV), and negative empty vector (pCDNA3.1-NC) were all prepared by Sangon. The above-synthesized oligonucleotides or plasmids were transfected with Lipofectamine®2000 (Thermo Fisher Scientific) at 50 nmol/ml. The culture medium was changed 6 h after transfection. After 48 h, VSMCs were assayed for transfection efficiency by RT-qPCR.

Luciferase reporter analysis

On the starBase 3.0 (http://starbase.sysu.edu.cn/), the binding sites shared by HMGB1 3′-UTR and miR-218-5p were discovered. Wild-type and mutant HMGB1 fragments containing miR-218-5p binding sites were synthesized by Sangon. Next, HMGB1 mutant (MUT) and wild type (WT) 3′-UTR were cloned into psiCHECK-2 (Promega Corp., Madison, WI, USA). The luciferase reporter (HMGB1 3′-UTR, 500 ng) was co-transfected into cells with miR-285-5p mimic or mimic-NC using Lipofectamine®2000. VSMCs were cultured in a humidified incubator at 37 °C and 5% CO_2 for 48 h and tested in the dual luciferase reporting Detection System (Promega Corp.). All plasmids were verified by sequencing.

EdU assay

VSMCs were cultured in 12-well plates at 2×10^5 cells/well and assayed by Clickit EdU kit (C10086, Invitrogen, Waltham, MA, USA). In simple terms, VSMCs were incubated with EdU solution for 12 h, washed with PBS, and fixed with 4% PFA, followed by nuclear staining with Hoechst 33342. Fluorescence signals were analyzed under a fluorescence microscope. EdU incorporation = EdU positive cells/(EdU positive + Hoechst positive cells) × 100%.

Scratch test

VSMCs were incubated in 12-well plates with 1×10^5 cells/well. The phenotypic transformation model was constructed using 10 ng/ml platelet-derived growth

factor-BB (PDGF-BB) (Biotechnology, Milton Keynes, UK) to stimulate VSMCs for more than 24 h [2]. At 80% confluence, cross-shaped marks were scratched with 1 ml tip in each bottom hole, and continued in the culture medium. At 0, 24, and 48 h, photos were taken and the scratch width was observed.

RT-qPCR

RNA from Willis circulus arteriosus and VSMCs was extracted using TRIzol® reagents (Invitrogen). After quantification of the RNA quality by Nanodrop 2000, cDNA synthesis of miRNA was executed using miRNA reverse transcription kit (TaKaRa, Shiga, Japan); and mRNA cDNA was synthesized using PrimeScript™RT Reagent kit (TaKaRa). PCR was executed using Light-Cycler 480 real-time PCR machine (Roche Diagnostics, Tokyo, Japan) in combination with TB Green®Fast qPCR Mix (TaKaRa), with U6 and GAPDH as endogenous reference genes. Gene expression was calculated by $2^{-\Delta\Delta Ct}$ method. Primer sequences of target genes were listed in Table S1.

Protein immunoblotting

Willis circulus arteriosus and VSMCs were put in cold RIPA lysis buffer (Solarbio, Beijing, China) containing protease inhibitor (36978, Thermo Fisher Scientific) and treated with the BCA kit (Beyotime, Shanghai, China). The protein was isolated by 15% SDS-PAGE and transferred to the PVDF membrane. At room temperature, it was covered with 5% skim milk powder for 1 h, then washed with TBST three times, and detected with the target primary antibody from Thermo Fisher Scientific: HMGB1, MA5-31967; SM-22α, MA5-17288; or OPN, PA5-34579. Antibodies were used at a working dilution of 1:1000. After TBST washing, the secondary antibody was labeled with HRP and incubated with the membrane. The blot was developed by the ECL detection system, and the target protein was quantified using Image J.

Statistical analysis

Data were statistically analyzed using SPSS21.0 software (Chicago, Illinois, USA) and expressed as mean ± standard deviation. Statistical evaluation was analyzed using Graphical Prism 8.0 (San Diego, California, USA). Multiple one-way ANOVA comparisons or bilateral t-test comparisons were performed, with a $p < 0.05$ considered significant.

RESULTS

Expression of miR-218-5p in tissues of IA rats

First, we performed bilateral carotid and bilateral renal artery posterior branch ligation surgeries on rats to establish an IA model. Inflammatory infiltration and aneurysm wall remodeling are considered two pathological features of IA. By RT-qPCR analysis, the results showed that the relative expression levels of pro-inflammatory-related genes Il-1β, TNF-α, and Il-6 mRNA were elevated in the IA tissues of rats in the IA group relative to the Sham group (Fig. 1A). In addition, the Victoria Blue staining of the IA tissues demonstrated the stretching and fracture of elastic fibers of the IA walls (Fig. 1B). To explore the expression of miR-218-5p in IA, we analyzed the results by RT-qPCR, which showed that the expression of miR-218-5p was significantly decreased in the IA tissues of rats (Fig. 2A). Subsequently, by RT-qPCR and protein immunoblotting analyses, the results showed that HMGB1 mRNA and protein expression levels were abnormally increased in the IA tissues of rat (Fig. 2A,B). These results suggested that miR-218-5p and HMGB1 were involved in IA in rats.

Effect of shRNA silencing of HMGB1 on the phenotype of VSMCs in the IA rat model

HMGB1 regulation of hypertensive VSMC phenotypic transformation has been demonstrated [20]. IA is influenced by the transformation of VSMC phenotype transformation from a contractile phenotype to a synthetic phenotype after vascular injury. Therefore, we first analyzed markers associated with VSMC phenotype in IA tissues by RT-qPCR. The results showed that the expression of contractile phenotypic markers α-SMA and SM-22α mRNA was decreased, and the expression of synthetic phenotypic markers MMP2 and OPN mRNA was increased in the IA tissues of rats (Fig. 3A). Immunoblot analysis also confirmed increased SM-22α protein expression and decreased OPN protein expression in IA (Fig. 3B). Next, to further explore the role of HMGB1 in IA, we performed a silencing assay. IA rats were injected with the interfering HMGB1 lentiviral plasmid vector and its control. RT-qPCR and immunoblotting confirmed the decreased expression of HMGB1 after silencing HMGB1. Subsequently, the effect of HMGB1 on the phenotypic transformation of VSMCs in the IA wall was analyzed by RT-qPCR analysis and immunoblotting. The results showed that knockdown of HMGB1 upregulated α-SMA and SM-22α expression and downregulated MMP2 and OPN expression (Fig. 3A,B). In addition, inhibition of HMGB1 expression alleviated the fracture of elastic fibers in IA tissues of rats (Fig. 3C). These results suggested that HMGB1 was involved in IA formation and that silencing of HMGB1 might inhibit the phenotypic transformation of VSMCs to a synthetic phenotype.

Role of miR-218-5p in the regulation of VSMC phenotype in IA rat model

Significant downregulation of miR-218-5p in patients with vascular diseases has been demonstrated [21]. To further investigate the role of miR-218-5p in IA, we performed miR-218-5p overexpression experiments. First, we injected the synthesized miR-218-5p agomir

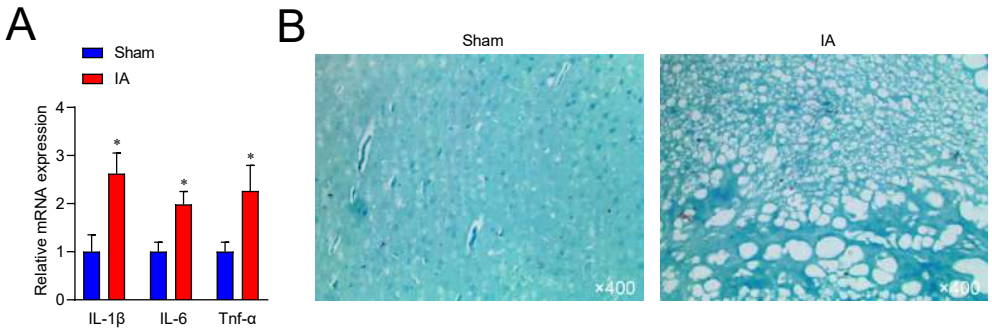

Fig. 1 Reduced elastic fibers of IA tissues in rats. A: Relative expression of inflammation-related genes Il-1β, TNF-α, and Il-6 mRNA in IA tissues of rats was detected by RT-qPCR; B: IA tissues of rats were stained using Victoria Blue to visualize representative images of elastic fibers. Data are expressed as mean ± SD ($n = 5$). * $p < 0.05$ compared with Sham group.

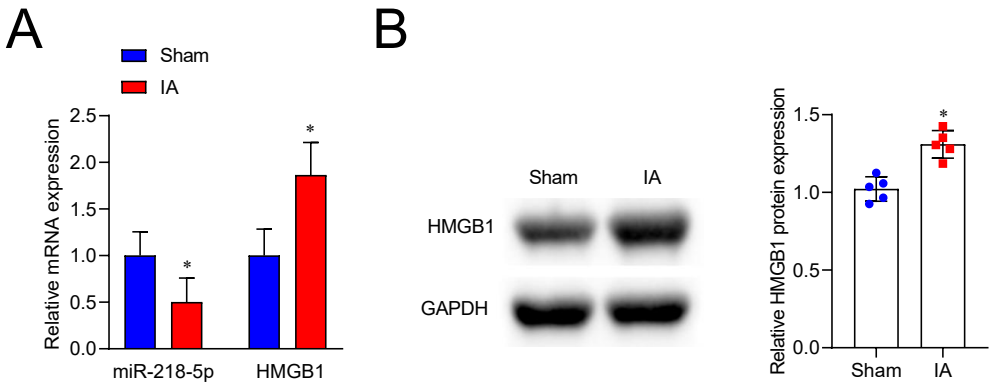

Fig. 2 Expression of miR-218-5p and HMGB1 in IA rats. A: Relative expression of HMGB1 and miR-218-5p in IA tissues of rats was detected by RT-qPCR; B: Relative expression of HMGB1 protein in IA tissues of rats was detected by immunoblotting, and the right side showing the bar graph of relative expression of HMGB1 protein, and each point representing a sample. Data are expressed as mean ± SD ($n = 5$). * $p < 0.05$ compared with Sham group.

plasmid or control agomir into IA rats. The expression of miR-218-5p in IA tissues was significantly increased after miR-218-5p agomir treatment in IA rats, as confirmed by RT-qPCR results (Fig. 4A). Subsequently, the effect of miR-218-5p overexpression on the expression levels of markers of phenotypic transformation in VSMCs was analyzed by RT-qPCR and immunoblotting. The results showed that overexpression of miR-218-5p upregulated α-SMA and SM-22α and downregulated MMP2 and OPN (Fig. 4A,B). In addition, the elastic fibers were assessed by Victoria Blue staining, which showed that miR-218-5p overexpression attenuated the elastic fiber fracture in IA rats (Fig. 4C). These results indicated that overexpression of miR-218-5p suppressed VSMC synthetic phenotypes in IA.w

Role of overexpression of miR-218-5p in phenotypic regulation of VSMCs

To explore the role of miR-218-5p in VSMCs, we stimulated rat VSMCs by PDGF-BB to transform from a contractile phenotype to a synthetic phenotype. Subsequently, the proliferative and migratory capacities of VSMCs were assessed by EdU method and scratch method. The results showed that overexpression of miR-218-5p inhibited the proliferation and migration ability of VSMCs, with the most obvious inhibition of cell migration after 48 h (Fig. 5A,B). Subsequently, the overexpression of miR-218-5p was verified by RT-qPCR results (Fig. 5C). In addition, RT-qPCR and immunoblotting analyses showed that miR-218-5p overexpression up-regulated α-SMA and SM-22α mRNA and down-regulated MMP2 and OPN (Fig. 5C,D). These results indicated that miR-218-5p overexpression inhibited PDGF-BB-induced proliferation, migratory ability, and conversion to a synthetic phenotype in VSMCs.

Interaction between HMGB1 and miR-218-5p

Regarding the expression patterns of HMGB1 and miR-218-5p in IA tissues, *in vitro* studies further analyzed the targeting relationship between miR-218-5p and HMGB1 according to the principle of miRNA regulation. HMGB1 was a potential target of miR-218-5p with a binding site on its 3′UTR through the Starbase 3.0 platform (Fig. 6A). In fact, in luciferase assays, when miR-218-5p mimics were co-transfected

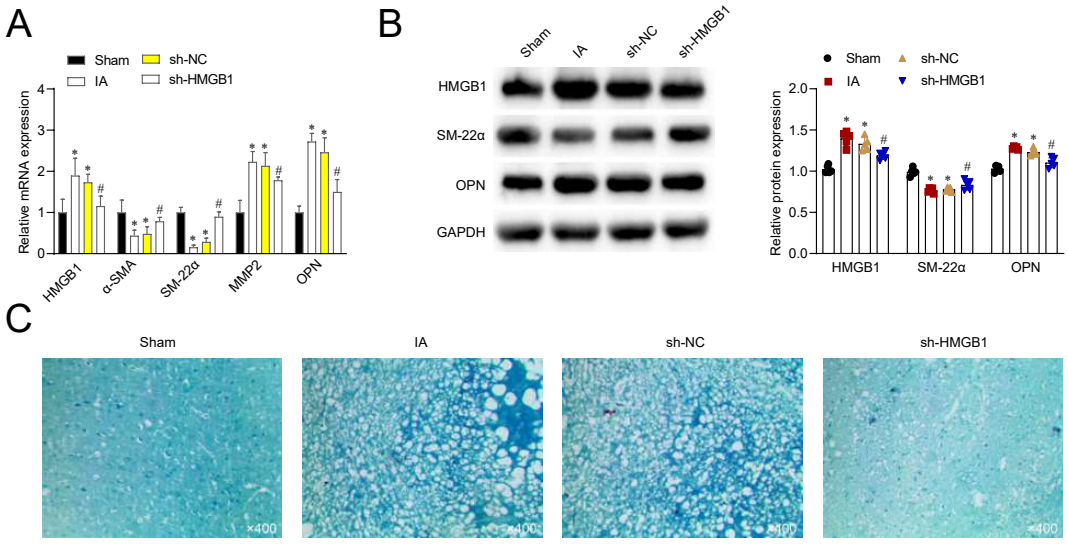

Fig. 3 Effect of sh RNA silencing of HMGB1 on VSMC phenotype in IA rat model. IA rats were infected using recombinant interfering HMGB1 lentivirus (PLVX-HMGB1-shRNA) plasmid vector or its control (PLVX-MOCK-shRNA). A: Relative expression of HMGB1, α-SMA, SM-22α, MMP2, and OPN mRNAs was detected by RT-qPCR; B: HMGB1, SM-22α and OPN protein expression was detected by immunoblotting, and the relative protein expression histogram was shown on the right, each point represented a sample; C: Rat IA tissues were stained with Victoria Blue to visualize representative images of elastic fibers. Data are expressed as mean ± SD ($n = 5$). * $p < 0.05$ compared with Sham group, # $p < 0.05$ compared with sh-NC group.

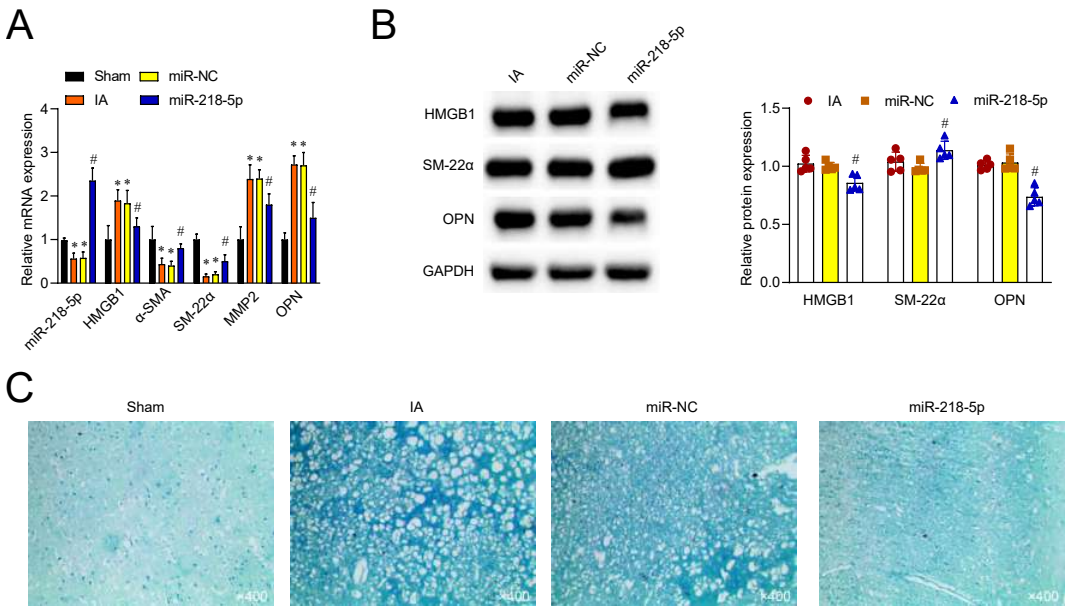

Fig. 4 Role of miR-218-5p on the regulation of VSMC phenotype in IA rat model. miR-218-5p agomir or its control miR-NC agomir was injected into IA rats through the tail vein. A: Relative expression of miR-218-5p, α-SMA, SM-22α, MMP2, and OPN mRNAs was detected in IA rats by RT-qPCR; B: Expression of HMGB1, SM-22α, and OPN proteins in IA rats was detected by immunoblotting, and a histogram of relative protein expression was shown on the right, with each point representing a sample; C: IA tissues of rats were stained using Victoria Blue to visualize a representative image of the elastic fibers. Data are expressed as mean ± SD ($n = 5$). * $p < 0.05$ compared with miR-NC group.

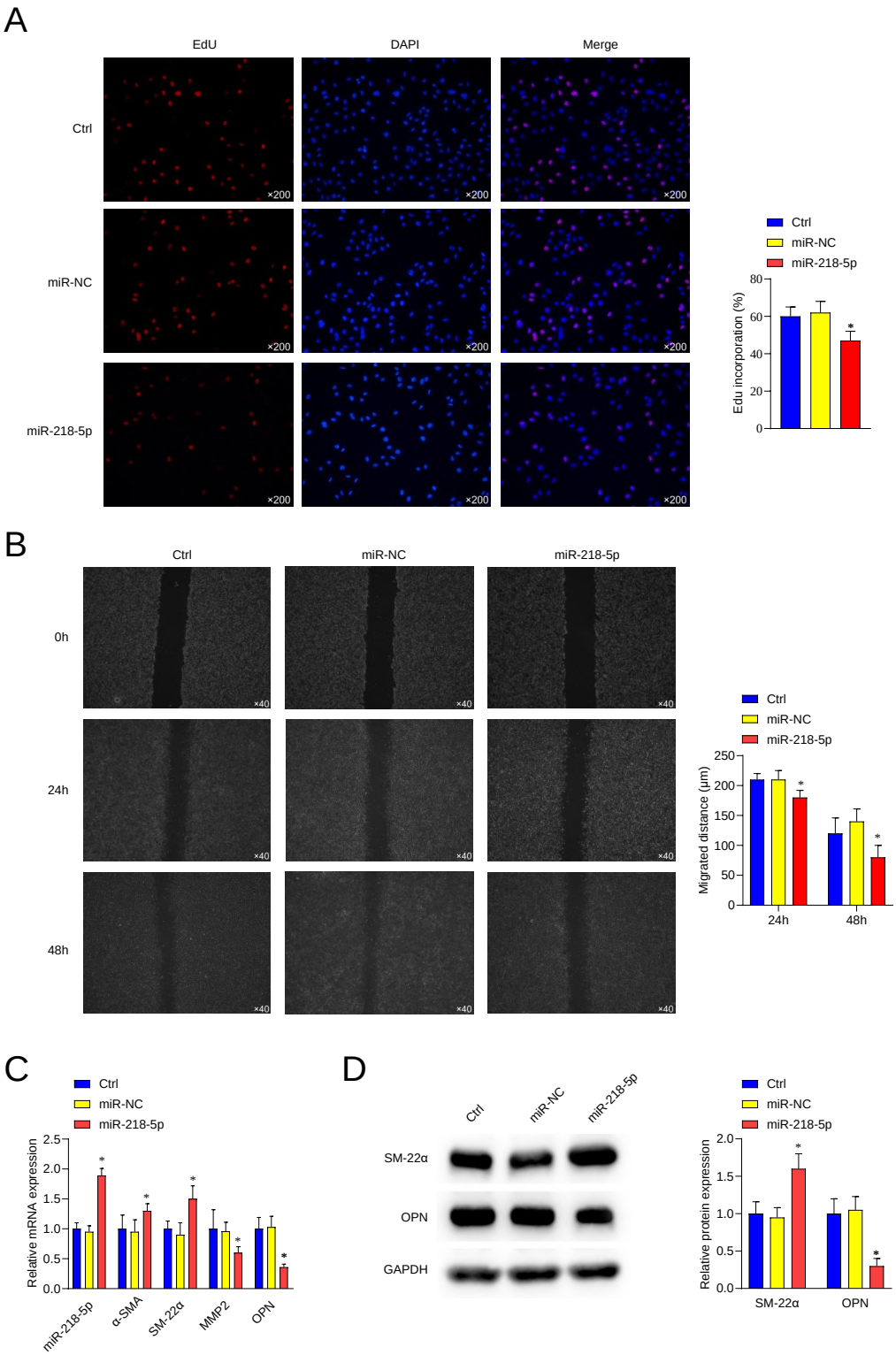

Fig. 5 Role of overexpression of miR-128-5p in the phenotypic regulation of VSMCs. Rat VSMCs were transfected with miR-128-5p mimic or their control miR-NC. A: Cell proliferation was assessed by EdU Cell Proliferation Detection Kit; B: Cell migration was detected by wound healing assay, and the various migratory distances were recorded at 24 h and 48 h; C: miR-218-5p, α SMA, SM-22α, MMP2, and OPN were detected by RT-qPCR; D: Expression of SM-22α and OPN in VSMCs were detected by immunoblotting, and the histogram of relative protein expression was shown on the right side. Data are expressed as mean ± SD ($n = 3$). * $p < 0.05$ compared with miR-NC group.

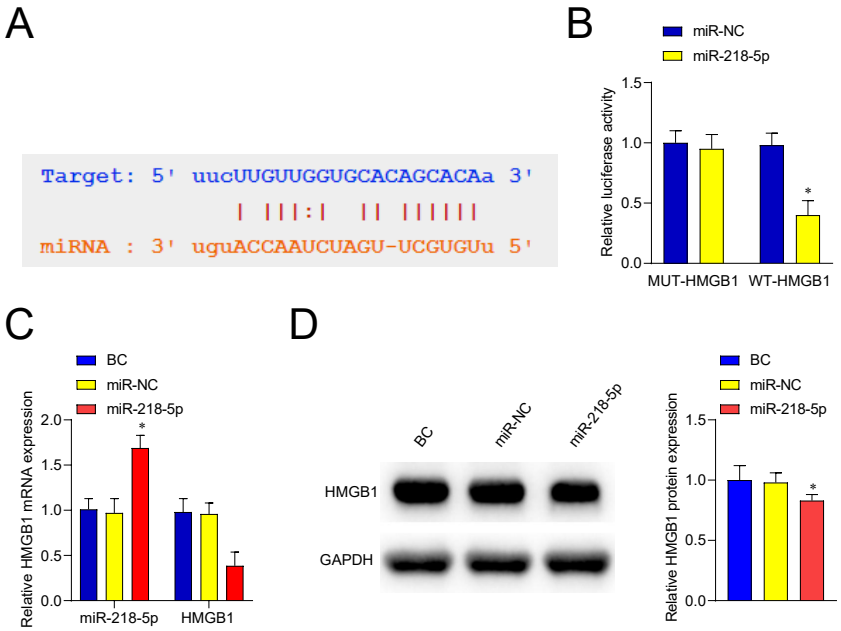

Fig. 6 Interaction between HMGB1 and miR-218-5p. A: Satarbase 3.0 predicted the targeting relationship between HMGB1 and miR-218-5p; B: Luciferase assay to identify direct targets between HMGB1 and miR-218-5p; C: Relative expression of miR-218-5p and HMGB1 were detected by RT-qPCR and immunoblotting, respectively; D: HMGB1 protein expression level was detected by immunoblotting. Data are expressed as mean ± SD ($n = 3$). * $p < 0.05$ compared with miR-NC group.

with HMGB1 WT sequences containing specific binding sites, VMSCs showed reduced luciferase activity. However, this result was not observed in reporters with HMGB1 MUT sequences (Fig. 6B). Overexpression of miR-218-5p was verified by RT-qPCR (Fig. 6C). When transfected with miR-218-5p mimics, it was found that miR-218-5p inhibited HMGB1 mRNA and protein (Fig. 6C,D).

Role of miR-218-5p/HMGB1 in the regulation of VSMC phenotypic transformation

To further investigate whether miR-218-5p overexpression regulated the proliferation, migration, and phenotypic transformation of VSMCs by targeting and inhibiting Hmngb1, VSMCs were transfected with HMGB1 overexpression plasmid vector alone or co-transfected with miR-218-5p mimic or miR-NC into VSMCs. The effects of HMGB1 overexpression on cell proliferation and migration were analyzed by EdU and wound healing assays. The results showed that HMGB1 overexpression induced an increase in the proliferative and migratory capacities of VSMCs compared with VSMCs that did not undergo any treatment (Fig. 7A). Regarding migration, HMGB1 overexpression caused VSMCs to show higher migratory capacity (Fig. 7B). HMGB1 overexpression was verified by RT-qPCR and immunoblotting analysis (Fig. 7C). Subsequently, RT-qPCR and protein immunoblotting showed that HMGB1 overexpression down-regulated α-SMA and SM-22α and up-regulated MMP2 and OPN (Fig. 7C,D). However, after co-transfection of VSMCs with miR-218-5p mimic and HMGB1 overexpression plasmid, overexpression of HMGB1 partially eliminated the inhibitory effect of miR-218-5p mimetic on proliferation, migration, and phenotype transformation of VSMCs (Fig. 7C,D). Taken together, miR-218-5p could regulate the proliferation, migration, and phenotypic transformation of VSMCs by targeting and inhibiting HMGB1.

DISCUSSION

IA involves complex pathological mechanisms. Subarachnoid hemorrhage caused by aneurysm rupture accounts for 5–10% of strokes, with a very high mortality and disability rate [22]. At present, little is known about IA molecular pathogenesis, among which internal factors such as vascular endothelial cell dysfunction, inflammation, and immune response are of great significance [23]. IA clipping and interventional intravascular embolization have become recognized treatment methods [24]. However, complications during endovascular therapy still occur. Therefore, it is of great clinical significance to prevent IA. Using miRNA microarray analysis and RT-qPCR technology, the possibility of using miRNA as a biomarker for IA has been improved [25, 26], indicating that miRNAs may be involved in IA development and have a certain early warning effect on IA rupture. In the present study, we demonstrated that miR-218-5p is a repressor that regulates IA. miR-218-5p overexpression inhibited the

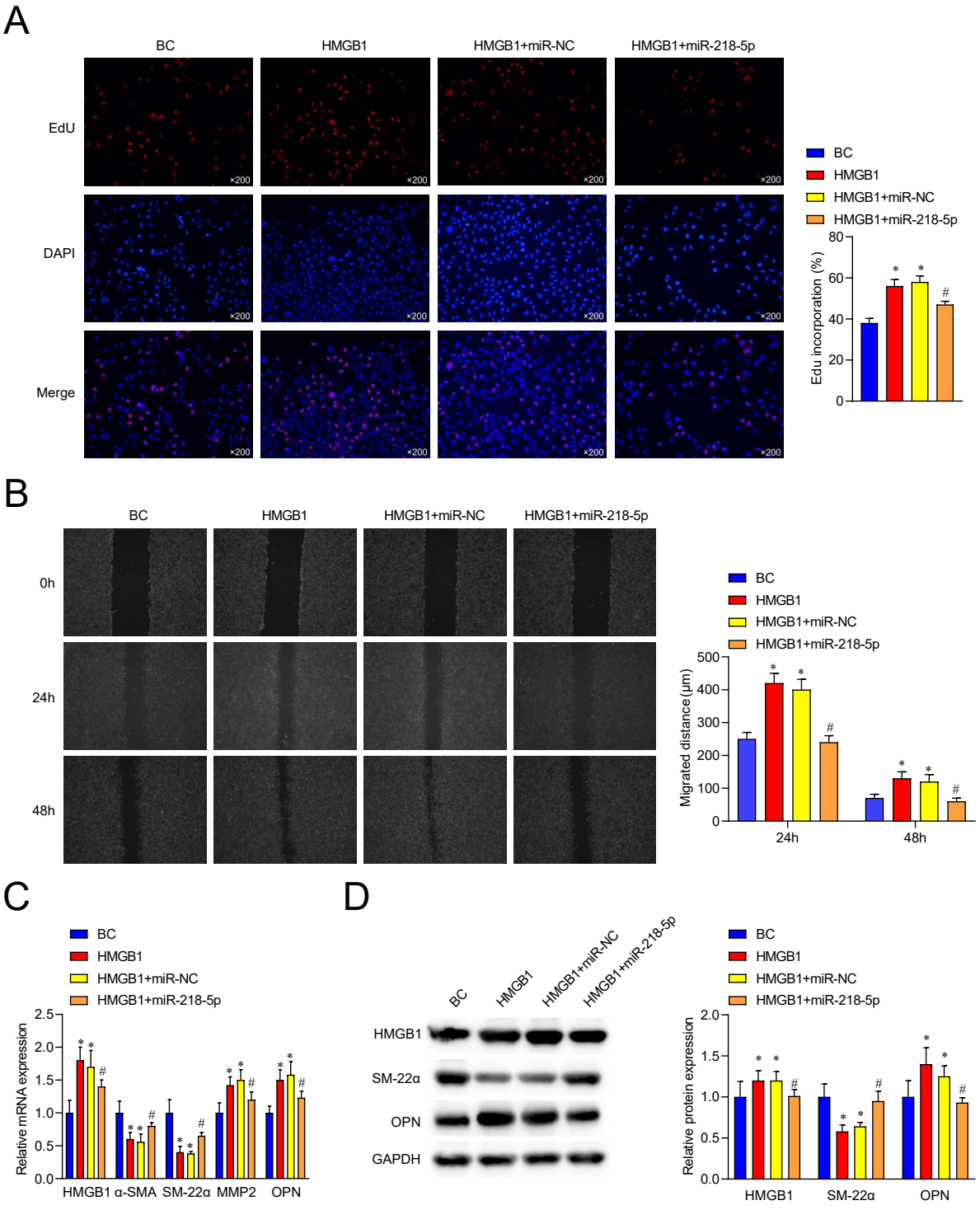

Fig. 7 Role of miR-128-5p/HMGB1 in the regulation of VSMC phenotypic transformation. VSMCs were transfected with pCDNA3.1 HMGB1 overexpression plasmid vector alone or co-transfected with miR-218-5p mimic or miR-NC. A: Cell proliferation was assessed by EdU Cell Proliferation Detection Kit; B: Cell migration was detected by wound healing assay, and the various migratory distances were recorded at 24 h and 48 h, respectively; C: miR-218-5p, α SMA, SM-22α, MMP2, and OPN were detected by RT-qPCR; E: Expression of SM-22α and OPN in VSMCs were detected by immunoblotting, and the histogram of relative protein expression was shown on the right side. Data are expressed as mean ± SD ($n = 3$). * $p < 0.05$ compared with BC group, # $p < 0.05$ compared with HMGB1 + miR-NC group.

phenotypic transformation of VSMCs by targeting and regulating HMGB1 expression in IA.

miR-218-5p is an inhibitory miRNA with low expression in a variety of tumor diseases, and studies have shown that it mainly inhibits cell proliferation and migration [27, 28]. miR-218-5p has also been reported to be associated with cardiovascular diseases. For example, by upregulating miR-218-5p, cardiac microvascular endothelial damage caused by preeclampsia or coronary heart disease can be alleviated [29, 30]. miR-218-5p regulates abdominal aortic aneurysms [31], suggesting that miR-218-5p may also be involved in regulating IA. Therefore, in the present study, we established a rat model of IA and confirmed that miR-218-5p expression was significantly reduced in IA. OPN and MMP have an important role in the pathogenesis of IA and may contribute to aneurysm rupture [32]. Nakajima et al confirmed that the phenotypic transformation of VSMCs was clearly shown in the tumor wall of IA, that is, the expression of contraction phenotypic markers of VSMCs in the tumor wall decreased (α-SMA and SM-22α), and that of synthetic phenotypic proteins increased (OPN and MMP2) [33]. VSMCs in cerebral arteries are an important component of the mesentery, and the main pathological cause of IA is lesions of the mesentery and elastic lamina of the cerebral blood vessels, which lead to localized dilatation or bulging of the arterial wall [34]. Subsequently, staining of elastic fibers of aneurysm tissues by Victoria Blue showed disorganized VSMC arrangement and broken elastic fibers in IA rats, which were attenuated by overexpression of miR-218-5p.

The early stages of IA are associated with abnormal proliferation of VMSCs that migrate to the endothelial layer. It has been shown that HMGB1 is involved in apoptosis and necrosis in inflammation-associated diseases, stimulating the proliferation of M1 pro-inflammatory macrophages [35]. Macrophages will further promote the contraction-to-synthesis phenotypic transformation of VSMCs by activating Notch signaling [36]. In addition, it has been shown that the HMGB1/TLR4 signaling pathway promotes the progression of abdominal aortic aneurysms in animals [37]. Therefore, we hypothesized that HMGB1 is involved in the phenotypic transformation of VSMCs in IA. In the present study, significant upregulation of HMGB1 in IA model rats was confirmed. Subsequently, inhibition of elastic fiber rupture and promotion of α-SMA and SM-22α were observed in an IA rat model following silencing of HMGB1. Further, HMGB1 was identified as a potential direct target gene of miR-218-5p. Moreover, both *in vivo* and *in vitro* studies confirmed that miR-218-5p overexpression led to a significant reduction in HMGB1 levels. Under pathological conditions, VSMCs accelerate the phenotypic transition from a contractile to a synthetic phenotype. This phenotypic switch significantly alters the function of VSMCs [38]. For example, VSMCs with a synthetic phenotype are more likely to migrate and proliferate than contractile VSMCs [39]. Therefore, we first transfected miR-218-5p overexpression plasmid into VSMCs, followed by PDGF-BB to stimulate the transformation of VSMCs from a contractile phenotype in the physiological state to a synthetic phenotype in the pathological state. Our results showed that miR-218-5p overexpression inhibited the phenotypic transformation of VSMCs and suppressed cell proliferation and migration functions. Overexpressing HMGB1 eliminated miR-218-5p overexpression-induced inhibition of VSMC proliferation, migration, and phenotypic transformation.

There was one major limitation of this study that needed to be recognized and addressed. Although the miR-218-5p/HMGB1 pathway was shown to be involved in IA rats and phenotypic transformation of VSMCs, clinical studies limited the validation of the involvement of the miR-218-5p/HMGB pathway in the transformation of VSMCs to a vascular phenotype in patients with IA. These issues could be addressed by recruiting patients in our future studies. In addition, we confirmed the involvement of HMGB1 in regulating the transformation of VSMC phenotypes, and then its downstream regulatory mechanisms would still need to be confirmed by more relevant studies.

In conclusion, the results herein demonstrate for the first time that HMGB1 is upregulated in IA. We also found that miR-218-5p is a negative regulator of HMGB1 expression. miR-218-5p/HMGB1 might form a novel pathway involved in the regulation of intracranial vascular remodeling. These results strongly suggest that overexpression of miR-218-5p could be a potential therapeutic strategy against IA.

Appendix A. Supplementary data

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request. Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2024.095.

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Appendix A. Supplementary data

Table S1 Primer sequences.

Gene	Primer	Primer
MiR-128-5p	F: CGAGTGCATTTGTGCTTGATCTA	R: TAATGGTCGAACGCCTAACGTC
HMGB1	F: TCTGTTCTGAGTACCGCCCA	R: TCGCAACATCACCAATGGA
IL-1β	F: GCCTCACCACATTCCCAAGA	R: GGGGTCTGGTTTCAGAGAGC
IL-6	F: CTTCCAGCCATGTGCCTTCT	R: GAGAGCATTGGAAGTTGGGG
TNF-α	F: AAAGCATGATCCGAGATGT	R: AGCAGGAATGAGAAGAGGC
α-SMA	F: AGTCGC-CATCAGGAACCTCGAG	R: ATCTTTTCGATGTCGTCCCAGTTG
SM-22a	F: GCATAAGAGGGAGTTCACAGACA	R: CTTCCCTTTCTAACTGATGATC
MMP2	F: CAGTCGATGTCCCTGACGG-3	R: GTTGCTGTCCTGATCAGAGG
GAPDH	F: GCGAGATCCCGCTAACATCA	R: CTCGTGGTTCACACCCATCA
U6	F: GCTTCGGCAGCACATATACTAAAAT	R: CGCTTCAGAATTTGCGTGTCAT