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Original Article

Exosomes from microRNA-199-3p-modified adipose-derived stem cells promote proliferation and migration of endothelial tip cells by downregulation of semaphorin 3A

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Abstract: Exosomes secreted by adipose-derived stem cells (ADSCs) have been shown to promote angiogenesis. This study aimed to investigate the effect of exosomes from ADSCs (ADSCs-Exos) on proliferation and migration of endothelial tip cells. In this study, ADSCs were analyzed by flow cytometry. The protein levels were examined by western blot. Cell proliferation and migration were assessed by CCK-8 assay, EdU cell proliferation assay and transwell migration assay. A luciferase reporter assay was performed to confirm whether sema3A was a direct target of miR-199a/b-3p. The results showed that ADSCs-Exos strikingly promoted the proliferation and migration of endothelial tip cells. The expression levels of miR-199a-3p and miR-199b-3p were strikingly increased in ADSCs and ADSCs-Exos. Compared to the $Exos^{scramble}$ group, the proliferation and migration of endothelial tip cells was dramatically increased in the $Exos^{199\ mimic}$ group, but remarkably decreased in the $Exos^{199\ inhibitor}$ group. Moreover, Sema3A was a target of miR-199-3p. The stimulatory effects of $Exos^{199\ mimic}$ on the proliferation and migration of endothelial tip cells were negated by Sema3A overexpression. Besides, the expression of tissue inhibitor of metalloproteinase 3 (TIMP3) was decreased, and the expression of matrix metalloproteinases 9 (MMP9) and proliferating cell nuclear antigen (PCNA) were increased in endothelial tip cells co-cultured with ADSCs-Exos, which were substantially enhanced by $Exos^{199\ mimic}$ treatment. However, the effect of $Exos^{199\ mimic}$ on the protein expression of TIMP3, MMP9 and PCNA were negated by upregulation of Sema3A. In conclusion, exosomes from miR-199-3p-modified ADSCs promote proliferation and migration of endothelial tip cells by downregulation of sema3A.

Keywords: Peripheral artery disease, endothelial tip cell, exosomes, adipose-derived stem cells

Introduction

Peripheral artery disease (PAD), also known as peripheral arterial occlusive disease or lower extremity arterial disease, is one of the most prevalent vascular diseases and occurs as a result of narrowing of the arteries which ultimately blocks blood flow to the extremities [1]. The causes for PAD include atherosclerosis, inflammation, thrombosis and embolism [2, 3]. PAD is a leading cause of morbidity, disability and death throughout the world [4]. Although the common signs of PAD include intermittent claudication, skin ulcers, bluish skin and cold skin, up to 50% of PAD patients are asymptomatic. In the present, the clinical treatment for PAD consists primarily of medication, angioplasty, atherectomy, bypass grafting, amputation, thrombolysis and thrombectomy. Unfortunately, these therapies have satisfactory results only in approximately 25% of patients with PAD [5]. Recently, therapeutic angiogenesis becomes one of the focuses of PAD therapy [6]. Therapeutic angiogenesis is considered a novel and promising treatment for PAD.

Angiogenesis is a dynamic process which induces the formation of new blood vessels in hope to reinstate the blood circulation of the artery [7]. Endothelial tip cells aresingle, highly

polarity, distinct and functionally specialized endothelial cells at the forefront of vascular sprouts during angiogenesis. During angiogenesis, endothelial tip cells extend long filopodia to sense the environment and direct the endothelial tip cell migration, while stalk cells elongate the sprout to form new branches [8, 9]. Endothelial tip cells have been documented to play a vital role in angiogenesis [10]. Previously, vascular endothelial growth factor (VEGF)-A guided retinal angiogenesis through promoting the directed migration of endothelial tip cells and the proliferation of stalk cells [11]. Therefore, promoting the directed migration of endothelial tip cells may become a new targeted therapy for PAD.

Recently, with further research of stem cells in cardiovascular disease, adipose-derived stem cells (ADSCs), as a representative of stem cells have attracted tremendous attention [12]. ADSCs derived from adipose tissues have the advantages of widespread source, less injury and minimal ethical consideration; moreover it harbors multiple potentials [13]. ADSCs have been reported to attenuate ischemic injury through secretion of paracrine factors and exosomes [14]. Therefore, ADSCs promise to become a therapeutic tool for cardiovascular disease, including PAD. Exosomes are small cell-derived vesicles (30-100 nm in diameter) and can be secreted by a variety of cell types and found in abundance in eukaryotic fluids [15]. Evidence is accumulating that exosomes as intercellular messengers participate in cell-to-cell signaling and impact the function of recipient cell by transferring biologically active molecules, such as proteins, mRNAs, and microRNAs (miRNAs) [16]. Exosomes derived by ADSCs (ADSCs-Exos) have been proved to play critical roles in cardiovascular disease and repair [17, 18]. However, the effects and mechanisms of ADSCs-Exos for PAD remain elusive.

In this study, we evaluated the effects of ADSCs-Exos on the proliferation and migration of endothelial tip cells, and the exact molecular mechanisms were further investigated. Our findings uncovered that ADSCs-Exos as a pro-angiogenic factor promoted the proliferation and migration of endothelial tip cells through microRNA (miR)-199a/b-3p/semaphorin 3A (Sema3A) axis.

Materials and methods

ADSCs culture

Human ADSCs were isolated from lipoaspirate tissue by enzymatic digestion method. Briefly, lipoaspirate tissue was washed in HBSS and digested with collagenase in HBSS for 40 min. Afterwards, the same volume of Dulbecco's modified Eagle's medium (Solarbio, Beijing, China) containing 10% FBS (Solarbio) was added and the supernatant, filtered and centrifuged to discard the floated lipid layer. After washing, the pellets were resuspended in DMEM medium contained with 10% FBS and 1% penicillin-streptomycin (Solarbio), plated into a 25 cm^2 dish and cultured in a humidified incubator at 37°C under 5% CO_2/95% air.

Endothelial tip cells culture

Human peripheral blood mononuclear cells were isolated from 4 ml heparinized blood from healthy adult volunteer by Discontinuous Ficoll Gradient Centrifugation. Human peripheral blood mononuclear cells were resuspended in EGM-2-MV medium (Lonza, Walkersville, Maryland) contained EGM-2-MV SingQuate (Lonza), seeded into flasks pre-coated with fibronectin and incubated in a humidified incubator at 37°C under 5% CO_2/95% air. Medium was exchanged every 2 days. After 4 weeks of incubation, endothelial tip cells was identified by inverted microscope (Nikon, Tokyo, Japan).

Isolation of exosomes

ADSCs transfected with scramble, miR-199-3p mimic or miR-199-3p inhibitor were used to obtain ADSCs-Exos, $Exos^{scramble}$, $Exos^{199\ mimic}$ and $Exos^{199\ inhibitor}$. Exosomes were isolated from ADSCs culture medium with total exosome isolation reagent (Invitrogen, Carlsbad, CA), following the manufacturer's specification. In brief, ADSCs culture medium was harvested, centrifuged at 2,000 *g* for 30 min at 4°C to remove cells and debris. The supernatant was mixed thoroughly with the total exosomes isolation reagent and incubated at 4°C overnight. Afterwards, the supernatant was centrifuged at 10,000 *g* for 1 h at 4°C and the pellets contained exosomes were resuspended in PBS. Endothelial tip cells were co-cultured with the different exosomes or PBS.

Table 1. Sequence of qRT-PCR primers used in this study

Gene name	Primer sequence
miR-199a-3p	ACAGUAGUCUGCACAUUGGUUA
miR-199b-3p	ACACTCCAGCTGGGAACTGGCCCTCAAAGTCCCG
miR-199-3p	CCC AGT GTT TAG ACT A stem-loop RT primer, GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACC TGC GGG AAC AGA
miR-145-5p	GTCCAGTTTTCCCAGGAATCCCT
miR-30a-5p	UGUAAACAUCCUCGACUGGAAG
miR-25-3p	ATTGCACTTGTCTCGGTCTG
miR-32-5p	CGGTATTGCACATTACTAAGTTGCA
U6	F: CTCGCTTCGGCAGCACA
	R: AACGCTTCACGAATTTGCGT
Sema3A	F: TGTTGGGACCGTTCTTAAAGTAGT
	R: TAGTTGTTGCTGCTTAGTGGAAAG
β-actin	F: TGAAGATCAAGATCATTGCTCCTC
	R: CAACTAAGTCATAGTCCGCCTAGA

Migration assay

The migration of endothelial tip cells was determined using transwell chamber (Corning, NY, USA). Cells were seeded into the upper chamber and the lower chamber is filled with EGM-2-MV medium contained 15% FBS. After 48 h of incubation, non-migrated cells were removed, and migrated cells were fixed with methanol and stained with 4', 6'-diamidino-2-phenylindole (DAPI; Solarbio). The migrated cells were pictured and quantified in five random fields per sample under a microscope.

Detection of cell proliferation capacity

The proliferation of endothelial tip cells was assessed using cell counting kit-8 (CCK-8, Solarbio) assay and 5-ethynyl-20-deoxyuridine (EdU, Beyotime, Shanghai, China) assay.

For the CCK-8 assay, cells were seeded in a 96-well plate and incubated with 10 μl CCK-8 reagent for 2 h in humidified incubator at 37°C with 5% CO_2. The optical density (OD) value was determined at 450 nm using a microplate reader.

For the EdU assay, endothelial tip cells were transfected with pcDNA-Sema3A or pcDNA, and then co-cultured with different exosomes. After 48 h of incubation, endothelial tip cells were incubated in medium supplemented with EdU for 2 h, and then fixed in 4% paraformaldehyde for 15 min at room temperature. After washing, endothelial tip cells were permeated with 0.3% Triton X-100 in PBS for 15 min at room temperature. Afterwards, endothelial tip cells were washed with PBS and stained with DAPI for 2 min in the dark. EdU positive cells were visualized under a fluorescence microscope and analyzed by Image J software (NIH Image, Bethesda, MD, USA).

Flow cytometry

ADSCs were collected, digested, washed and resuspended in PBS. ADSCs were probed with FITC-conjugated antibodies against CD31, CD45, CD90, CD105 and immunoglobulin G (IgG, all from Abcam, Cambridge, UK) for 3 h at 4°C. Subsequently, ADSCs were washed in PBS, followed by fixation with 4% buffered formalin. ADSCs were analyzed with FACSCalibur flow cytometry (Becton Dickinson, San Diego, CA, USA).

Luciferase reporter assay

The 3'UTR sequence (contained the putative miR-199-3p recognition site) of wild type Sema3A (WT UTR) and the mutant (Mut UTR) were amplified and subcloned to the luciferase reporter vector, respectively. Endothelial tip cells were transfected with Mut UTR or WT UTR, and then co-cultured with ADSCs-Exos or Exos derived from ADSCs transfected with scramble, miR-199-3p mimic or miR-199-3p inhibitor ($Exos^{scramble}$, $Exos^{199\ mimic}$ or $Exos^{199\ inhibitor}$). At 24 h after incubation, the relative luciferase activity was determined by the dual luciferase reporter assay system (Promega, Madison, WI).

Cell transfection

Scramble, miR-199 mimic and miR-199 inhibitor were synthesized and purchased by GenePharma (Shanghai, China). The fragment of Sema3A was amplified and inserted into pcDNA-3.1 vector to obtain the pcDNA-Sema3A

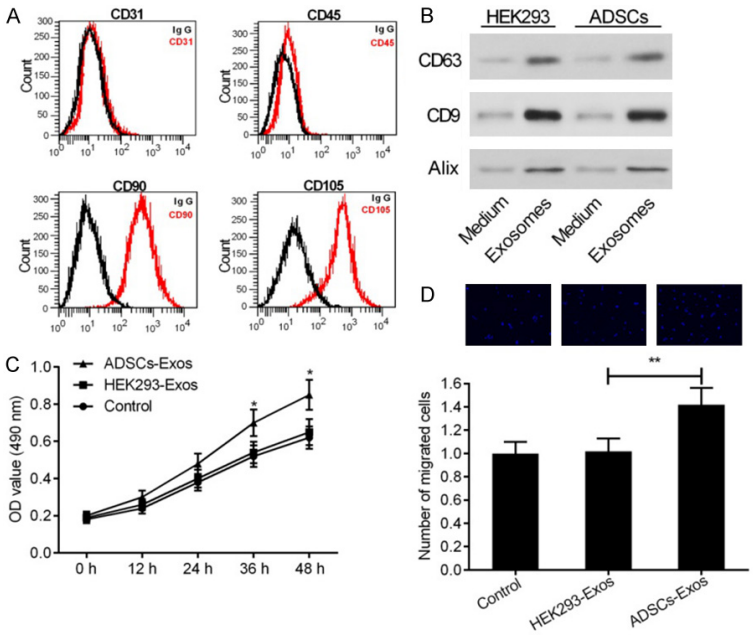

Figure 1. ADSCs-Exos contributes to proliferation and migration of endothelial tip cells. A. Flow cytometric analysis of surface markers (positive for CD90 and CD105, and negative for CD31 and CD 45) expressed on ADSCs. IgG was selected as isotype controls. B. Western blot analysis of exosome marker proteins (CD63, CD9, and Alix) expressed on HEK293-Exos and ADSCs-Exos. C and D. Endothelial tip cells were co-cultured with HEK293-Exos or ADSCs-Exos for 12, 24, 36 or 48 h, the angiogenesis of endothelial tip cells was evaluated by CCK-8 assay and transwell migration assay. *P < 0.05 and **P < 0.01.

plasmid. Cell transfection was conducted using Lipofectamine 2000 in accordance with the manufacturer's specification.

Western blot analysis

Cells were collected and lysed in ice-cold RIPA buffer contained with protease inhibitor cocktail, and the concentration of protein was measured by use of Enhanced BCA Protein Assay Kit (Beyotime). Cell lysates were separated by 14% SDS-PAGE gel, followed by electro-transfer onto a PVDF membrane. Membranes were blocked with 5% skim milk in PBS and immunoblotted with antibodies against CD63 (Boster, Wuhan, China), CD9 (Boster), Alix (Boster), Sema3A (Boster), tissue inhibitor of metalloproteinase 3 (TIMP3; Novus, Littleton, Colorado, USA), matrix metalloproteinases 9 (MMP9; Novus) and proliferating cell nuclear antigen (PCNA; Novus) at 4°C overnight. Subsequently, membranes were probed with horseradish peroxidase-conjugated secondary antibody (Boster) for 2 h at room temperature. After washing, brands were developed using BeyoECL Plus (Beyotime) and analyzed by Image J software.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells using TRIzol reagent (Invitrogen) and reverse-transcribed to generate a double-stranded cDNA using PrimeScript™ RT reagent kit (Takara, Dalian, China). qRT-PCR assay was conducted using SYBR-Green qPCR Master mix (Takara) on a 7300 PCR System (ABI, Carlsbad, CA, USA). β-actin was selected as a loading control for Sema3A. For detection of miRNA expression, cDNA was generated using Hairpin-itTM miRNAs qPCR Quantitation Kit (Genepharma), and U6 was employed as a housekeeping gene for miRNAs. The $2^{-\Delta\Delta Ct}$ method was applied to estimate the relative expression of miRNAs and Sema3A. Gene-special primes used in this study were listed in **Table 1**.

Statistical analysis

All results are expressed as means ± SD of at least three independent experiments and statistical comparisons between two groups was assessed by one-way ANOVA followed by Tukey post hoc test with the help of SPSS 20.0 software (IBM, Armonk, NY, USA). *P* value less than 0.05 was considered significant.

Results

ADSCs-Exos contributes to proliferation and migration of endothelial tip cells

First, ADSCs and ADSCs-Exos were isolated and identified by flow cytometry. As expected, CD90 and CD105 were highly expressed in ADSCs, whereas CD31 and CD 45 were hardly expressed in ADSCs (**Figure 1A**). Similarly,

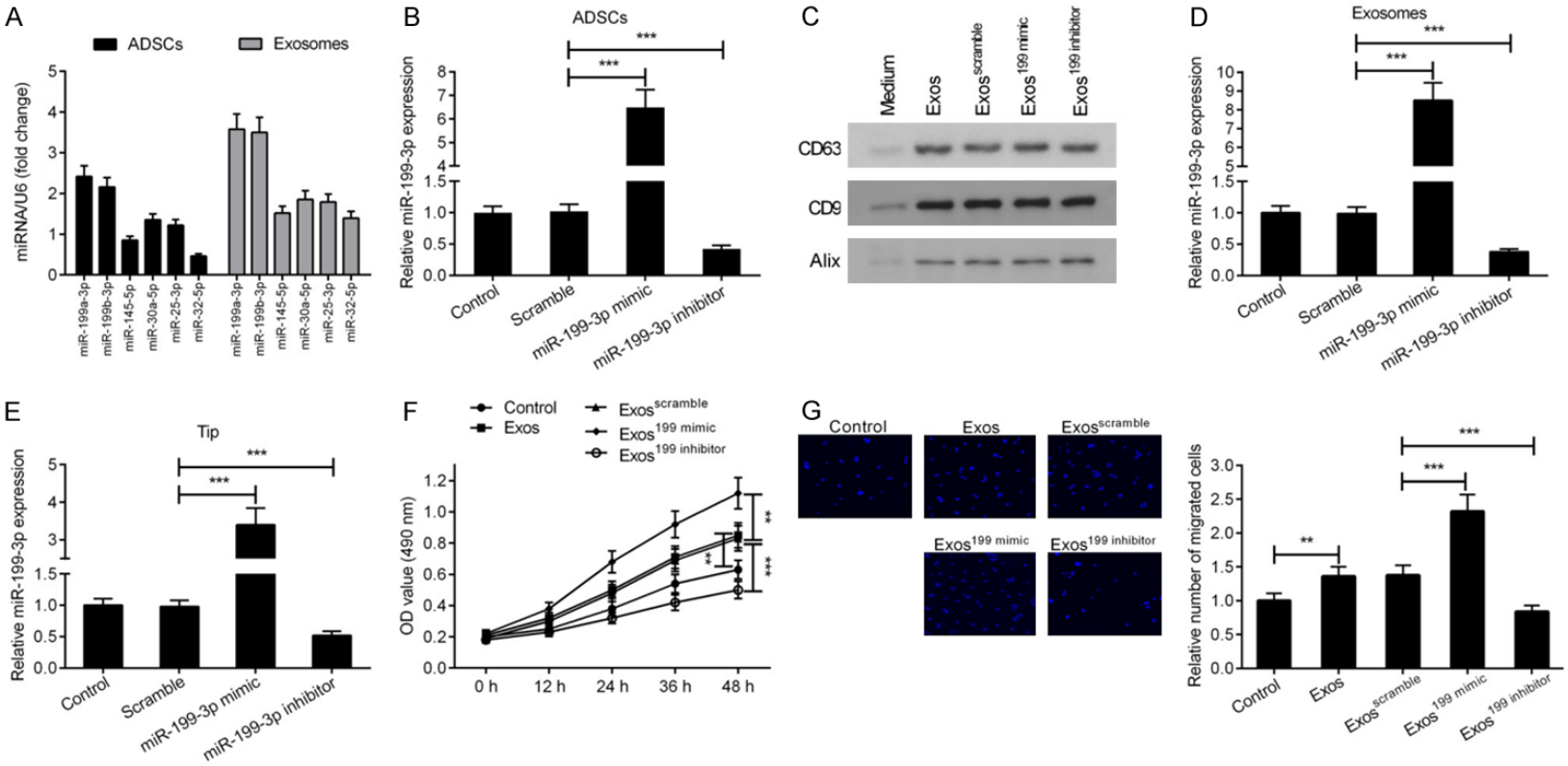

Figure 2. Exosomes from miR-199-3p-modified ADSCs contributes to proliferation and migration of endothelial tip cells. A. qRT-PCR analysis of exosomal miRNAs expression in ADSCs and ADSCs-Exos. B. ADSCs were transfected with scramble, miR-199-3p mimic or miR-199-3p inhibitor, and the expression of miR-199-3p were examined by qRT-PCR. C. The protein levels of CD9, CD63 and Alix were determined by western blot. D. Exosomes were isolated from ADSCs transfected with scramble, miR-199-3p mimic or miR-199-3p inhibitor, and then the expression of miR-199-3p in different exosomes were determined. E. Endothelial tip cells were co-cultured with $Exos^{scramble}$, $Exos^{199\ mimic}$ or $Exos^{199\ inhibitor}$. After 48 h of incubation, the expression of miR-199-3p was determined by qRT-PCR. F and G. The proliferation and migration of endothelial tip cells were evaluated by CCK-8 assay and transwell migration assay. $^{**}P < 0.01$ and $^{***}P < 0.001$.

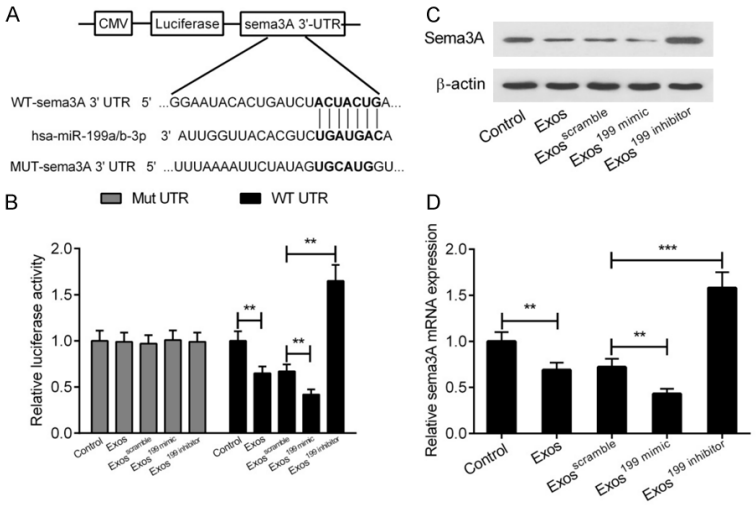

Figure 3. Sema3A is a target of miR-199a/b-3p. A. The predicted miR-199a/b-3p binding site within the Sema3A 3'-UTR was shown. B. Endothelial tip cells were transfected with Mut UTR or WT UTR, and then co-cultured with/without ADSCs-Exos, Exos^{scramble}, Exos$^{199\ mimic}$ and Exos$^{199\ inhibitor}$. At 48 h after transfection, the luciferase activity was measured. C and D. The protein and mRNA levels of Sema3A were determined by western blot and qRT-PCR. **P < 0.01 and ***P < 0.001.

western blot showed that the protein markers of exosomes, such as CD63, CD9, and Alix were highly expressed in exosomes derived from HEK293 or ADSCs cells (**Figure 1B**). To investigate the effect of ADSCs-Exos on the angiogenesis of endothelial tip cells, endothelial tip cells were co-cultured with HEK293-Exos or ADSCs-Exos for indicated times and the angiogenesis of endothelial tip cells was evaluated by CCK-8 assay and transwell migration assay. We found that the proliferation of endothelial tip cells was increased in the ADSCs-Exos group compared with that in the HEK293-Exos group (**Figure 1C**). Meanwhile, the migration of endothelial tip cells co-cultured with ADSCs-Exos was markedly elevated compared with that stimulated with HEK293-Exos (**Figure 1D**). Collectively, these data revealed that ADSCs-Exos promoted the proliferation and migration of endothelial tip cells.

Exosomes from miR-199-3p-modified ADSCs contributes to proliferation and migration of endothelial tip cells

To study the mechanisms by which ADSCs-Exos contributes to angiogenesis of endothelial tip cells, the expression of miR-199a-3p, miR-199b-3p, miR-145-5p, miR-30a-5p, miR-25-3p and miR-32-5p in ADSCs and ADSCs-Exos were measured by qRT-PCR. As a result, the expression of miR-199a-3p and miR-199b-3p were strikingly increased in ADSCs and ADSCs-Exos (**Figure 2A**). To investigate the role of exosomal miR-199-3p in angiogenesis of endothelial tip cells, we altered the expression of miR-199-3p in ADSCs by transfecting with scramble, miR-199-3p mimic or miR-199-3p inhibitor. After 24 h of transfection, the transfection efficiency was confirmed by qRT-PCR. Compared to the scramble group, the expression of miR-199-3p was markedly increased in the miR-199-3p mimic group, but decreased in the miR-199-3p inhibitor group (**Figure 2B**). At 24 post transfection, ADSCs-Exos were isolated and identified, and the results of western blot suggested that CD63, CD9, and Alix were highly expressed in ADSCs-Exos and ADSCs-Exos, Exos^{scramble}, Exos$^{199\ mimic}$ and Exos$^{199\ inhibitor}$ (**Figure 2C**). Furthermore, the expression of miR-199-3p was obviously elevated in Exos$^{199\ mimic}$ compared with that in Exos^{scramble}. Conversely, the expression of miR-199-3p was obviously decreased in Exos$^{199\ inhibitor}$ compared with that in Exos^{scramble} (**Figure 2D**). Endothelial tip cells were co-cultured with Exos^{scramble}, Exos$^{199\ mimic}$ or Exos$^{199\ inhibitor}$. After 48 h of incubation, the expression of miR-199-3p was obviously increased in endothelial tip cells co-cultured with Exos$^{199\ mimic}$, but obviously decreased in endothelial tip cells co-cultured with Exos$^{199\ inhibitor}$ (**Figure 2E**). Compared to the Exos^{scramble} group, the proliferation and migration of endothelial tip cells was dramatically increased in the Exos$^{199\ mimic}$ group, but remarkably decreased in the Exos$^{199\ inhibitor}$ group (**Figure 2F**, **2G**). Together, these results indicated that exosomes from miR-199-3p-modified ADSCs contributed to proliferation and migration of endothelial tip cells.

Sema3A is a target of miR-199a/b-3p

Bioinformatic analysis predicted that Sema3A sequence harbors a putative binding site for miR-199a/b-3p (**Figure 3A**). To identify whether

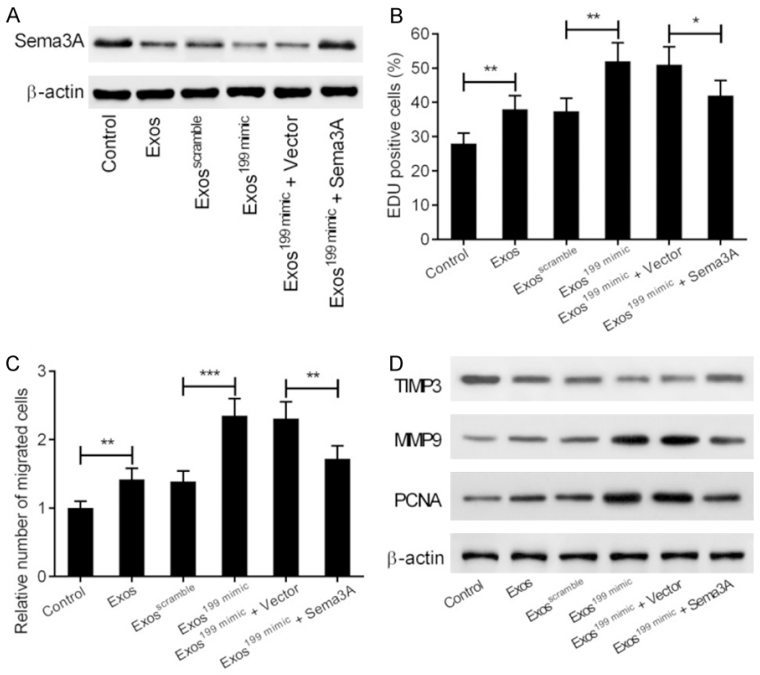

Figure 4. Exosomes from miR-199-3p-modified ADSCs contribute to proliferation and migration of endothelial tip cells by targeting Sema3A. Endothelial tip cells were transfected with pcDNA-Sema3A or pcDNA, and then co-cultured with ADSCs-Exos, Exos^{scramble}, or Exos$^{199\ mimic}$. A. The expression of Sema3A was evaluated by western blot. B and C. The proliferation and migration of endothelial tip cells was determined by EdU assay and transwell migration assay. D. The protein levels of TIMP3, MMP9, and PCNA were examined by western blot. *P < 0.05, **P < 0.01 and ***P < 0.001.

Sema3A is a target of miR-199a/b-3p, endothelial tip cells were transfected with Mut UTR or WT UTR, and then co-cultured with ADSCs-Exos, Exos^{scramble}, Exos$^{199\ mimic}$ or Exos$^{199\ inhibitor}$. ADSCs-Exos markedly reduced the luciferase activity of luciferase reporter containing WT UTR. Moreover, the luciferase activity of luciferase reporter containing WT UT was inhibited by Exos$^{199\ mimic}$, but enhanced by Exos$^{199\ inhibitor}$. However, the luciferase activity of luciferase reporter containing Mut UTR was barely affected (**Figure 3B**). In parallel, the expression of Sema3A was determined by western blot and qRT-PCR. The protein and mRNA leves of Sema3A were markedly decreased in endothelial tip cells co-cultured with ADSCs-Exos, which was substantially enhanced by Exos$^{199\ mimic}$ treatment. In contrast, the protein and mRNA levels of Sema3A were prominently increased in endothelial tip cells co-cultured with Exos$^{199\ inhibitor}$ (**Figure 3C**, **3D**). Taken together, our results suggested that Sema3A was a target of miR-199a/b-3p.

Exosomes from miR-199-3p-modified ADSCs accelerate proliferation and migration of endothelial tip cells by targeting Sema3A

To further elucidate the mechanisms by which exosomes from miR-199-3p-modified ADSCs contribute to angiogenesis of endothelial tip cells, we elevated the expression of Sema3A in endothelial tip cells by transfecting with pcDNA-Sema3A. Then, endothelial tip cells were co-cultured with ADSCs-Exos, Exos^{scramble} or Exos$^{199\ mimic}$. As a result, the protein level of Sema3A was markedly elevated in endothelial tip cells transfected with pcDNA-Sema3A compared with that in endothelial tip cells transfected with pcDNA in the presence of Exos$^{199\ mimic}$ (**Figure 4A**). Furthermore, the stimulatory effect of Exos$^{199\ mimic}$ on the proliferation and migration of endothelial tip cells was notably abrogated by Sema3A overexpression (**Figure 4B**, **4C**). Also, the protein levels of TIMP3, MMP9 and PCNA were detected by western blot. The expression of TIMP3 was decreased, and the expression of MMP9 and PCNA were increased in endothelial tip cells co-cultured with ADSCs-Exos, which were substantially enhanced by Exos$^{199\ mimic}$ treatment. However, the regulatory effects of Exos$^{199\ mimic}$ on the protein expression of TIMP3, MMP9 and PCNA were conversed by overexpressing Sema3A (**Figure 4D**). In conclusion, these findings exhibited that exosomes from miR-199-3p-modified ADSCs promoted the proliferation and migration of endothelial tip cells by targeting Sema3A.

Discussion

Numerous studies over the past decades have revealed that ADSCs-Exos acts as a key modulator in angiogenesis. Previously, ADSCs-Exos accelerated the migration and tube formation of brain microvascular endothelial cells follow-

ing oxygen-glucose deprivation/reoxygenation through miRNA-181b/TRPM7 axis [19]. ADSCs-Exos facilitated the proliferation and tube formation capability, and inhibited the apoptosis of endothelial progenitor cell in the presence of high glucose. Moreover, ADSCs-Exos reduced the intracellular levels of ROS and decreased the levels of inflammatory cytokine IL-1β, IL-6, and TNF-α in endothelial progenitor cell treated with high glucose [20]. In the present study, we found that ADSCs-Exos remarkably promoted the proliferation and migration of endothelial tip cells.

miRNAs, a set of long single-strand non-coding RNA with 20-22 nucleotides in length, serve as pivotal regulators of various biological processes [21]. miRNAs are capable of binding to the 3'-untranslated regions of their target gene, resulting in the degradation of target mRNA and inhibiting its translation [22]. miRNAs have been shown to be closely associated with the development of numerous diseases, including PAD [23]. Several reports have shown that miRNA-199 acts as a crucial regulator of angiogenesis. For instance, Ghosh et al. showed that miR-199a-3p was downregulated in hepatocellular carcinoma tissues and overexpression of miR-199a-3p repressed the angiogenesis and migration of hepatocellular carcinoma cells by inhibiting the expression of VEGF-A, VEGF receptor 1 (VEGFR1), VEGFR2, hepatocyte growth factor, and matrix metalloproteinase-2 [24]. Liu et al. found that VEGFA is a target of miR-199a, and upregulation of miRNA-199 reduced the expression of VEGF and then repressed the VEGF-mediated angiogenesis and tumor growth [25]. Duan *et al.* suggested that miR-199a-3p was underexpressed in human osteosarcoma cell lines and tissue specimens. Overexpression of miR-199a-3p repressed the migration and proliferation of KHOS and U-2OS cell lines [26]. Dai *et al.* documented that forced expression of miR-199a strongly inhibited the proliferation, migration, invasion and tube formation ability of EA hy926 cells under hypoxia through suppression of the HIF-1α/VEGF pathway [27]. In our study, miR-199a-3p and miR-199b-3p were dramatically elevated in ADSCs and ADSCs-Exos, indicating that exosomal miR-199-3p may implicated in the stimulatory effect of ADSCs-Exos on the angiogenesis of endothelial tip cells. Further study revealed that exosomes from miR-199-3p-modified ADSCs contributed to proliferation and migration of endothelial tip cells.

Sema3A belonging to the semaphorin family, acts as an axon guidance molecule that can regulate axonal re-growth and plays a significant role in a great deal of physiological and pathogenic processes, including immune response, angiogenesis, and neural development [28, 29]. As an example, deletion of Sema3A promoted tumor growth and angiogenesis in breast cancer by downregulating p-phosphatase and tensin homolog and MelCAM, and upregulating p-forkhead box O3a and VEGF [30]. Endothelial cell-derived Sema3A has been postulated to suppress the formation of tip cell filopodia induced by VEGF-A [31]. Inhibition of the Sema3A/PlexinD1 signaling pathway promoted the migration of endothelial cells and vessel sprouting formation [32]. Here, we identified that Sema3A was a target of miR-199a/b-3p. The promotory effect of $Exos^{199\ mimic}$ on the proliferation and migration of endothelial tip cells was abrogated by Sema3A overexpression. Also, the expression of TIMP3 was decreased, and the expression of MMP9 and PCNA were increased in endothelial tip cells co-cultured with ADSCs-Exos, which were substantially enhanced by $Exos^{199\ mimic}$ treatment. However, the regulator effects of $Exos^{199\ mimic}$ on the protein expression of TIMP3, MMP9 and PCNA were conversed by upregulation of Sema3A.

In summary, the results of our study revealed that ADSC-Exos promoted the proliferation and migration of endothelial tip cells through miR-199a/b-3p/Sema3A axis. Our findings uncovered that ADSCs-Exos as a pro-angiogenic factor and possibly a new targeted therapy for PAD. However, this conclusion needs to be further verified by *in vivo* studies.

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Disclosure of conflict of interest

None.

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