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Up-regulation of exosomal miR-106a may play a significant role in abdominal aortic aneurysm by inducing vascular smooth muscle cell apoptosis and targeting TIMP-2, an inhibitor of metallopeptidases that suppresses extracellular matrix degradation

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Abstract. – OBJECTIVE: Abdominal aortic aneurysm (AAA) rupture is a dramatic and lethal clinical condition with a high risk of death. Emerging evidence indicates a role for miRNAs in AAA pathogenesis, therefore we aimed to identify miRNAs that differently expressed in exosomes from AAA patients and explore the underlying mechanisms of how miR-106a plays its role in this disease.

PATIENTS AND METHODS: Exosomes were isolated from plasma of AAA patients, as well as from the tissue-conditioned culture medium. The exosomal expression profiles of several miRNAs including miR-106a were analyzed by quantitative RT-PCR. To determine the potential role of miR-106a in the pathogenesis of AAA, miR-106a was overexpressed in vascular smooth muscle cells (VSMCs), and then cell viability and apoptosis were evaluated by performing CCK-8 assay and flow cytometry, respectively. Afterwards, enzyme-linked immunosorbent assay (ELISA) was applied to assess the expression levels of some proteins involved in the modulation of extracellular matrix (ECM) homeostasis. Furthermore, the target gene of miR-106a was predicted and verified through Dual-Luciferase reporter assay.

RESULTS: MiR-106a was up-regulated in exosomes from plasma of those patients with AAA as compared with healthy peers. Likely, increased level of miR-106a was observed in exosomes released from AAA tissue in comparison to those from adjacent normal tissues. Enhanced expression of miR-106a in VSMCs suppressed cell viability but promoted cellular apoptosis, whereas inhibition of miR-106a in VSMCs resulted in a significant decrease in the percentage of apoptotic cells compared to the control group. In addition, the protein levels of matrix metalloproteinases (MMPs, including MMP-2 and MMP-12) secreted from VSMCs were significantly up-regulated, while their inhibitor TIMP-2 was down-regulated due to miR-106a overexpression. Finally, TIMP-2 was validated subsequently as the direct target of miR-106a through Dual-Luciferase reporter assay.

CONCLUSIONS: In aggregate, our results suggest that increased expression of miR-106a promotes VSMC cell apoptosis and down-regulates TIMP-2 through directly targeting its 3'-UTR, which in turn restores MMP production and ultimately accelerates ECM degradation. Therefore miR-106a is proposed to play a crucial role in AAA development and this will provide an update on the understanding of the clinical value of miRNAs as novel therapeutic targets for the treatment of this disease.

Key Words:
Abdominal aortic aneurysm, Exosome, MicroRNA-106, Smooth muscle cell apoptosis, Extracellular matrix.

Introduction

Abdominal aortic aneurysm (AAA) is mainly featured by gradual degradation of the arterial wall and focal enlargement of the abdominal aorta that exceeds more than 50% of normal aortic diameter[1]. Rupture of the dilated segments usually leads to severe internal bleeding, which

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is recognized as the major cause of mortality[1]. Patients with AAA are mostly asymptomatic and the progression of this disease towards rupture is not predictable. Moreover, surgical intervention is recommended if the aneurysm is greater than 55 mm in diameter, but so far there is no acknowledged therapy for those patients with an aneurysm less than 50 mm[2,3]. Therefore, it is imperative to discover reliable biomarkers for the prediction of aortic events or therapeutic targets in the management of patients with AAA.

Exosomes are microvesicles that are generally described to be originated from endosomes and mainly contribute to communication between cells[4,5]. Exosomes are secreted in vitro by various cell types, such as dendritic cells, platelets, immunocytes, endothelial cells, epithelial cells, mesenchymal stem cells, and several cancer cells[6,7]. They are also detected in sorts of body fluids including plasma, cerebrospinal, urine, saliva, etc.[5,6]. Given that exosomes encompass numerous proteins and non-coding RNAs which participate in the regulation of intercellular signaling pathways, aberrant alterations in exosomes are supposed to be responsible for the pathogenesis of multiple human diseases[8-11]. Indeed, exosome dysregulation has been widely reported to promote cancer progression by facilitating metastasis, mediating cell resistance to chemotherapy, or maintaining a carcinogenic environment inside the human body[8,9]. Additionally, exosomes can function as delivery vehicles in several autoimmune and nervous system disorders[10]. However, it remains elusive regarding how the contents of exosomes change during the occurrence or development of AAA, and the pertinence of these abnormal changes to AAA pathogenesis.

In the present study, we identified a novel miRNA hitherto unreported to be responsible for AAA pathogenesis that its abundance was increased in exosomes secreted from plasma and tissue-conditioned medium of AAA patients. Furthermore, findings of this study investigated ex vivo the underlying mechanisms by which the increased expression of miR-106a plays a role in the pathological process of AAA development.

Patients and Methods

Patients

A total of 21 AAA patients and 8 healthy peers were recruited at our hospital from Mar 2018 to Jan 2020 before the operation. The selection of AAA populations was executed according to American Society for Vascular Surgery (ASVS) Guidelines on the Diagnosis and Management of Abdominal Aortoiliac Artery Aneurysms (2018). AAA patients were eligible if the AAA diameter, measured by computed tomography angiography (CTA), exceeded 5.5 cm. For those patients undergoing open surgical repair, AAA tissues (containing thrombus and wall) and adjacent normal aortic tissues were obtained during surgery, and tissue samples were immediately frozen in liquid nitrogen. Informed consent was obtained for all participants, and the procedures for human blood and tissue samples were approved by the Ethics Review Board of our hospital.

Precipitation of Exosomes From Tissue-Conditioned Medium

Both AAA and adjacent normal aortic samples were cut into small pieces (~5 mm^2) and separately immersed in serum-free Roswell Park Memorial Institute-1640 (RPMI-1640) medium and incubated at 37°C for 24 h. The tissue-conditioned culture medium was centrifuged at 3000 g for 15 min and the supernatants were transferred to a fresh tube, followed by centrifugation at increasing speeds (20,000 g for 30 min, twice and 100,000 g for 70 min) to precipitate exosomes, which were resuspended for further analysis.

Blood Sample Collection and Exosome Isolation

Blood samples were collected prior to CTA imaging for all patients. Briefly, 10 ml of blood was collected in a vacuum tube and centrifuged at 3,000 rpm for 10 min to separate plasma. The plasma was centrifuged again at 1500 ×g for 10 min to discard dead cells, and then supernatant was collected before processed by centrifugation at 17,000 ×g for 15 min to remove debris. Afterwards, the supernatant was subjected to ultracentrifugation at 100,000 ×g for 70 min to obtain pellets containing exosomes. Relative exosome abundance was determined by measuring acetylcholinesterase activity, with a change in absorbance at 412 nm.

MiRNA Extraction and Quantification

RNA was isolated from exosomal pellet using TRIZOL reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Reverse transcription was performed with TaqMan miRNA Reverse Transcription Kit (Thermo Labsystem, Waltham, MA, USA). Real-time PCR was

carried out using and TaqMan MicroRNA Assays (Thermo Labsystem, Waltham, MA, USA) in a 7900 HT Real-Time PCR system (Applied Biosystems, Foster City, CA, USA). The data were normalized to U6 snRNA to calculate fold changes using the method of $2^{-\Delta\Delta Ct}$.

Cell Culture

Human vascular smooth muscle cell (VSMC) and HEK293T cell lines were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and propagated in Dulbecco's Modified Eagle's Medium (DMEM'F-12) media (Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin (100 U/ml)/streptomycin (100 μg/ml). Cells were maintained in 5 % CO_2 at 37°C prior to further experiment procedures.

Transfection

The VSMCs cells were seeded in serum-free media at a density of 5×10^5 cells/ml. Following 24 h- incubation, cells were transfected with miRNA-106a mimic, inhibitor or negative controls (NC mimics and NC inhibitor) using Lipofectamine 2000 (Life Technologies, Carlsbad, CA, USA) according to manufacturer's instructions. After another 24 h- incubation cells were harvested for further analysis.

Cell Counting Kit-8 (CCK-8) Assay

Cell viability after transfection was examined by performing CCK-8 (Dojindo Molecular Technologies, Kumamoto, Japan) assay. 24h after transfection, cells were seeded into a 96-well plate at 1×10^4 cells/well, followed by incubation at 37°C and 5% CO_2. Subsequently, 10 μl of CCK-8 solution was added to each well of culture plate, and cells were incubated at 37°C for another 1 h. The absorbance at 450 nm was measured using a microplate reader (Multiskan MK3, Thermo Labsystem, Waltham, MA, USA).

Apoptosis Assay

To detect the apoptosis rate, cells were collected in phosphate-buffered saline (PBS) buffer after transfection, and then centrifuged and resuspended again with 195 μL Annexin V-PE binging buffer. Afterwards, 5 μL Annexin V- propidium iodide (PI) agent was added, and cells were incubated at the room temperature in the dark for 15 min. Cell apoptosis was then immediately analyzed by flow cytometry (Beckman Coulter, Fullerton, CA, USA).

Western Blotting

The harvested cells were disrupted in radio immunoprecipitation assay (RIPA) lysis buffer on ice, followed by centrifugation at 15,000 rpm for 5 min at 4°C to obtain protein supernatants. Total proteins were subjected to separation via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene difluoride (PVDF) membrane. The membrane was blocked with 5% silk milk at room temperature for 2 h and then incubated overnight at 4°C with primary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA; or Cell Signaling Technology, Danvers, MA, USA), followed by subsequent incubation with HRP-conjugated secondary goat anti-mouse or anti-rabbit IgG (Beijing Solarbio Science & Technology Co., Ltd. Beijing, China) at room temperature for 1 hour. Then, the membrane was washed for three times before detected using enhanced chemiluminescence (ECL) agents.

Enzyme-Linked Immunosorbent Assay (ELISA)

After transfection, conditioned culture medium was collected and centrifuged to remove cell debris. Protein concentrations in the supernatants were measured by using corresponding ELISA kits (R&D Systems, Abingdon, UK) following the manufacturer's instructions. In brief, monoclonal antibodies were applied to each well of a 96-well microplate and coated overnight. Wells were then washed 3 times and blocked with PBS containing 1% BSA for 30 min, followed by incubation with test samples for 2 hours at 37°C. After washing 3 times, each well was incubated with diluted biotinylated polyclonal antibodies for 1 h and then washed 3 times. After staining with streptavidin–horseradish peroxidase for 30 min, TMB was added to each well and incubated for 15 min in the dark. The reaction was then stopped by 2 M H_2SO_4. Data analysis was performed on a microplate reader based on the absorbance at 450 nm. The experiment was performed in triplicate.

Dual-Luciferase Reporter Assay

The wild-type (WT) or mutant full-length 3'-UTR of TIMP-2 containing miR-106a binding site was cloned into the downstream of pMIR report vector, which was co-transfected with miR-106a mimics or Scr-miR into HEK293T cells. Luciferase activities were determined by

Table I. Demographic and clinical features of the involved population.

	Patient (n = 21)	Healthy peer (n = 8)	*p*-value
Age, years	67.2 ± 5.53	64.3 ± 2.20	0.938
Gender, mal: female	19:2	22:3	0.732
BMI, kg/m^2	24.6 ± 3.20	21.7 ± 2.39	0.630
Hypertension, n (%)	7 (33.3%)	2 (8.0%)	< 0.05
Smoking, n (%)	4 (19.0%)	2 (8.0%)	< 0.05
Statins, n (%)	4 (19.0%)	1 (4.0%)	< 0.05

using the Dual-Luciferase Reporter Assay System (Promega, Madison, WI, USA) following the manufacturers' protocol. All the reactions were performed in triplicates.

Statistical Analysis

Data were analyzed with SPSS (version 22.0, IBM Statistics, Armonk, NY, USA). Differences between two groups were compared using the Student's *t*-test. All experiments included at least 3 replicates per group. Data are presented as means ± SD. A value of $p < 0.05$ was considered statistically significant.

Results

MiR-106 Is Down-Regulated in Exosomes from Patients With AAA

The basic characteristics of AAA patients and healthy cohorts enrolled in this study were presented in Table I. There were no statistical differences between two groups in the demographic data, including age, gender, and BMI value, whereas hypertension and smoking were significant risk factors for AAA occurrence ($p<0.05$).

We detected the expression profiles of several indicated microRNAs in exosomes isolated from tissue-conditioned medium and plasma of AAA patients. These microRNAs were aberrantly expressed in AAA and reported to participate in the pathogenesis of this disease[12-15], including miR-29, miR-33, miR-133, miR-204, and miR-24. As shown in Figure 1A, the expression of miR-29 and miR-33 was up-regulated in the plasmic exosomes from patients with AAA in comparison with that from healthy counterparts, whereas miR-24 and miR-204 were significantly down-regulated ($p<0.05$ and $p<0.01$). In exosomes derived from aortic aneurysm tissues, miR-133, miR-24, and miR-204 were decreased while miR-29 and miR-33 were elevated compared with the adjacent normal tissues ($p < 0.05$ and $p < 0.01$, Figure 1B).

Among the investigated miRNAs, miR-106a was also identified as a novel biomarker increased in exosomes from both patients' plasma ($p < 0.01$) and aneurysmal tissue-conditioned medium ($p < 0.05$), and the augment in expression was more significant in plasmic exosomes (Figure 1A

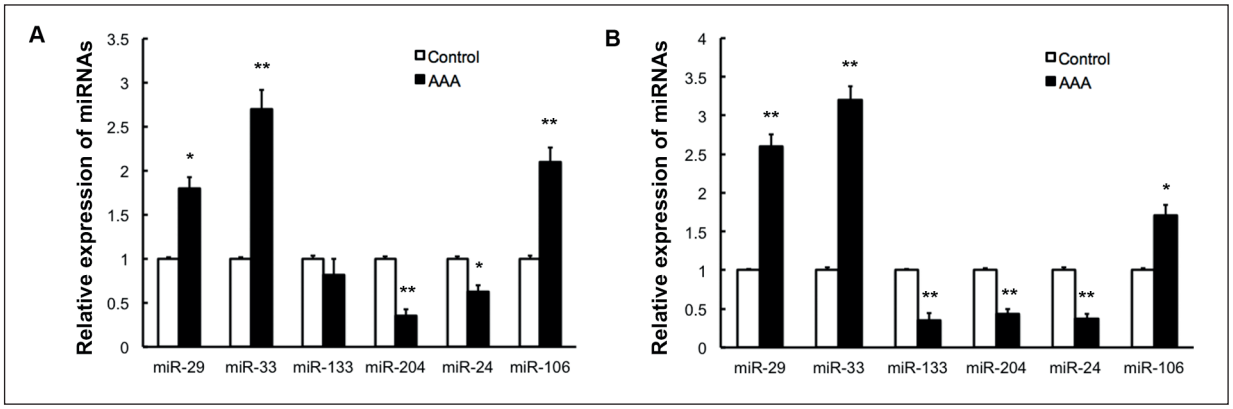

Figure 1. Expression alterations in exosomal miRNAs from AAA patients. The expression profiles of the indicated miRNAs were detected in exosomes isolated from plasma (**A**) and tissue-conditioned medium (**B**) of AAA patients by performing qRT-PCR assay. Data were presented as means ± SD. *$p<0.05$ and **$p<0.01$, compared with the control.

and B). To date, miR-106 has not been reported in published studies concerning the pathogenetic mechanism of AAA.

MiR-106a Inhibits the Viability of Vascular Smooth Muscle Cells

To determine the potential involvement of miR-106a in AAA pathogenesis, vascular smooth muscle cells (VSMCs) were used in this study. VSMC is a predominant component of the aortic media and this cell type plays a crucial role in AAA formation by phenotypic switching. We first enhanced the expression of miR-106a in VSMCs by transfecting with miR-106a mimics, while the expression was suppressed via transfection of miR-106a inhibitor. The transfection efficiency was assessed through quantitative RT-PCR (Figure 2A).

The impact of miR-106a on cellular viability of VSMCs was determined by performing a CCK-8 assay. As shown in Figure 2B, overexpression of miR-106a caused a marked decrease in cell viability ($p < 0.01$), in contrast to the fact that the number of viable VSMC cells was remarkably augmented after inhibition of miR-106a ($p < 0.01$). These results demonstrate that miR-106a exhibited an inhibitory effect on the viability of VSMCs.

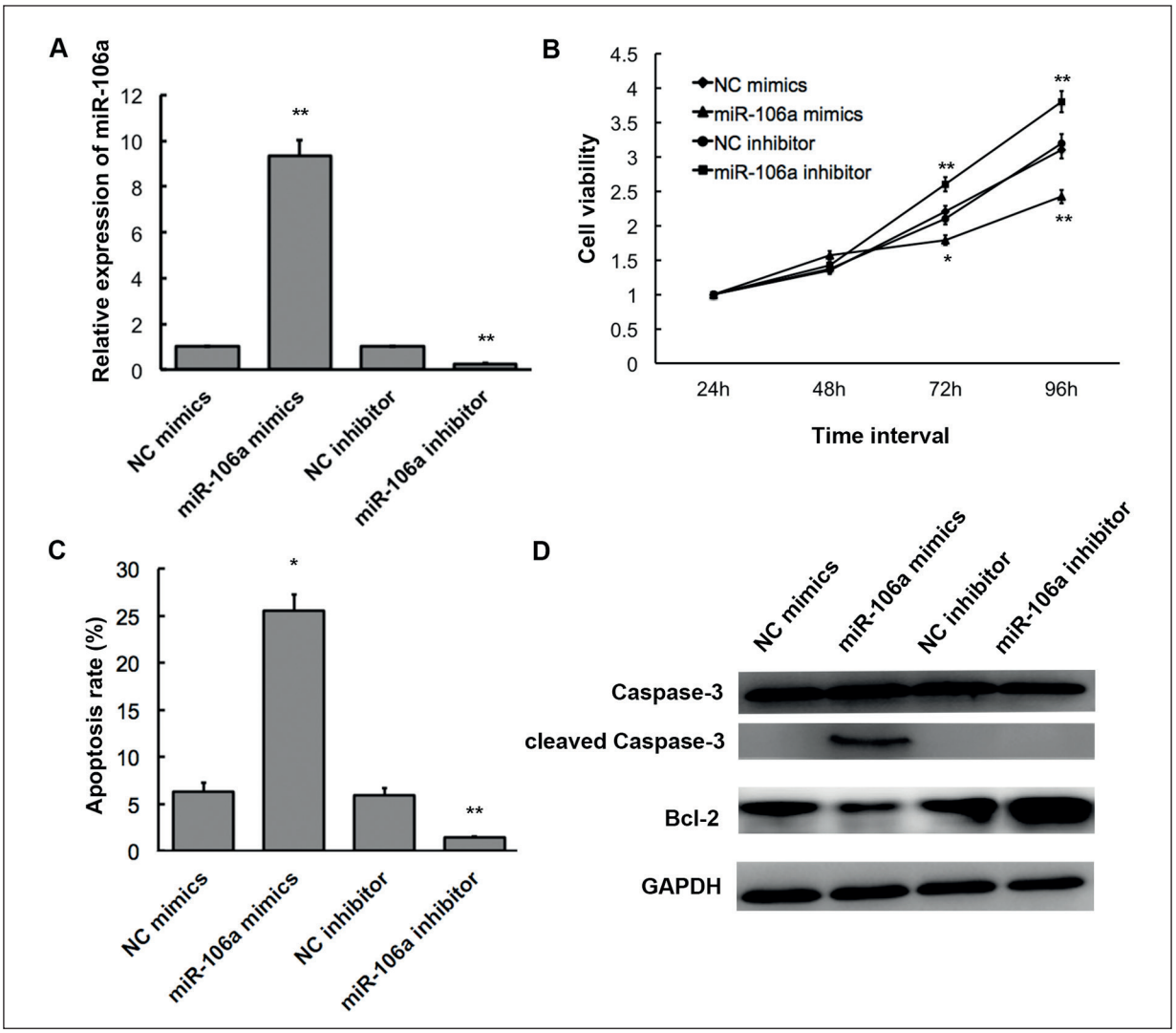

Figure 2. MiR-106a induces the apoptosis of VSMC cells. **A**, The expression level of miR-106a in VSMCs was determined via qRT-PCR, after transfection of miR-106a (or NC) mimics and inhibitor. **B**, The CCK-8 assay was used to evaluate cell viability. **C**, Flow cytometry was conducted to evaluate cell apoptosis. **D**, The protein levels of Caspase3, cleaved Caspase-3, and Bcl-2 were tested through Western blot assay. Data were presented as means ± SD. *p<0.05 and **p<0.01, compared with the control.

MiR-106 Promotes VSMC Cell Apoptosis

The flow cytometry was conducted to evaluate the impact of miR-106a on the apoptosis of VSMCs. We found that the percentage of apoptotic cells was increased by enhanced expression of miR-106a ($p < 0.05$, Figure 2C), which was otherwise suppressed after the inhibition of miR-106a ($p < 0.01$).

As the activated form of Caspase3, cleaved Caspase-3 was markedly increased in VSMC cells transfected with miR-106a mimics. Meanwhile, the anti-apoptotic protein Bcl-2 was significantly reduced in these cells (Figure 2D). When miR-106a was depleted in VSMC cells, cleaved Caspase-3 was down-regulated while protein expression of Bcl-2 was aggrandized. The flow cytometric analysis and Western blot results demonstrate that miR-106a promoted cell apoptosis of VSMCs, which contributes significantly to the progression of AAA.

MiR-106a Modulates MMPs Expression Profiles

On the other hand, AAA is an inflammatory process histologically characterized by extracellular matrix (ECM) degradation and increased production of matrix metalloproteinases (MMPs). In this context, we investigated the role of miR-106a in the regulation of several MMPs related to AAA development, including MMP-2, MMP-9, and MMP-12. Alternations in MMPs expression profiles were examined in vitro by using ELISA. As shown in Figure 3A, while MMP-9 section from VSMCs was slightly down-regulated in response to miR-106a overexpression ($p > 0.05$), expression of MMP-2 and MMP-12 was markedly up-regulated ($p < 0.01$ and $p < 0.05$).

Of note, we also observed a significant decrease in the protein level of metallopeptidase inhibitor 2 (TIMP-2) after miR-106a overexpression, whereas TIMP-2 secretion was aggrandized due to inhibition of miR-106a (Figure 3B). However, the difference in TIMP-1 production resulted from miR-106a overexpression or knockdown was barely detectable.

MiR-106b Directly Targets the 3'-UTR of TIMP2 Gene

The putative target genes of miR-106a were predicted by utilizing online tools such as TargetScan, miRanda and PicTar. Among the target candidates, TIMP-2 could bind to miR-106a via the 3'-UTR region and the interaction sites were illustrated in Figure 4A. Dual-Luciferase reporter assay was carried out to verify the direct interaction between TIMP-2 and miR-106a. The 3'-UTR of TIMP-2 gene was cloned into a Luciferase reporter vector, which was co-transfected into HEK-293T cells with the miR-106a mimics. In addition, 3'-UTR of TIMP-2 was mutated and co-transfected with miR-106a as a negative control. The results showed that miR-106a remarkably suppressed the relative Luciferase activity of the wild-type TIMP-2 3'-UTR,

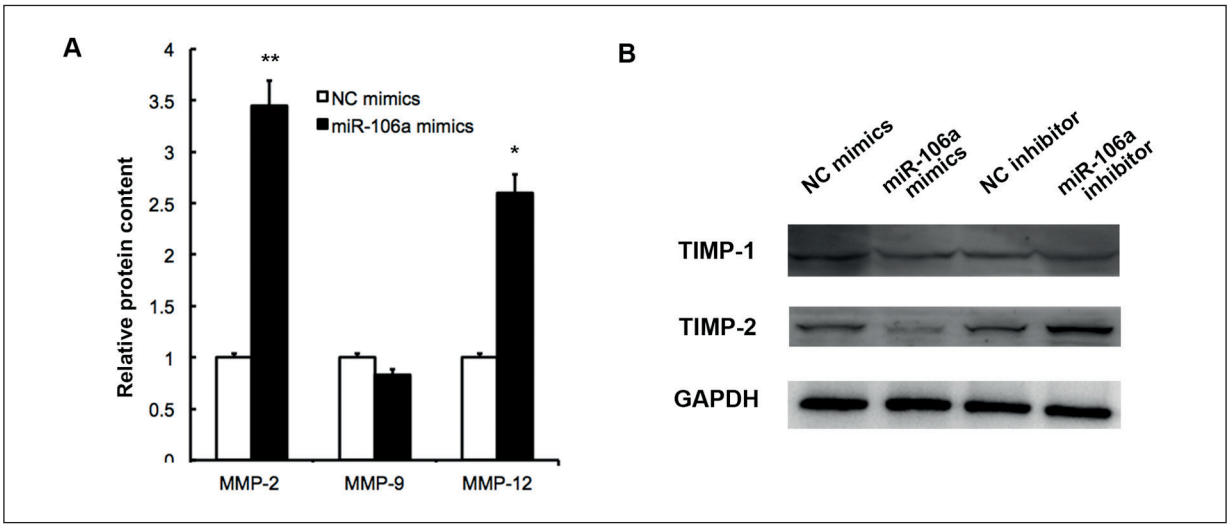

Figure 3. MiR-106a modulates the expression profiles of MMPs and TIMPs. **A**, Alternations in the expression of MMPs (MMP-2, MMP-9, and MMP-12) in response to miR-106a overexpression were examined using ELISA. **B**, The protein levels of TIMP-1 and TIMP-2 were measured through Western blotting. Data were presented as means ± SD. *p<0.05 and **p<0.01, compared with the control.

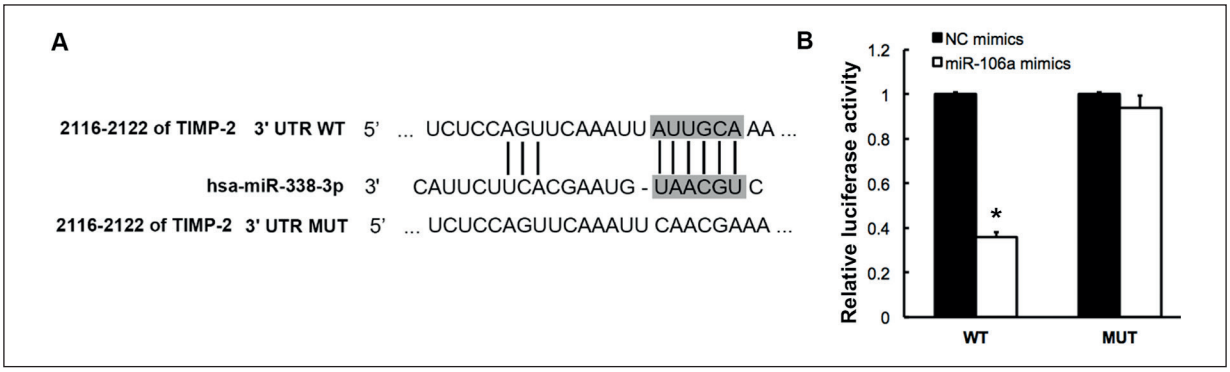

Figure 4. MiR-106b directly targets the 3'-UTR of TIMP2 gene. **A**, The putative interaction sites between miR-106a and 3'-UTR of TIMP-2 were shown. **B**, The Dual-Luciferase reporter assay was carried out to verify the direct interaction between miR-106a and the wild-type (WT) or mutant (MUT) 3'-UTR region of TIMP-2. *$p<0.05$, compared with the control.

without effect on its mutant (Figure 4B, $p > 0.05$). Taken into account the aforementioned observation that increased expression of miR-106a inhibited TIMP-2 protein level, while miR-106a knockdown exerted opposite effects (Figure 3B), we concluded that miR-106a mitigated TIMP-2 expression through directly targeting its 3'-UTR, thereafter restoring the secretion of MMP-2 and MMP-12 from ASMCs and ultimately leading to the proteolysis of ECM components.

Discussion

Studies aimed at understanding the etiopathology of AAA are important as they may identify novel targets for therapy. Although the molecular basis of AAA pathogenesis can generally be analyzed by profiling gene expression of the whole aneurysms, characterization of exosomal molecular changes can provide more valuable information since they play an essential role in the mediation of intercellular communication. By identifying the expression profiles of exosomal miRNAs from patients with AAA, we detected an increased expression of miR-106 that was previously unreported considering the potential connections with AAA development.

In the whole history of miRNAs investigation, miR-17~92 cluster has been famous for its angiogenesis-related functions and, as its paralogues in human, miR-106a~363 and miR-106b-25 clusters also play a significant role in the angiogenesis during the occurrence of both atherosclerosis and tumors. While miR-106b-25 cluster merely encodes for miR-106b, miR-93 and miR-25, miR-106a-363 cluster encodes for multiple miRNAs including miR-106a, miR-20b, miR-18b, miR-19b, miR-92a and miR-363[16]. Previous researches revealed that miR-106a was abnormally expressed in different types of carcinoma, functioning as a tumor suppressor or an oncogene. Of note, miR-106a induces apoptosis of glioma cells independent of p53 status and inhibits cell proliferation and glucose uptake by targeting various downstream molecules[17,18]. In non-small cell lung cancer (NSCLC) miR-106a could also inhibit cell growth[19]. On the contrary, miR-106a promotes pancreatic cancer progression by enhancing the processes of invasion and epithelial-mesenchymal transition (EMT) by repressing expression of TIMP-2[20]. Therefore, we assumed that miR-106 might exert similar effects on other cell types that were involved in the pathogenesis of AAA.

Depletion of medial smooth muscle cells (SMC), generally caused by apoptosis, is predominantly responsible for the pathological remodeling of vessel wall during AAA development[21], because SMC is a cell population capable of directing connective tissue repair. In the present study we found that enhanced expression of miR-106a decreased the viability of VSMC cells and enhanced cell apoptosis. In fact, the pro-apoptosis activity of miR-106 has been corroborated in human umbilical vein endothelial cells (HUVECs) considering ischaemic stroke[22,23]. We also evaluated the impact of miR-106a on cell apoptosis in HUVECs, and the results demonstrated that the survival of HUVECs was mitigated in response to miR-106a overexpression (data not shown). These data suggest that miR-106a can exert a pro-apoptosis effect on cells. Given that the expression of miR-106a was markedly increased in exosomes isolated from the plasma of patients

with AAA, these results imply an important role of miR-106a in the etiology or progression of AAA.

In addition to apoptosis of smooth muscle cells, AAA is characterized by ECM degradation, an obvious inflammatory response, and elevated oxidative stress in the aortic wall[24,25]. Previous studies describing the contribution of dysregulated miR-106 to atherosclerosis and ischaemic stroke[26,27] have revealed its role in cardiovascular diseases. It has been well established that miR-106 impairs cellular cholesterol efflux by targeting ABCA1 and other lipoprotein receptors[27,28], ultimately exacerbating the formation of atherosclerotic plaques. Faulty regulation of cholesterol metabolism promotes AAA development because lipid deposition in SMCs may result in the switch of SMCs phenotype from contractile to synthetic form[29,30]. On the other hand, ECM as one of the components in atherosclerotic plaques is previously found to participate in the regulation of lipid metabolism, particularly in the ABCA1-dependent cholesterol removal, and ECM depletion contributed largely to increased lipid accumulation in macrophages[31,32]. Then, it will be intriguing to know whether miR-106 is also implicated in ECM degradation related to AAA development. Our results showed that enhanced expression of miR-106a significantly increased the section of MMP-2 and MMP-12 from VSMC cells and suppressed the production of their inhibitor TIMP-2. Additionally, Dual-Luciferase reporter assay indicated the direct interaction between miR-106a and the 3'-UTR region of TIMP-2. This study firstly reveals the potential involvement of miR-106a in the pathogenesis of AAA and investigates the exosomal miRNAs hitherto unreported in AAA disease.

Conclusions

Aberrant expression of miR-106 as well as its potential involvement in human AAA was first proposed by Spear et al[33], reporting that miR-106 could be detected in the samples of adventitial tertiary lymphoid organs rather than the whole aortic aneurysmal tissues. However, the authors did not further verify this observation or investigate the action of miR-106 *ex vivo* in AAA pathogenesis. Our data in this study demonstrate that miR-106a promotes VSMC cell apoptosis and accelerates ECM degradation by mitigating TIMP-2 expression and restoring the secretion of matrix metallopeptidases. Therefore, the increased expression of miR-106a in exosomes from patients with AAA may play a crucial role in the pathogenesis and progression of this disease.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Weintraub NL. Understanding abdominal aortic aneurysm. N Engl J Med 2009; 361: 1114-1116.
2) Kent KC. Clinical practice. Abdominal aortic aneurysms. N Engl J Med 2014; 371: 2101-2108.
3) Filardo G, Powell JT, Martinez MA, Ballard DJ. Surgery for small asymptomatic abdominal aortic aneurysms. Cochrane Database Syst Rev 2015; 2015: CD001835.
4) Yu B, Zhang X, Li X. Exosomes derived from mesenchymal stem cells. Int J Mol Sci 2014; 15: 4142-4157.
5) Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol 2013; 200: 373-383.
6) Kalluri R, Lebleu VS. The biology, function, and biomedical applications of exosomes. Science 2020; 367: eaau6977.
7) Théry C, Zitvogel L, Amigorena S. Exosomes: composition, biogenesis and function. Nat Rev Immunol 2002; 2: 569-579.
8) Gangoda L, Boukouris S, Liem M, Kalra H, Mathivanan S. Extracellular vesicles including exosomes are mediators of signal transduction: are they protective or pathogenic? Proteomics 2015; 15: 260-271.
9) Azmi AS, Bao B, Sarkar FH. Exosomes in cancer development, metastasis, and drug resistance: A comprehensive review. Cancer Metastasis Rev 2013; 32: 623-642.
10) Bellingham SA, Guo BB, Coleman BM, Hill AF. Exosomes: Vehicles for the transfer of toxic proteins associated with neurodegenerative diseases? Front Physiol 2012; 3: 124.
11) Yellon DM, Davidson SM. Exosomes: nanoparticles involved in cardioprotection? Circ Res 2014; 114: 325-332.
12) Pahl MC, Derr K, Gäbel G, Hinterseher I, Elmore JR, Schworer CM, Peeler TC, Franklin DP, Gray JL, Carey DJ, Tromp G, Kuivaniemi H. MicroRNA expression signature in human abdominal aortic aneurysms. BMC Med Genomics 2012; 5: 25.
13) Fu XM, Zhou YZ, Cheng Z, Liao XB, Zhou XM. MicroRNAs: novel players in aortic aneurysm. Biomed Res Int 2015; 2015: 831641.
14) Maegdefessel L, Spin JM, Raaz U, Eken SM, Toh R, Azuma J, Adam M, Nakagami F, Heymann HM, Cher-

nogubova E, Jin H, Roy J, Hultgren R, Caidahl K, Schrepfer S, Hamsten A, Eriksson P, McConnell MV, Dalman RL, Tsao PS. miR-24 limits aortic vascular inflammation and murine abdominal aneurysm development. Nat Commun 2014; 5: 5214.

15) Nakao T, Horie T, Baba O, Nishiga M, Nishino T, Izuhara M, Kuwabara Y, Nishi H, Usami S, Nakazeki F, Ide Y, Koyama S, Kimura M, Sowa N, Ohno S, Aoki H, Hasegawa K, Sakamoto K, Minatoya K, Kimura T, Ono K. Genetic ablation of microRNA-33 attenuates inflammation and abdominal aortic aneurysm formation via several anti-inflammatory pathways. Arterioscler Thromb Vasc Biol 2017; 37: 2161-2170.

16) Tanzer A, Stadler PF. Molecular evolution of a microRNA cluster. J Mol Biol 2004; 339: 327-335.

17) Yang G, Zhang R, Chen X, Mu Y, Ai J, Shi C, Liu Y, Shi C, Sun L, Rainov NG, Li H, Yang B, Zhao S. MiR-106a inhibits glioma cell growth by targeting E2F1 independent of p53 status. J Mol Med (Berl) 2011; 89: 1037-1050.

18) Dai DW, Lu Q, Wang LX, Zhao WY, Cao YQ, Li YN, Han GS, Liu JM, Yue ZJ. Decreased miR-106a inhibits glioma cell glucose uptake and proliferation by targeting SLC2A3 in GBM. BMC Cancer 2013; 13: 478.

19) Xie X, Liu H, Mei J, Ding F, Xiao H, Hu F, Hu R, Wang M. miR-106a promotes growth and metastasis of non-small cell lung cancer by targeting PTEN. Int J Clin Exp Pathol 2015; 8: 3827–3834.

20) Li P, Xu Q, Zhang D, Li X, Han L, Lei J, Duan W, Ma Q, Wu Z, Wang Z. Upregulated miR-106a plays an oncogenic role in pancreatic cancer. FEBS Lett 2014; 588: 705-712.

21) Thompson RW, Liao S, Curci JA. Vascular smooth muscle cell apoptosis in abdominal aortic aneurysms. Coron Artery Dis 1997; 8: 623-631.

22) Li P, Shen M, Gao F, Wu J, Zhang J, Teng F, Zhang C. An antagomir to microRNA-106b-5p ameliorates cerebral ischemia and reperfusion injury in rats via inhibiting apoptosis and oxidative stress. Mol Neurobiol 2017; 54: 2901-2921.

23) Maimaiti A, Maimaiti A, Yang Y, Ma Y. MiR-106b exhibits an anti-angiogenic function by inhibiting STAT3 expression in endothelial cells. Lipids Health Dis 2016; 15: 51.

24) McCormick ML, Gavrila D, Weintraub NL. Role of oxidative stress in the pathogenesis of abdominal aortic aneurysms. Arterioscler Thromb Vasc Biol 2007; 27: 461-469.

25) Longo GM, Xiong W, Greiner TC, Zhao Y, Fiotti N, Baxter BT. Matrix metalloproteinases 2 and 9 work in concert to produce aortic aneurysms. J Clin Invest 2002; 110: 625-632.

26) Khoshnam SE, Winlow W, Farbood Y, Moghaddam HF, Farzaneh M. Emerging roles of microRNAs in ischemic stroke: as possible therapeutic agents. J Stroke 2017; 19: 166-187.

27) Semo J, Chernin G, Jonas M, Shimoni S, George J. Deletion of the Mir-106b~25 MicroRNA cluster attenuates atherosclerosis in Apolipoprotein E knockout mice. Lipids Health Dis 2019; 18: 208.

28) Kim JK, Yoon H, Ramírez CM, Lee SM, Hoe HS, Fernández-Hernando C, Kim JS. MiR-106b impairs cholesterol efflux and increases Aβ levels by repressing ABCA1 expression. Exp Neurol 2012; 235: 476-483.

29) Martínez-López D, Cedó L, Metso J, Burillo E, García-León A, Canyelles M, Lindholt JS, Torres-Fonseca M, Blanco-Colio LM, Vázquez J, Blanco-Vaca F, Jauhiainen M, Martín-Ventura JL, Escolà-Gil JC. Impaired HDL (high-density lipoprotein)-mediated macrophage cholesterol efflux in patients with abdominal aortic aneurysm. Arterioscler Thromb Vasc Biol 2018; 38: 2750-2754.

30) Mourmoura E, Vasilaki A, Giannoukas A, Michalodimitrakis E, Pavlidis P, Tsezou A. Evidence of deregulated cholesterol efflux in abdominal aortic aneurysm. Acta Histochem 2016; 118: 97-108.

31) Lu L, Liu H, Peng J, Gan L, Shen L, Zhang Q, Li L, Zhang L, Su C, Jiang Y. Regulations of the key mediators in inflammation and atherosclerosis by aspirin in human macrophages. Lipids Health Dis 2010; 9: 16.

32) Burgess JW, Kiss RS, Zheng H, Zachariah S, Marcel YL. Trypsin-sensitive and lipid-containing sites of the macrophage extracellular matrix bind apolipoprotein A-I and participate in ABCA1-dependent cholesterol efflux. J Biol Chem 2002; 277: 31318-31326.

33) Spear R, Boytard L, Blervaque R, Chwastyniak M, Hot D, Vanhoutte J, Staels B, Lemoine Y, Lamblin N, Pruvot FR, Haulon S, Amouyel P, Pinet F. Adventitial Tertiary Lymphoid Organs as Potential Source of MicroRNA Biomarkers for Abdominal Aortic Aneurysm. Int J Mol Sci 2015; 16: 11276-11293.