MicroRNA-203a-3p suppresses endothelial cell proliferation and invasion, and promotes apoptosis in hemangioma by inactivating the VEGF-mediated PI3K/AKT pathway

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Abstract. Our previous study demonstrated that microRNA-203a-3p (miR-203a-3p) was involved in the regulation of long non-coding RNA MEG8-mediated the progression of hemangioma, which is a benign tumor characterized by endothelial hyperplasia in the blood vessels and primarily occurring in infants and females. Therefore, the present study aimed to further investigate the effects of miR-203a-3p on endothelial cell proliferation, invasion and apoptosis, as well as its underlying mechanism in hemangioma. Human hemangioma endothelial cells (HemECs) were first transfected with either miR-203a-3p mimics or a miR-203a-3p inhibitor. Subsequently, vascular endothelial growth factor A (VEGFA) was overexpressed in these cells. Cell proliferation (by Cell Counting Kit-8 assay), apoptosis (by TUNEL assay), invasion (by Transwell assay) and PI3K/AKT signaling (by western blot) were assessed following transfection of these cells. Notably, transfection with miR-203a-3p mimics caused a reduction in cell proliferation, invasion and in the phosphorylation levels of PI3K and AKT, and promoted cell apoptosis in HemECs. By contrast, transfection with the miR-203a-3p inhibitor exerted the opposite effects compared with those of the miR-203a-3p mimics. miR-203a-3p was revealed to directly suppress VEGFA expression in HemECs. VEGFA overexpression alone increased cell proliferation and invasion, but decreased apoptosis. Furthermore, VEGFA co-transfection reversed the effects mediated by miR-203a-3p mimics transfection in HemECs. Mechanistically, miR-203a-3p was demonstrated to inactivate the PI3K/AKT pathway, whereas VEGFA overexpression produced the opposite effect. VEGFA co-transfection also attenuated the miR-203a-3p mimics-induced inactivation of PI3K/AKT signaling in HemECs. In conclusion, these data suggested that miR-203a-3p may inhibit endothelial cell proliferation and invasion, and promote apoptosis by inactivating VEGFA and PI3K/AKT signaling in hemangioma. These findings also implicated miR-203 as a possible treatment option for this disease.

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Abbreviations: miR, microRNA; RGS17, regulator of G-protein signaling 17; RUNX2, runt-related transcription factor 2; HemECs, human hemangioma endothelial cells; NC, negative control; VEGFA, vascular endothelial growth factor A; RT-qPCR, reverse transcription-quantitative PCR; WT, wild type; MT, mutant type

Key words: miR-203a-3p, hemangioma, cellular function, VEGFA, PI3K/AKT pathway

Introduction

Hemangioma is a benign tumor that is characterized by endothelial hyperplasia in the blood vessels, which primarily occurs in infants and female patients (1,2). Patients with hemangioma may suffer from disease-related syndromes such as PHACE and LUMBAR syndromes, or exhibit skin lesions during the disease growth phase (2,3). To reduce the risk of complications experienced by patients with hemangioma, early identification by physical examination, ultrasound or MRI allows for immediate intervention with appropriate measures (3,4). Propranolol treatment, laser therapy and surgical resection are among the potential treatment options available for patients with hemangioma. However, certain patients with hemangioma do not respond well to these treatment strategies (2,5,6). Therefore, to optimize the management protocols of patients with hemangioma, it is necessary to explore the pathogenic mechanism of this disease to identify novel treatment targets.

MicroRNAs (miRNAs/miRs) are a class of endogenous, non-coding RNAs ~22 nucleotides in length, which mainly mediate the post-transcriptional regulation of mRNA (7). Among miRNAs, miR-203 has been shown to serve a key role in cell proliferation, migration and/or invasion in several malignancies (8-10). By decreasing the expression of regulator

of G-protein signaling 17 (RGS17), miR-203 has been shown to suppress cell proliferation and invasion in non-small cell lung cancer (8). In addition, miR-203 has been reported to inhibit the proliferation and invasion of osteosarcoma cells, but promote their apoptosis, by targeting runt-related transcription factor 2 (RUNX2) expression (9). miR-203 has also been reported to inhibit epithelial-mesenchymal transition and invasion of renal cell carcinoma cells by inactivating the PI3K/AKT signaling pathway (10). However, whether miR-203a-3p exhibits similar effects on hemangioma remains unclear and the molecular mechanism requires further investigation.

Vascular epithelial growth factor A (VEGFA) is a critical regulator of a number of cellular processes, including proliferation, apoptosis and invasion (11-13). Notably, it has been demonstrated that VEGFA can serve a role in the pathogenesis of hemangioma in several ways, including through the promotion of angiogenesis (14). In addition, GG genotype of +405 G/C VEGFA polymorphism is associated with a lower risk for infantile hemangioma (15). The PI3K/AKT pathway has been shown to regulate various biological processes, including cell survival, mobility and malignant transformation (16,17). In particular, this signaling pathway has been reported to be associated with the pathogenesis and progression of hemangioma (18).

Our previous study revealed that miR-203a-3p was involved in the regulation of hemangioma progression mediated by long non-coding RNA MEG8 (19). Therefore, the present study aimed to explore the effects of miR-203a-3p on endothelial cell proliferation, apoptosis and invasion in hemangioma, with specific focus on its downstream targets and associated signaling pathways.

Materials and methods

Cell lines. Human primary hemangioma endothelial cells (HemECs) and the 293T cell line were purchased from the BeNa Culture Collection. HemECs were maintained in complete endothelial cell medium (Gibco; Thermo Fisher Scientific, Inc.), whereas the 293T cells were maintained in DMEM (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.). All cells were cultured in a humidified incubator with 5% CO_2 at 37˚C. Ethics approval for the use of HemECs was waived by the Ethics Committee of the Affiliated Hospital of Hebei University of Engineering (Handan, China).

Cell transfection. miR-203a-3p mimics, the miR-203a-3p inhibitor and their corresponding negative controls (NCs) were purchased from Changchun Changsheng Gene Biotechnology Co., Ltd. The VEGFA overexpression plasmid (pEX2-VEGFA) and the empty NC plasmid (pEX2-NC) were purchased from Shenzhen Morecell Biomedical Co., Ltd. HemECs ($1x10^6$ cells) were transfected with 50 nM miR-203a-3p mimics, 50 nM miR-203a-3p inhibitors, 50 nM NC mimics, 50 nM NC inhibitors, 0.8 μg pEX2-VEGF or 0.8 μg pEX2-NC by Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol at 37˚C for 6 h. The sequences for miR-203a-3p or NC mimics and inhibitors used were as follows: miR-203a-3p mimics, 5'-GUGAAAUGU UUAGGACCACUAG-3'; miR-203a-3p inhibitors, 5'-CUA GUGGUCCUAAACAUUUCAC-3'; NC mimics, 5'-UUGUAC UACACAAAAGUACUG-3' and NC inhibitors, 5'-CAGUAC UUUUGUGUAGUACAA-3'.

miR-203a-3p manipulation. The cultured HemECs were divided into the following five groups: i) Control group (without any treatment); ii) NC mimic group (transfected with NC mimics); iii) miR mimic group (transfected with miR-203a-3p mimics); iv) NC inhibitor group (transfected with the NC inhibitor); and v) miR inhibitor group (transfected with the miR-203a-3p inhibitor). Reverse transcription-quantitative PCR (RT-qPCR), cell proliferation, apoptosis, invasion and western blotting assays were performed following transfection.

Luciferase reporter assay. The binding sites between miR-203a-3p and VEGF were predicted using RNAhybrid v22 (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid). The 3'untranslated regions of VEGFA containing miR-203a-3p-binding wild type (WT) and mutant type (MT) sites were inserted into the pGLuc vector, which was synthesized by Beyotime Institute of Biotechnology. The 0.8 μg VEGFA-WT or 0.8 μg VEGFA-MT plasmids were then co-transfected with the 50 nM miR-203a-3p mimics or 50 nM NC mimics into $1x10^6$ 293T cells using Lipofectamine 2000. Following cell culture for 48 h, the cells were harvested and assessed using a luciferase assay kit (Beyotime Institute of Biotechnology) according to the kit's protocol. The luciferase enzyme activity was normalized to *Renilla* luciferase enzyme activity.

VEGFA regulation experiment. HemECs were cultured and divided into the following five groups: i) Control group (without any treatment), ii) NC mimic + pEX2-NC group (co-transfected with NC mimics and the pEX2-NC plasmid), iii) miR mimic + pEX2-NC group (co-transfected with miR-203a-3p mimics and the pEX2-NC plasmid), iv) NC mimic + pEX2-VEGFA group (co-transfected with NC mimics and the pEX2-VEGFA plasmid) and v) miR mimic + pEX2-VEGFA group (co-transfected with miR-203a-3p mimics and the pEX2-VEGFA plasmid). Further experiments, namely RT-qPCR, western blotting, cell proliferation, apoptosis and invasion assays, were performed following transfection.

RT-qPCR. Total RNA was extracted from the HemECs using a RNeasy Mini kit (Qiagen China Co. Ltd.) 48 h after transfection. The RNA was reverse transcribed into cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the kit's protocol. qPCR was performed using a SYBR®-Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.). The qPCR was carried out with following procedure: 95˚C hold for 10 min; 40 cycles of denaturation at 95˚C for 15 sec and annealing/extension at 61˚C for 30 sec. The expression levels of miR-203a-3p and VEGFA were evaluated using the $2^{-\Delta\Delta Cq}$ method (20) with U6 or β-actin used as endogenous controls, respectively. The primer sequences are listed in Table SI.

Cell proliferation assay. Cell Counting Kit-8 (CCK-8; MilliporeSigma) was utilized to assess the proliferation of HemECs. Briefly, HemECs were plated in 96-well plates

($2x10^3$ cells/well). At 0, 24, 48 and 72 h following transfection, 10 μl CCK-8 reagent was added before the mixture was incubated for an additional 2 h at 37˚C. The optical density values at 450 nm were subsequently evaluated using an automated enzyme immunoassay analyzer (BioTek China).

Cell apoptosis assay. A TUNEL apoptosis kit (Beyotime Institute of Biotechnology) was used for detecting the apoptosis of HemECs following transfection. Briefly, transfected HemECs were plated into 24-well plates ($1x10^4$ cells/well) and cultured for 48 h. The HemECs were then fixed with 4% paraformaldehyde (Beyotime Institute of Biotechnology) for 30 min at 37˚C and incubated with 0.1% Triton X-100 (Beyotime Institute of Biotechnology) for 5 min at 4˚C. Subsequently, 50 μl TUNEL solution was added and the cells were incubated for 1 h at 37˚C, followed by DAPI (Sangon Biotech Co., Ltd.) staining for 15 min at room temperature. After adding Antifade Mounting Medium (Beyotime Institute of Biotechnology), the cells were sealed and observed under an inverted fluorescence microscope (Motic China Group Co., Ltd.) with 3 random fields being chosen.

Cell invasion assay. The invasive ability of HemECs following transfection was assessed using Transwell chambers. Briefly, 48 h following transfection, HemECs, which were suspended in 1% FBS containing complete endothelial cell medium, were seeded into the upper Matrigel-coated chambers (Corning, Inc.) at a density of $5x10^4$ cells/well, whilst complete endothelial cell medium containing 10% FBS was added into the lower chamber. The upper chamber was coated by 70 μl Matrigel, which was diluted with complete endothelial cell medium at a ratio of 1:8, at 37˚C for 1 h. The cells were cultured for 24 h before their invasive ability was assessed by staining with 0.5% crystal violet (MilliporeSigma) for 15 min at room temperature after being fixed with 4% paraformaldehyde (MilliporeSigma). The images were taken under an inverted light microscope (Motic China Group Co., Ltd.).

Western blot analysis. Total proteins from HemECs at 48 h following transfection were lysed using RIPA buffer (MilliporeSigma) and quantified using bicinchoninic acid assay (Thermo Fisher Scientific, Inc.). Of the extracted proteins, 20 μg per lane were separated by 4-20% SDS-PAGE and transferred onto nitrocellulose membranes (Pall Life Sciences). After blocking with 5% BSA (MilliporeSigma) at 37˚C for 1 h, the membranes were incubated at 4˚C overnight with primary antibodies (all obtained from Invitrogen; Thermo Fisher Scientific, Inc.) against Bcl-2 (1:1,000 dilution; cat. no. PA5-11379), cleaved (C)-caspase 3 (1:1,000; cat. no. PA5-96077), VEGFA (1:1,000; cat. no. PA5-16754), phosphorylated (p)-PI3K (1:1,000; cat. no. PA5-118549), PI3K (1:1,000; cat. no. PA5-99518), p-AKT (1:1,000; cat. no. MA5-38243), AKT (1:1,000; cat. no. 44-609G) and β-actin (1:2,000; cat. no. PA5-85291). Subsequently, the membranes were incubated with a HRP-conjugated goat anti-rabbit secondary antibody (1:10,000; cat. no. 65-6120; Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 1 h. The bands were visualized using the ECL-PLUS reagent (Thermo Fisher Scientific, Inc.). Densitometric quantification was completed by ImageJ 1.8 (National Institutes of Health).

Statistical analysis. GraphPad Prism 7.0 (GraphPad Software, Inc.) was used to analyze the data, which were triplicates and exhibited as mean ± standard deviation. One-way ANOVA followed by Tukey's post-hoc test was used for comparisons among multiple groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effects of miR-203a-3p on the proliferation, apoptosis and invasion of HemECs. The expression levels of miR-203a-3p were significantly increased following transfection of the cells with miR-203a-3p mimics compared with those in the NC mimics group ($P<0.001$; Fig. 1A). By contrast, miR-203a-3p expression was significantly decreased following the transfection of HemECs with the miR-203a-3p inhibitor compared with that in the NC inhibitor group ($P<0.05$; Fig. 1A). These observations suggested that transfection was successful. In addition, compared with those in their corresponding NC groups, miR-203a-3p mimics and miR-203a-3p inhibitor transfection induced significant decreases and increases in cell proliferation at 48 and 72 h, respectively (all $P<0.05$; Fig. 1B). Subsequently, it was revealed that apoptosis was significantly increased by transfection with the miR-203a-3p mimics ($P<0.001$) but significantly alleviated by the miR-203a-3p inhibitor ($P<0.05$; Fig. 1C and D), compared with that in their corresponding NC groups. Furthermore, the expression levels of apoptotic markers Bcl-2 and C-caspase 3 were measured; miR-203a-3p mimics transfection significantly suppressed Bcl-2 expression whilst increasing that of C-caspase 3, whereas transfection with the miR-203a-3p inhibitor resulted in the opposite effects compared with miR-203a mimics (all $P<0.05$; Fig. 1E-G). The number of invasive cells was significantly decreased in cells transfected with miR-203a-3p mimics ($P<0.01$) but significantly elevated in cells transfected with the miR-203a-3p inhibitor ($P<0.05$; Fig. 2A and B), compared with that in their corresponding NC groups.

miR-203a-3p regulates VEGFA expression and the PI3K/AKT signaling pathway in HemECs. A binding site on the 3'untranslated region of VEGFA mRNA for miR-203a-3p was predicted (Fig. 3A). Therefore, VEGFA-MT and VEGFA-WT sequences were designed based on this binding site. The relative luciferase activity was significantly decreased ($P<0.001$) in cells co-transfected with VEGFA-WT and miR-203a-3p mimics compared with that in cells transfected with the NC mimics, suggesting direct binding between VEGFA mRNA and miR-203a-3p; however, the relative luciferase activity did not differ between cells co-transfected with VEGFA-MT and miR-203a-3p mimics compared with that in cells transfected with the NC mimics (Fig. 3B). In addition, miR-203a-3p mimics transfection significantly decreased the expression levels of VEGFA, whereas transfection with the miR-203a-3p inhibitor exerted opposite effects (all $P<0.05$; Fig. 3C-E), compared with those in their corresponding NC groups. These results suggested that miR-203a-3p may negatively regulate VEGFA expression. In addition, compared with those in their corresponding NC groups, miR-203a-3p

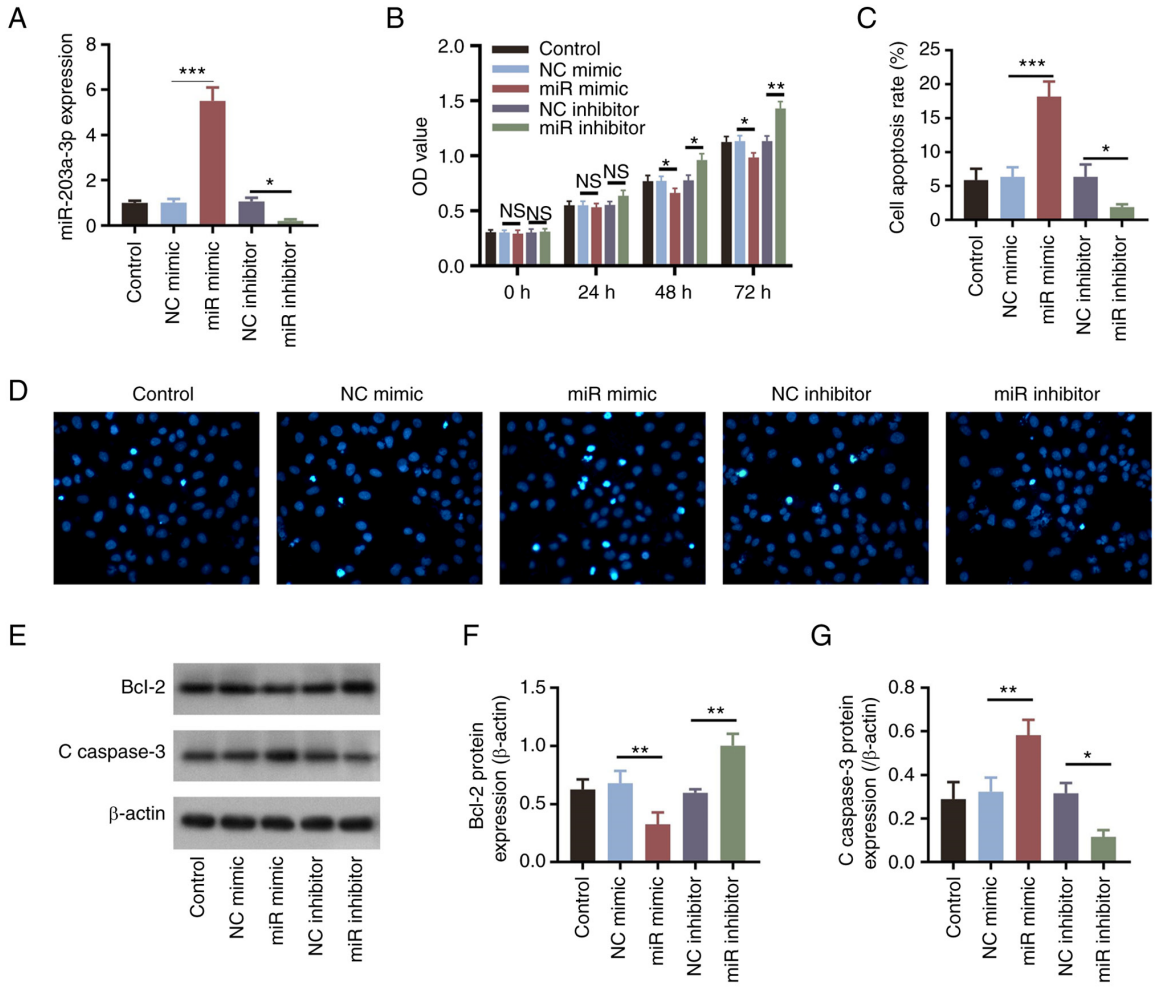

Figure 1. miR-203a-3p suppresses the proliferation and promotes apoptosis of HemECs. (A) miR-203a-3p expression, (B) cell proliferation and (C) cell apoptosis rate were measured in HemECs following transfection. (D) Representative images of TUNEL staining in C (magnification, x200). (E) Representative images of Bcl-2 and C-caspase 3 expression by western blot analysis. Relative expression levels of (F) Bcl-2 and (G) C-caspase 3 were semi-quantified. *P<0.05, **P<0.01 and ***P<0.001. miR-203a-3p, microRNA-203a-3p; HemECs, human hemangioma endothelial cells; NS, non-significant; C-, cleaved; NC, negative control; OD, optical density.

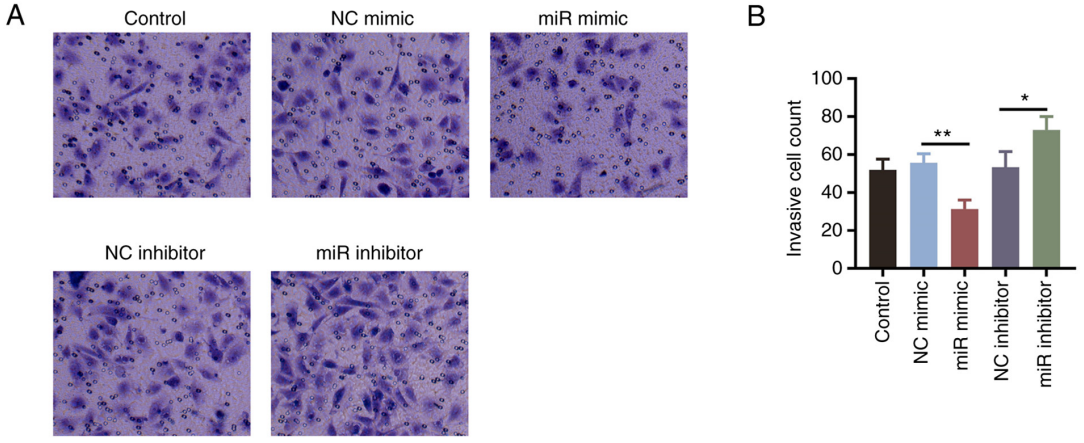

Figure 2. miR-203a-3p decreases the number of invasive HemECs. (A) Representative images of invasive cells by crystal violet staining (magnification, x200). (B) Invasive cell count in HemECs following transfection. *P<0.05 and **P<0.01. miR-203a-3p, microRNA-203a-3p; HemECs, human hemangioma endothelial cells; NC, negative control.

overexpression significantly decreased the phosphorylation levels of PI3K and AKT, whereas miR-203a-3p knockdown resulted in the opposite effects (all P<0.05; Fig. 3F and G).

miR-203a-3p regulates proliferation, apoptosis and invasion by inhibiting VEGFA in HemECs. Transfection of HemECs with the VEGFA overexpression plasmids significantly

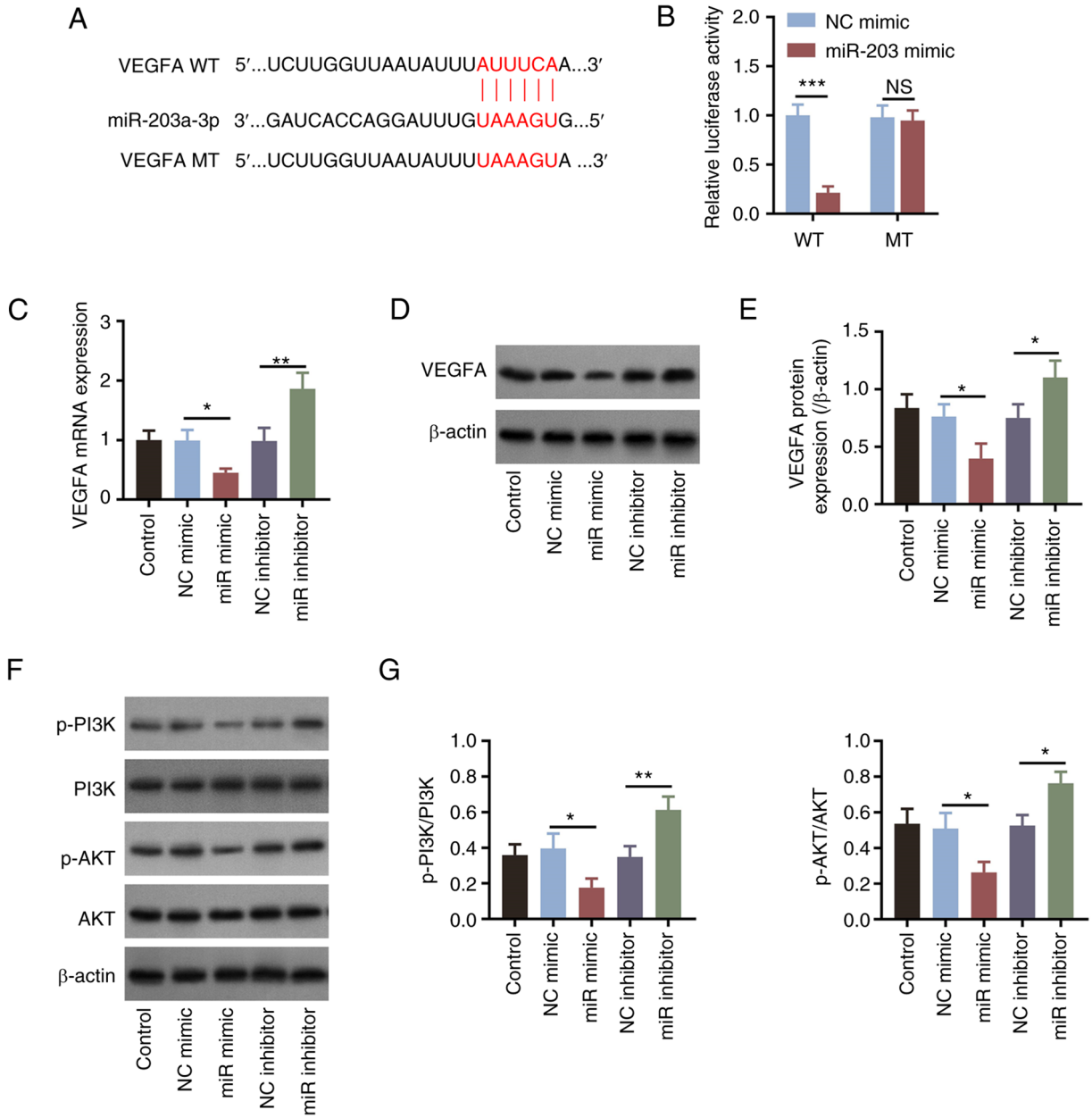

Figure 3. miR-203a-3p downregulates VEGFA expression and PI3K/AKT signaling in HemECs. (A) Design of the WT and MT sequences of VEGFA for luciferase reporter gene assays. (B) Comparison of relative luciferase activity among the WT and MT groups after miR-203 mimics co-transfection. (C) Comparison of VEGFA mRNA expression levels among the groups after the manipulation of miR-203 expression. (D) Detection of the VEGFA protein expression by western blotting after the manipulation of miR-203 expression, (E) which was semi-quantified. (F) Detection of PI3K and AKT phosphorylation by western blot analysis. (G) Semi-quantification of the p-PI3K/PI3K and p-AKT/AKT ratios among the groups in HemECs following the manipulation of miR-203 expression. *P<0.05, **P<0.01 and ***P<0.001. miR-203a-3p, microRNA-203a-3p; VEGF, vascular endothelial growth factor; WT, wild type; MT, mutant type; p-, phosphorylated; HemECs, human hemangioma endothelial cells; NS, non-significant; NC, negative control.

increased the expression levels of VEGFA (all P<0.001; Fig. S1A-C) and attenuated miR-203a-3p mimics-induced reduction of VEGFA (all P<0.001; Fig. 4A-C), suggesting successful transfection. Subsequently, overexpression of VEGFA significantly increased cell proliferation (all P<0.05; Fig. S1D) and also significantly reversed the miR-203a-3p mimics-induced decreases in cell proliferation after 48 and 72 h in HemECs (all P<0.01; Fig. 4D). By contrast, VEGFA overexpression significantly inhibited cell apoptosis (P<0.01; Fig. S1E and F), in addition to significantly reversing the miR-203a-3p mimics-induced increases in HemEC apoptosis (P<0.001; Fig. 4E and F). The number of invasive cells was found to be significantly increased by VEGFA overexpression (P<0.01; Fig. S1G and H), which also significantly reversed the miR-203a-3p mimics-induced reductions in invasive cell numbers (P<0.01; Fig. 5A and B).

miR-203a-3p inhibits the PI3K/AKT signaling pathway by targeting VEGFA in HemECs. To further explore if miR-203a-3p could regulate the PI3K/AKT signaling pathway through VEGFA, additional experiments were performed. Overexpression of VEGFA significantly increased the phosphorylation levels of PI3K (P<0.01) and AKT (P<0.05; Fig. S1I-K). In addition, overexpression of VEGFA

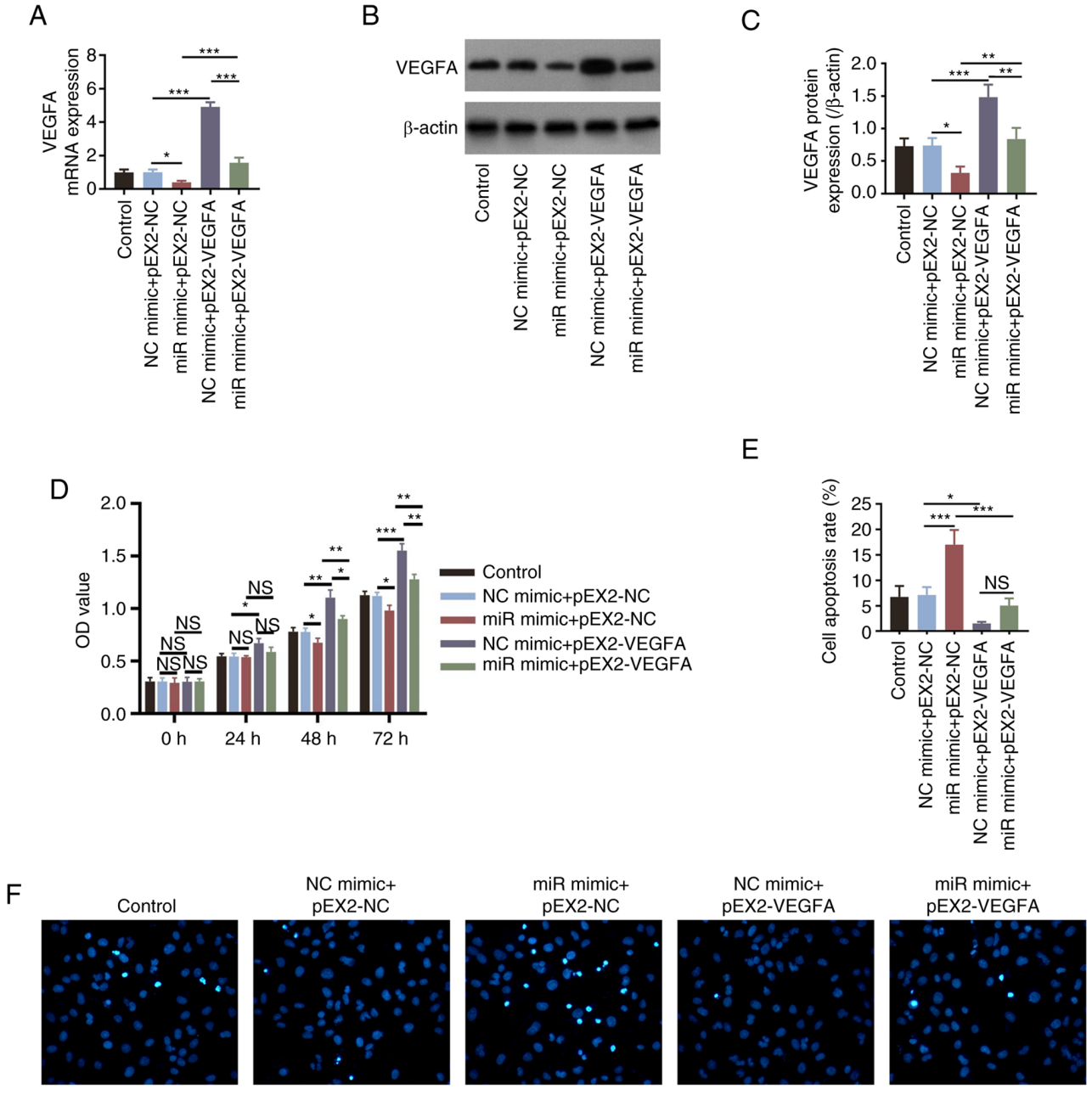

Figure 4. VEGFA is implicated in miR-203a-3p-mediated HemEC proliferation and apoptosis. (A) Comparison of VEGFA mRNA expression levels among groups after miR-203-3p and VEGFA co-transfection. (B) Measurement of VEGFA protein expression by western blotting. (C) Semi-quantification of VEGFA protein expression among groups after miR-203-3p and VEGFA co-transfection. Comparison of (D) cell proliferation and (E) cell apoptosis rates after miR-203-3p and VEGFA co-transfection. (F) Representative TUNEL staining images of HemECs in E (magnification, x200). *P<0.05, **P<0.01 and ***P<0.001. miR-203a-3p, microRNA-203a-3p; VEGF, vascular endothelial growth factor; HemECs, human hemangioma endothelial cells; NS, non-significant; NC, negative control; OD, optical density.

significantly reversed the miR-203a-3p mimics-induced decreases in PI3K and AKT phosphorylation in HemECs (all P<0.01; Fig. 6A and B).

Discussion

A number of miRNAs have been reported to be involved in the progression of hemangioma. In particular, miR-200c-3p has been reported to promote the proliferation of vascular endothelial cells by targeting the Notch signaling pathway, which serves a key role in the pathogenesis of hemangioma (21). In another previous study, downregulation of miR-556-3p expression was reported to suppress hemangioma cell proliferation whilst accelerating apoptosis by targeting VEGFC expression (22). Furthermore, miR-139-5p may negatively modulate cell proliferation and migration in hemangioma stem cells by targeting insulin-like growth factor 1 (23). Other studies have also reported that some miRNAs can regulate the progression of hemangioma, including miR-382-5p and miR-125b-5p (24,25). Although these previous studies have revealed miRNAs can regulate the proliferation and apoptosis of hemangioma cells, it remains necessary to explore alternative biological pathways that are involved in the biological functions of hemangioma cells. This information could then

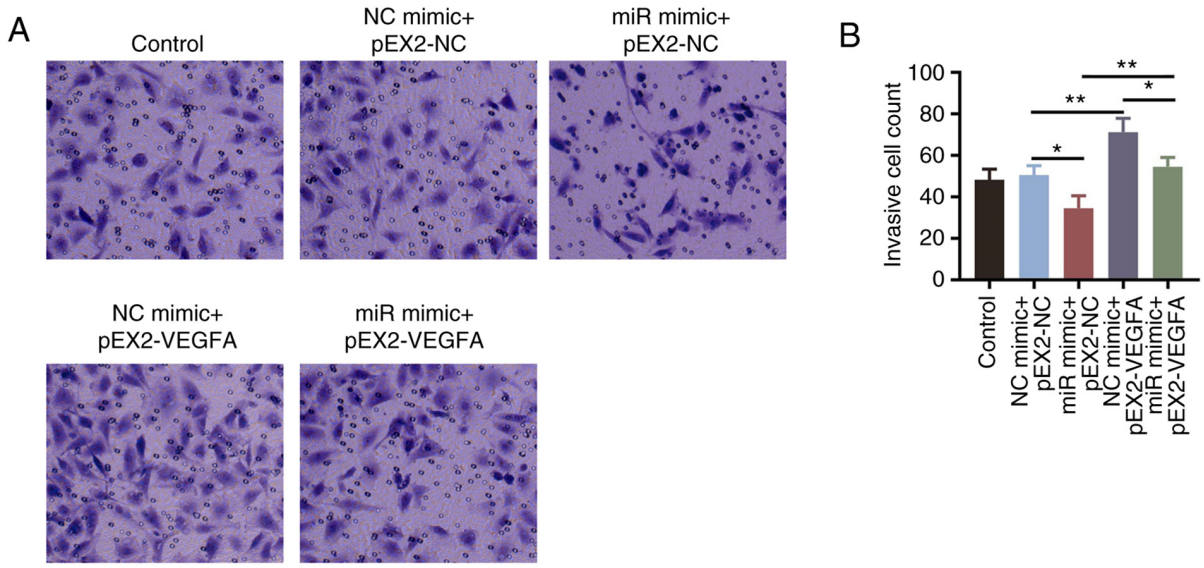

Figure 5. VEGFA is involved in miR-203a-3p-modulated HemEC invasion. (A) Representative images of invasive cells by crystal violet staining (magnification, x200). (B) Invasive cell count among groups following miR-203-3p and VEGFA co-transfection. *P<0.05 and **P<0.01. VEGF, vascular endothelial growth factor; miR-203a-3p, microRNA-203a-3p; HemECs, human hemangioma endothelial cells; NC, negative control.

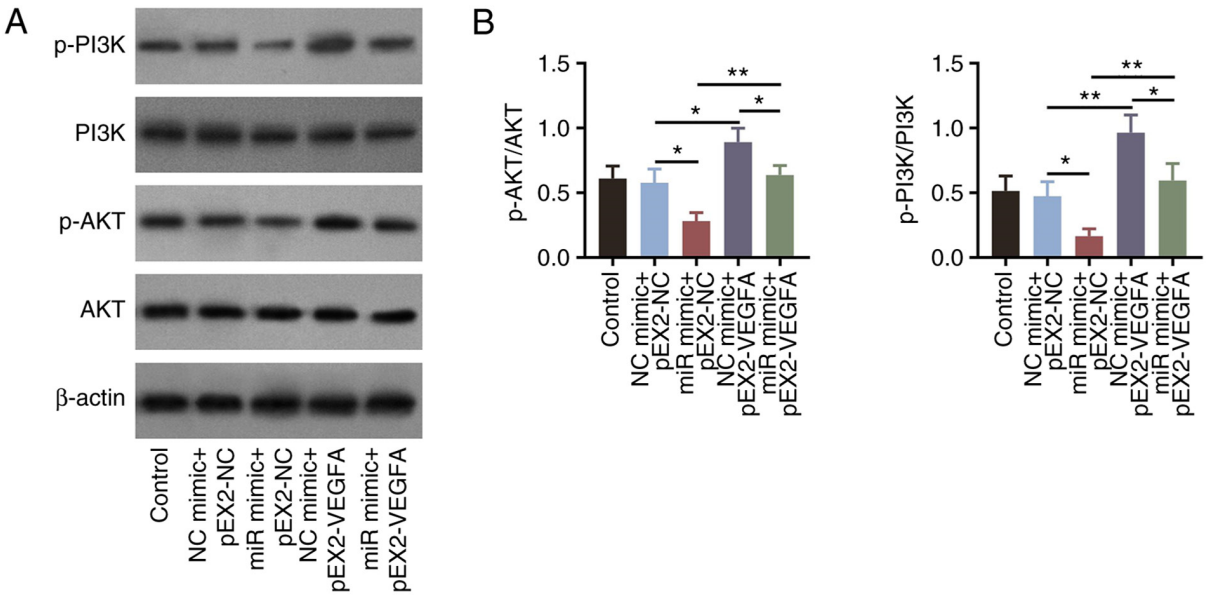

Figure 6. VEGFA is implicated in the miR-203a-3p-mediated PI3K/AKT signaling inhibition in HemECs. (A) Detection of PI3K and AKT phosphorylation by western blot analysis after miR-203-3p and VEGFA co-transfection. (B) Semi-quantification of p-PI3K/PI3K and p-AKT/AKT ratios among the different groups after miR-203-3p and VEGFA co-transfection. *P<0.05 and **P<0.01. VEGF, vascular endothelial growth factor; miR-203a-3p, microRNA-203a-3p; p-, phosphorylated; HemECs, human hemangioma endothelial cells.

be applied for designing potential treatment strategies for hemangioma. Our preliminary study revealed that miR-203 expression can be regulated by the lncRNA MEG8 and may participate in the modulation of hemangioma progression via inhibiting the Notch signaling pathway (19). However, the molecular mechanism by which miR-203a-3p can participate in modulation of hemangioma progression remains unclear. As a miRNA that has been extensively studied, miR-203 functions as a tumor suppressor in a variety of malignancies (26). Overexpression of miR-203 has been found to inhibit cell invasion and proliferation in hepatocellular carcinoma cells (27), whereas the expression levels of miR-203 have been revealed to be downregulated during lung cancer tumorigenesis (8). In lung cancer cells, miR-203 can suppress the invasion and proliferation of the A549 and Calu-1 cell lines by targeting RGS17 expression (8). In estrogen-dependent breast cancer cells, miR-203 has been reported to inhibit their viability and migration by suppressing the protein expression of estrogen receptor α (28). In addition, miR-203 knockdown has been revealed to inhibit cisplatin-induced cell apoptosis in osteosarcoma cells *in vitro* by targeting RUNX2 expression (29). Therefore, it is reasonable to hypothesize that miR-203 may participate in the regulation of cell viability, apoptosis and migration in hemangioma. In the present study, overexpression

of miR-203a-3p decreased the proliferation and invasion of HemECs, whilst increasing apoptosis of these cells. By contrast, knocking down miR-203a-3p expression resulted in the opposite effects. Possible reasons for these findings could be that miR-203a-3p regulated activation of the VEGFA-mediated PI3K/AKT pathway upstream of cell proliferation, apoptosis and invasion, or that miR-203a-3p could have regulated cell invasion and proliferation by targeting RGS17 expression (8).

The VEGF family consists of cytokines known to be angiogenic factors that participate in blood vessel formation and lymphangiogenesis (30). VEGFA is the main member of this family of proteins, which has been revealed to regulate cell proliferation, apoptosis and invasion (11-13,31,32). In particular, VEGFC has also been reported to impact the progression of hemangioma (22). A previous study suggested that VEGFA serves a key role in the pathogenesis of hemangioma, specifically through the proliferation and invasion physiological pathways (24). In addition, the miR-206/VEGFA axis has been documented to serve an important role in the suppression of HemEC proliferation after the expression of metastasis-associated lung adenocarcinoma transcript 1 has been knocked down (33). It has also been shown that VEGFA is a master regulator of angiogenesis in hemangioma (14,34). In the present study, it was demonstrated that VEGFA could regulate hemangioma proliferation, apoptosis and invasion. According to previous studies, VEGFA is one of the targets of miR-203a-3p and has been shown to be a key regulatory mechanism during the pathogenesis of laryngeal carcinoma and progression of cervical cancer (35,36). In the present study, it was found that miR-203a-3p regulated the cellular functions of hemangioma through VEGFA. The reason could be attributed to VEGFA being a direct target of miR-203a-3p as previously reported (35).

AKT is a serine threonine kinase acting downstream of PI3K, which regulates a high number of critical cellular processes (37). Inhibition of the PI3K/AKT/mTOR signaling pathway has been shown to restore the autophagic process in epidermal keratinocyte cells from psoriatic mice (38). In addition, suppression of AKT/mTOR signaling has been observed to promote chemo-radiotherapy resistance in lung squamous cell carcinoma cells (39). In another study, AKT was found to serve a critical role during the life cycle of acute and persistent murine norovirus strains in macrophages (40). In addition to these cellular activities, it has been reported that the PI3K/AKT signaling pathway serves a key role in hemangioma. According to an earlier study, activation of the PI3K/AKT signaling pathway may affect tumorigenesis of lung hemangioma through the downstream mTOR (41). Furthermore, the PI3K/AKT pathway has been demonstrated to suppress hemangioma proliferation by downregulating the expression of proliferating cell nuclear antigen, which may further influence the progression of hemangioma (42). In the present study, it was found that the PI3K/AKT signaling pathway was regulated by miR-203a-3p in hemangioma, which was in line with the data previously reported in other tumors, such as renal cell carcinoma, papillary thyroid cancer and glioblastoma (10,43,44). In addition, it was confirmed that miR-203a-3p can directly suppress VEGFA expression and the PI3K/AKT pathway, which could suppress the proliferation of hemangioma, thereby uncovering the miR-203a-3pVEGFA/PI3K/AKT axis.

In conclusion, in the present study, miR-203a-3p overexpression was found to inhibit endothelial cell proliferation, suppress invasion and promote apoptosis by inactivating the VEGFA-mediated PI3K/AKT pathway in hemangioma. These findings may assist in identifying suitable targets for the management of hemangioma.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZH and LZ designed the present study. Material preparation and data collection were performed by YL and DD. ZH, LZ and JG analyzed the data. All authors contributed to the interpretation of data, methodology and reviewing and editing of the manuscript. ZH and LZ confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

Ethics approval for the use of HemECs was waived for the present study by the Ethics Committee of the Affiliated Hospital of Hebei University of Engineering (Handan, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Jung HL: Update on infantile hemangioma. Clin Exp Pediatr 64: 559-572, 2021.
2. Sebaratnam DF, Rodriguez Bandera AL, Wong LF and Wargon O: Infantile hemangioma. Part 2: Management. J Am Acad Dermatol 85: 1395-1404, 2021.
3. Valdebran M and Wine Lee L: Hemangioma-related syndromes. Curr Opin Pediatr 32: 498-505, 2020.
4. DeHart A and Richter G: Hemangioma: Recent advances. F1000Res 8: F1000, 2019.
5. Ji Y, Chen S, Wang Q, Xiang B, Xu Z, Zhong L, Yang K, Lu G and Qiu L: Intolerable side effects during propranolol therapy for infantile hemangioma: frequency, risk factors and management. Sci Rep 8: 4264, 2018.

6. Kowalska M, Debek W and Matuszczak E: Infantile hemangiomas: An update on pathogenesis and treatment. J Clin Med 10: 4631, 2021.
7. Rolle K, Piwecka M, Belter A, Wawrzyniak D, Jeleniewicz J, Barciszewska MZ and Barciszewski J: The sequence and structure determine the function of mature human miRNAs. PLoS One 11: e0151246, 2016.
8. Chi Y, Jin Q, Liu X, Xu L, He X, Shen Y, Zhou Q, Zhang J and Jin M: miR-203 inhibits cell proliferation, invasion, and migration of non-small-cell lung cancer by downregulating RGS17. Cancer Sci 108: 2366-2372, 2017.
9. Lin W, Zhu X, Yang S, Chen X, Wang L, Huang Z, Ding Y, Huang L and Lv C: MicroRNA-203 inhibits proliferation and invasion, and promotes apoptosis of osteosarcoma cells by targeting Runt-related transcription factor 2. Biomed Pharmacother 91: 1075-1084, 2017.
10. Han N, Li H and Wang H: MicroRNA-203 inhibits epithelial-mesenchymal transition, migration, and invasion of renal cell carcinoma cells via the inactivation of the PI3K/AKT signaling pathway by inhibiting CAV1. Cell Adh Migr 14: 227-241, 2020.
11. Ma M, Zhang J, Gao X, Yao W, Li Q and Pan Z: miR-361-5p Mediates SMAD4 to promote porcine granulosa cell apoptosis through VEGFA. Biomolecules 10: 1281, 2020.
12. Zhang T, Qian Y, Yuan C, Wu Y, Qian H, Lu H, Hu C and Li W: Propranolol suppresses proliferation and migration of HUVECs through regulation of the miR-206/VEGFA axis. Biomed Res Int 2021: 7629176, 2021.
13. Geng A, Luo L, Ren F, Zhang L, Zhou H and Gao X: miR-29a-3p inhibits endometrial cancer cell proliferation, migration and invasion by targeting VEGFA/CD C42/PAK1. BMC Cancer 21: 843, 2021.
14. Makkeyah SM, Elseedawy ME, Abdel-Kader HM, Mokhtar GM and Ragab IA: Vascular endothelial growth factor response with propranolol therapy in patients with infantile hemangioma. Pediatr Hematol Oncol 39: 215-224, 2022.
15. Oszajca K, Szemraj J, Wyrzykowski D, Chrzanowska B, Salamon A and Przewratil P: Single-nucleotide polymorphisms of VEGF-A and VEGFR-2 genes and risk of infantile hemangioma. Int J Dermatol 57: 1201-1207, 2018.
16. Sanaei MJ, Razi S, Pourbagheri-Sigaroodi A and Bashash D: The PI3K/Akt/mTOR pathway in lung cancer; oncogenic alterations, therapeutic opportunities, challenges, and a glance at the application of nanoparticles. Transl Oncol 18: 101364, 2022.
17. Barzegar Behrooz A, Talaie Z, Jusheghani F, Los MJ, Klonisch T and Ghavami S: Wnt and PI3K/Akt/mTOR survival pathways as therapeutic targets in glioblastoma. Int J Mol Sci 23: 1353, 2022.
18. Ji Y, Chen S, Li K, Li L, Xu C and Xiang B: Signaling pathways in the development of infantile hemangioma. J Hematol Oncol 7: 13, 2014.
19. Hu Z, Liu X, Guo J, Zhuo L, Chen Y and Yuan H: Knockdown of lncRNA MEG8 inhibits cell proliferation and invasion, but promotes cell apoptosis in hemangioma, via miR-203-induced mediation of the Notch signaling pathway. Mol Med Rep 24: 872, 2021.
20. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
21. Hu X, Bai S, Li L, Tian P, Wang S, Zhang N, Shen B, Du J and Liu S: MiR-200c-3p increased HDMEC proliferation through the notch signaling pathway. Exp Biol Med (Maywood) 246: 897-905, 2021.
22. Jin W, Chen L, Gao F, Yang M, Liu Y and Wang B: Down-regulation of miR-556-3p inhibits hemangioma cell proliferation and promotes apoptosis by targeting VEGFC. Cell Mol Biol (Noisy-le-grand) 66: 204-207, 2020.
23. Wu Y, Li H, Xie J, Wang F, Cao D and Lou Y: miR1395p affects cell proliferation, migration and adipogenesis by targeting insulinlike growth factor 1 receptor in hemangioma stem cells. Int J Mol Med 45: 569-577, 2020.
24. Yuan X, Xu Y, Wei Z and Ding Q: CircAP2A2 acts as a ceRNA to participate in infantile hemangiomas progression by sponging miR-382-5p via regulating the expression of VEGFA. J Clin Lab Anal 34: e23258, 2020.
25. Wang T, Zhang FL, Zhao Y, Guo DD and Yang R: Effects of miR-125b-5p on the proliferation and apoptosis of human hemangioma endothelial cells HemES and its mechanism. Zhongguo Ying Yong Sheng Li Xue Za Zhi 37: 247-253, 2021 (In Chinese).
26. Zong M, Feng W, Wan L, Yu X and Yu W: miR-203 affects esophageal cancer cell proliferation, apoptosis and invasion by targeting MAP3K1. Oncol Lett 20: 751-757, 2020.
27. Chen H, Kong M, Chen Y, Jiang Y, Wen M and Zhang X: Prognostic significance of miR-203 and ZEB1 expression in early-stage hepatocellular carcinoma. J Cancer 12: 4810-4818, 2021.
28. Lin J, Wang L, Gao J and Zhu S: MiR-203 inhibits estrogen-induced viability, migration and invasion of estrogen receptor alpha-positive breast cancer cells. Exp Ther Med 14: 2702-2708, 2017.
29. Huang Z, Huang L, Liu L, Wang L, Lin W, Zhu X, Su W and Lv C: Knockdown of microRNA-203 reduces cisplatin chemo-sensitivity to osteosarcoma cell lines MG63 and U2OS in vitro by targeting RUNX2. J Chemother 33: 328-341, 2021.
30. Shibuya M and Claesson-Welsh L: Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. Exp Cell Res 312: 549-560, 2006.
31. Iyer S and Acharya KR: Tying the knot: The cystine signature and molecular-recognition processes of the vascular endothelial growth factor family of angiogenic cytokines. FEBS J 278: 4304-4322, 2011.
32. Ye X, Gaucher JF, Vidal M and Broussy S: A structural overview of vascular endothelial growth factors pharmacological ligands: From macromolecules to designed peptidomimetics. Molecules 26: 6759, 2021.
33. Wang S, Ren L, Shen G, Liu M and Luo J: The knockdown of MALAT1 inhibits the proliferation, invasion and migration of hemangioma endothelial cells by regulating MiR-206/VEGFA axis. Mol Cell Probes 51: 101540, 2020.
34. Cai C, Böttcher MC, Werner JA and Mandic R: Differential expression of VEGF121, VEGF165 and VEGF189 in angiomas and squamous cell carcinoma cell lines of the head and neck. Anticancer Res 30: 805-810, 2010.
35. Xu L, Shen B, Chen T and Dong P: miR-203 is involved in the laryngeal carcinoma pathogenesis via targeting VEGFA and Cox-2. Onco Targets Ther 9: 4629-4637, 2016.
36. Zhu X, Er K, Mao C, Yan Q, Xu H, Zhang Y, Zhu J, Cui F, Zhao W and Shi H: miR-203 suppresses tumor growth and angiogenesis by targeting VEGFA in cervical cancer. Cell Physiol Biochem 32: 64-73, 2013.
37. Dimri M and Satyanarayana A: Molecular signaling pathways and therapeutic targets in hepatocellular carcinoma. Cancers (Basel) 12: 491, 2020.
38. Tang ZL, Zhang K, Lv SC, Xu GW, Zhang JF and Jia HY: LncRNA MEG3 suppresses PI3K/AKT/mTOR signalling pathway to enhance autophagy and inhibit inflammation in TNF-α-treated keratinocytes and psoriatic mice. Cytokine 148: 155657, 2021.
39. Shen C, Shyu DL, Xu M, Yang L, Webb A, Duan W and Williams TM: Deregulation of AKT-mTOR signaling contributes to chemoradiation resistance in lung squamous cell carcinoma. Mol Cancer Res 20: 425-433, 2022.
40. Owusu IA, Passalacqua KD, Mirabelli C, Lu J, Young VL, Hosmillo M, Quaye O, Goodfellow I, Ward VK and Wobus CE: Akt plays differential roles during the life cycles of acute and persistent murine norovirus strains in macrophages. J Virol 96: e0192321, 2022.
41. Amin RM, Hiroshima K, Miyagi Y, Kokubo T, Hoshi K, Fujisawa T and Nakatani Y: Role of the PI3K/Akt, mTOR, and STK11/LKB1 pathways in the tumorigenesis of sclerosing hemangioma of the lung. Pathol Int 58: 38-44, 2008.
42. Ou JM, Qui MK, Dai YX, Dong Q, Shen J, Dong P, Wang XF, Liu YB and Fei ZW: Combined blockade of AKT/mTOR pathway inhibits growth of human hemangioma via downregulation of proliferating cell nuclear antigen. Int J Immunopathol Pharmacol 25: 945-953, 2012.
43. Yang X, Li X, Lin Q and Xu Q: Up-regulation of microRNA-203 inhibits myocardial fibrosis and oxidative stress in mice with diabetic cardiomyopathy through the inhibition of PI3K/Akt signaling pathway via PIK3CA. Gene 715: 143995, 2019.
44. Czechowicz JA, Benjamin T, Bly RA, Ganti SN, Balkin DM, Perkins JA, Frieden IJ and Rosbe KW: Airway hemangiomas in PHACE syndrome: A multicenter experience. Otolaryngol Head Neck Surg 165: 182-186, 2021.