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Research

Inhibition of hemangioma development by regulating the VEGF/VEGFR autocrine loop via the miR-494/PTEN pathway

Jingyu Peng[1] · Feifei Li[2] · Mingke Qiu[1] · Xinjie Xu[1] · Guanghua Liu[1] · Jingmin Ou[1]

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Abstract

Background Infantile hemangioma (IH) is the most common type of benign vascular tumor found in infants and young children. Hemangioma-derived endothelial cells within the lesion from birth to three months of age are the primary characteristic of IH. (hemangioma-derived endothelial cells, HemECs) proliferated rapidly and formed hemangioma masses, most of which gradually regressed spontaneously within the next 1 to 5 years of age and continued to improve until the age of 6 to 12 years. But 10–15% of IH cases can still result in ulcerative, obstructive, deformity, and even potentially fatal consequences and sequelae. These conditions seriously affect children's physical and mental health as well as their growth and development, necessitating prompt and efficient medical attention of IH is known.

Objective The purpose of this work. HemECs are crucial to the development of IH as a result. Not all of the pathophwork is to examine the impact of OMT on HemECs, with a specific emphasis on its role in cell migration, proliferation, cell cycle regulation, and apoptosis. Additionally, we will research the influence of OMT on the VEGFA/VEGFR-2 signaling pathway in HemECs and assess the impact of OMT on the miR-494/PTEN axis.

Methods The Cell Counting Kit-8 (CCK-8) assay was employed to evaluate the influence of Oxymatrine (OMT) on the proliferation of Hemangioma Endothelial Cells (HemECs). The Transwell Assay was employed to detect cell invasion and migration. The cell cycle and apoptosis were analyzed through flow cytometry. The impact of OMT on the expression of apoptosis markers (cleaved caspase-3) and proteins associated with the cell cycle (Cyclin D1, Bcl-2, Bax) was examined using Western Blot and Reverse Transcription Polymerase Chain Reaction (RT-PCR).

Results OMT treatment significantly inhibited the proliferation of HemECs, especially when combined with the miR-494 inhibitor. Additionally, OMT administration raised the proportion of cells entering the G2 phase, accelerated apoptosis, and decreased HemECs' capacity for migration and invasion. The results of Western Blot and RT-PCR demonstrated that OMT decreased the expression levels of Bax and Cleaved Caspase-3 while increasing the expression of Bcl-2 and Cyclin D1. OMT and miR-494 inhibitors have distinct impacts on the phosphorylated versions of VEGFR-2, PTEN, and ERK signaling pathways. OMT may control cell survival and proliferation through the miR-494/PTEN pathway, as evidenced by the fact that PTEN expression was dramatically upregulated in the miR-1297 inhibitor with OMT treatment group, while p-ERK expression was markedly reduced in that group.

Conclusions OMT effectively inhibits the growth, migration, and apoptosis of hemangioma endothelial cells, likely by regulating key proteins involved in the cell cycle and apoptosis. The combination with miR-494 inhibitors enhances its

Jingyu Peng and Feifei Li are the co-first author.

✉ Jingmin Ou, oujingmin@xinhuamed.com.cn | [1]Department of Intervention and Vascular Surgery, XinHua Hospital, Shanghai Jiaotong University School of Medcine, 1665 Kongjiang Road, Yangpu District, Shanghai, China. [2]Department of Health Vascular Surgery and Intervention, Chongming Hospital Affiliated to Shanghai University of Medicine and Health Sciences, Shanghai, China.

therapeutic effect, suggesting a potential new approach for hemangioma treatment. These findings support the development of OMT-based strategies for hemangiomas and its potential use in cancer therapy.

Keywords Oxymatrine · miR-494 · VEGFVEGFR autocrine loop · Hemangioma

1 Introduction

Hemangiomas are benign vascular tumors, predominantly found in infants and young children. While these tumors are self-limiting, they can lead to complications such as ulcers, bleeding, and dysfunction, and in severe cases, can be life-threatening [1]. Current treatments, including hormone therapy, laser treatment, and surgical excision, have limitations and side effects, highlighting the need for safer and more effective treatment options [2].

Oxymatrine (OMT), a natural alkaloid from Sophora flavescens, has shown anti-inflammatory, anti-viral, and anti-tumor properties [3]. Recent studies have suggested that OMT can inhibit angiogenesis, induce apoptosis, and suppress tumor cell proliferation [4, 5]. However, its role in hemangioma treatment remains unclear and warrants further exploration.

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression by binding to target mRNAs, influencing their translation or degradation [6]. miR-494 is involved in various cellular processes, including migration, apoptosis, and proliferation, and has been implicated in the progression of several cancers. In different cancer types, miR-494 can act as both an oncogene and a tumor suppressor, depending on the context. Notably, miR-494 regulates the Phosphoinositide 3-Kinase/Protein Kinase B (PI3K/Akt) signaling pathway by targeting PTEN, thereby influencing tumor cell growth and survival [7–9].

PTEN, a key tumor suppressor [10], dephosphorylates and inactivates PI3K/Akt signaling, affecting cell proliferation, apoptosis, and migration [11, 12]. Loss of PTEN function is often observed in cancers, leading to aberrant activation of the PI3K/Akt pathway [13]. Restoring PTEN function has been proposed as a therapeutic strategy for cancer treatment [14]. Moreover, PTEN expression is regulated by several miRNAs [15, 16], including miR-494, which can further modulate the PI3K/Akt pathway [17, 18].

Vascular endothelial growth factor (VEGF) and its receptor VEGFR play crucial roles in angiogenesis by promoting endothelial cell migration and proliferation. Dysregulated VEGF/VEGFR signaling contributes to tumor angiogenesis [19, 20]. miR-494, through its regulation of PTEN, can activate the PI3K/Akt pathway and enhance VEGF expression, which, in turn, activates VEGFR and its downstream signaling. This autocrine loop may be essential for the formation and progression of hemangiomas [21, 22–24].

The aim of this study is to systematically investigate the specific mechanism by which oxymatrine regulates VEGF/VEGFR autocrine loop through miR-494/PTEN axis to inhibit hemangioma, so as to reveal its potential role in the treatment of hemangioma. In addition, cell proliferation and apoptosis detection, Western blot, qRT-PCR, and immunofluorescence will be employed to comprehensively analyze the role and molecular mechanism of OMT in the cells of hemangioma. This study will also explore the therapeutic effect of OMT in combination with other anti-tumor medications, providing a new theoretical basis for the clinical application of OMT. See Fig. 1 for the flow chart.

2 Materials and methods

2.1 Experimental groupings

The study was designed to evaluate the effects of Oxymatrine (OMT) on the proliferation, migration, apoptosis, and cell cycle regulation of hemangioma endothelial cells (HemECs), as well as the impact of combining OMT with miR-494 inhibitors. The experimental groups were as follows:

Control Group (Vehicle Treatment): HemECs were cultured under standard conditions without any treatment to serve as the baseline control.

OMT Treatment Group: HemECs were treated with varying concentrations of Oxymatrine (OMT) for 24, 48, and 72 h to assess its effects on cell proliferation, migration, apoptosis, and the cell cycle.

miR-494 Inhibitor Group: HemECs were treated with miR-494 inhibitors alone to evaluate the impact of miR-494 inhibition on cell proliferation, apoptosis, and migration.

Combination Treatment Group (OMT + miR-494 Inhibitors): HemECs were co-treated with OMT and miR-494 inhibitors to assess any synergistic effects on the cell cycle, apoptosis, and migration.

Each experimental assay, including the CCK-8 assay, Transwell migration assay, and flow cytometry, included a negative control group where cells were treated with the vehicle (e.g., DMSO or PBS) without OMT or miR-494 inhibitors. Each experimental group was performed in triplicate, and data were collected at multiple time points (24, 48, and 72 h) for comprehensive analysis.

2.2 Cell culture

This study used the hemangioma endothelial cells (HemECs) provided by American Type Culture Collection (ATCC) cell bank. The cells were grown at 37 °C in a humidified environment containing 5% CO_2, using RPMI-1640 medium (Catalog No. 11875-093, Gibco™, Thermo Fisher Scientific, Waltham, MA, USA) enriched with 10% FBS (Catalog No. 16140–071, Gibco™, Thermo Fisher Scientific) and 1% penicillin/streptomycin (Catalog No. 15140–122, Gibco™, Thermo Fisher Scientific).

2.3 Drug treatment

Oxymatrine (OMT) was purchased from MCE (Catalog No. HY-15235, MCE, Monmouth Junction, NJ, USA). Cells were seeded to a suitable density 24 h before the experiment. Subsequently, cells were treated with different concentrations of OMT (0, 0.5, 1.0, 1.5 mg/mL) for 24 or 48 h. Control cells were only supplemented with an equal volume of medium.

2.4 Treatment with miRNA inhibitors

miR-494 inhibitor (5′-AGAGGUUUCCCGUGUAUGUUUCA-3′) and miR-494 inhibitor NC (5′-CAGUACUUUUGUGUAGUACAA-3′) were provided by Sigma-Aldrich (St. Louis, MO, USA; Catalog No. HPA042039). In line with the manufacturer's protocol, the cells were transfected with the matching miRNA inhibitors using Lipofectamine 2000 (Catalog No. 11668027, Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) 24 h after inoculation. OMT treatment was performed 24 h after transfection.

2.5 CCK-8 proliferation assay

The cell proliferation was assessed using the Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Japan). The treated cells were seeded at 5000 cells per well within 96-well plates. After 0, 24, and 48 h of incubation, 10 µL of CCK-8 reagent was applied, and after 4 h, the absorbance at 450 nm was recorded by a microplate reader.

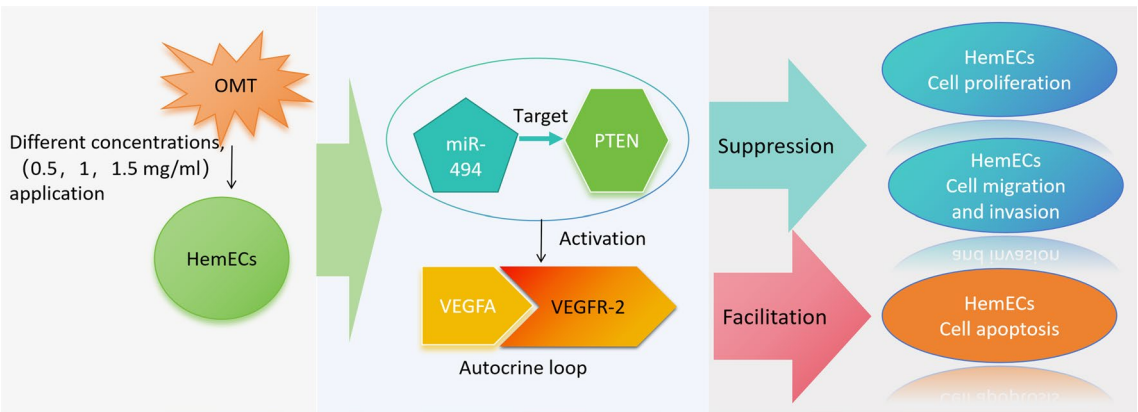

Fig. 1 Flow chart

2.6 Transwell migration and invasion assays

The migration assay was performed using a Transwell device (8 μm, Corning) without a matrix coating. For the invasion assay, the Transwell membrane was pre-coated with Matrigel (Corning, Catalog No. 354234). Matrigel was diluted to a concentration of 1 mg/mL in cold serum-free medium and 100 μL of the solution was applied to the upper chamber. The chamber was incubated at 37 °C for 2 h to allow the Matrigel to solidify. For both assays, the cell suspension was prepared in serum-free medium and added to the upper chamber. The lower chamber contained culture medium with 10% FBS as a chemotactic agent.

After 24 h of incubation, cells that migrated or invaded through the membrane were fixed with 4% formaldehyde (Sigma-Aldrich, Merck, St. Louis, MO, USA; Catalog No. F8775) for 20 min at room temperature, stained with 0.1% crystal violet (Sigma-Aldrich, Merck, St. Louis, MO, USA; Catalog No. C3886) for 15 min, and then counted under a light microscope.

2.7 Analysis of cell cycle and apoptosis

Following collection, cells were fixed with 70% ethanol at 4 °C for 24 h to prepare for cell cycle analysis. For cell cycle evaluation, cells were stained with Propidium Iodide (PI) (Waltham, MA, USA; Catalog No. P3566) at a final concentration of 50 μg/mL for 30 min at 37 °C in the dark. Flow cytometry was performed using a BD FACSCanto II flow cytometer, and the cell cycle distribution was analyzed using FlowJo software.

For apoptosis analysis, cells were stained with Annexin V-FITC and Propidium Iodide (PI) (BD Biosciences, Franklin Lakes, NJ, USA; Catalog No. 556547) according to the manufacturer's protocol. Cells were incubated with Annexin V-FITC (1:10 dilution) for 15 min at room temperature, followed by PI staining (50 μg/mL) for 5 min in the dark. Apoptotic and necrotic cell populations were then assessed by flow cytometry using the BD FACSCanto II flow cytometer.

2.8 RNA extraction and RT-PCR analysis

Reverse transcription suite (Takara Bio, Shiga, Japan; Catalog No. RR047A) is used to synthesize cDNA following the extraction of total RNA with TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA; Catalog No. 15596026). Using GAPDH as an internal reference, real-Time PCR was performed using SYBR Green PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA; Catalog No. 4309155). A particular miRNA quantitative PCR kit (Qiagen, Hilden, Germany, Catalog No. 217204) was used to quantify miRNA. U6 was used as an internal control. The primer used for reverse transcription PCR was: miR-494 forward 5′-AGCTGAAACATACACGGGA-3′; U6 forward 5′-CTCGCTTCGGCA GCACA-3′; U6 reverse 5′-AACGCTTCACGAATTTGCGT-3′; PTEN forward 5′-CAGGCGAGGGAGATGAGAGACGG-3′; PTEN reverse 5′-CTGGACCGCAGCCGGGTAAT-3′.

2.9 Western Blot analysis

Total cellular protein was extracted, and protein concentration was determined using the BCA Protein Quantification kit (Pierce, Thermo Fisher Scientific, Waltham, MA, USA; Catalog No. 23225). Equal amounts of proteins were electrophoresed by SDS-PAGE and transferred to PVDF membranes (Millipore, Merck, Darmstadt, Germany; Catalog No. IPVH00010). The cells were blocked with 5% skim milk powder for 1 h, followed by the addition of primary antibodies Cyclin D1 (Catalog No. 2926, 1:1000), Bcl-2 (Catalog No. 2870, 1:1000), Bax (Catalog No. 2772, 1:1000), Cleaved Caspase-3 (Catalog No. 9661, 1:1000), PTEN (Catalog No. 9188, 1:1000), VEGFR-2 (Catalog No. 2479, 1:1000), p-VEGFR-2 (Catalog No. 2478, 1:1000), ERK (Catalog No. 9102, 1:1000), p-ERK (Catalog No. 4370, 1:1000), and β-actin (Catalog No. 4970, 1:1000), purchased from Cell Signaling Technology) and incubated overnight at 4° C. The corresponding HRP-labeled secondary antibody was added and incubated for 1 h at room temperature. Visualization was performed using ECL chemiluminescence reagent (Thermo Fisher Scientific, Waltham, MA, USA; Catalog No. 34580), and band gray values were photographed and analyzed using a gel imaging system.

2.10 Immunofluorescence staining

The treated cells were seeded on coverslips, fixed, permeabilized with 0.1% Triton X-100, and blocked with 5% bovine serum albumin for 1 h. Primary anti-PTEN (Catalog No. 9188, 1:500) or VEGFR-2 (Catalog No. 2479, 1:500) antibodies were added and incubated overnight at 4° C. Fluorescently labeled secondary antibodies were added the next day and incubated for 1 h at room temperature in the dark. The nuclei were stained with DAPI (Invitrogen, Waltham, MA, USA; Catalog No. D1306) and observed under a fluorescence microscope and photographed.

2.11 Data analysis

Standard error (SEM) is the mean ± standard deviation for experimental data. Groups were compared by Tukey's multiple comparison test and one-way ANOVA, with $P \geqq 0.05$ considered statistically significant. SPSS 22.0 software was utilized for the statistical analysis. The original western blot figures are shown in supplementary file.

3 Results

3.1 Effects of OMT on proliferation and apoptosis of hemangioma endothelial cells

Hemangioma endothelial cells (HemECs) were treated for 24 h at different dosages of OMT (0, 0.5, 1.0, and 1.5 mg/mL). The outcomes demonstrated that OMT had a proliferation-promoting effect on HemECs, and as the concentration of OMT increased, cell proliferation activity showed a marked rise (Fig. 2A). In comparison to the group under control, the cell proliferation activity was noticeably higher at high concentrations of 1.0 mg/mL and 1.5 mg/mL OMT. Impact of varying OMT concentrations on HemECs' cell cycle. The findings demonstrated that while the proportion of S phase dropped, the percentage of G2 phase showed an increasing trend after OMT treatment, suggesting that OMT may promote HemECs cells to enter the G2 phase (Fig. 2B). Using Annexin V-FITC labeling, the effects of various OMT doses on HemECs' early apoptosis were assessed. In HemECs, in comparison to the group under control, OMT administration dramatically raised the rate of early apoptosis, and as OMT concentration rose, so did the rate of early apoptotic cells (Fig. 2C).

3.2 Effect of OMT on the expression of proteins associated with proliferation and apoptosis

To explore the molecular mechanism by which OMT promotes cell proliferation and inhibits apoptosis, the expression levels of key proteins related to cell cycle and apoptosis were analyzed. The results showed that the expression levels of Cyclin D1 and Bcl-2 proteins were significantly up-regulated in the OMT treatment group, which reached the highest level at 1.0 mg/mL concentration comparing with other groups (Fig. 3). In contrast, the expression levels of the pro-apoptotic proteins Bax and Cleaved caspase-3 were significantly down-regulated after OMT treatment (Fig. 3). These results suggest that OMT promotes cell proliferation and survival by up-regulating the expression of Cyclin D1 and Bcl-2, while down-regulating the expression of Bax and Cleaved caspase-3 and inhibiting cell apoptosis.

3.3 Effect of OMT on miR-494 expression and prediction analysis of miR-494 targeting PTEN

To explore whether OMT affects the biological characteristics of HemECs by regulating the expression of miR-494, we first performed bioinformatics prediction analysis of the target genes of miR-494 using miRDB and TargetScan Human 7.1 databases. The results showed that miR-494 could target and regulate the expression of PTEN gene (Fig. 4A). In addition, the results showed that the expression of miR-494 was significantly down-regulated after treatment with OMT at a concentration of 1 mg/mL for 24 h comparing to NC group (Fig. 4B). These results suggest that OMT may relieve the inhibition of PTEN by down-regulating the expression of miR-494, thereby affecting the biological

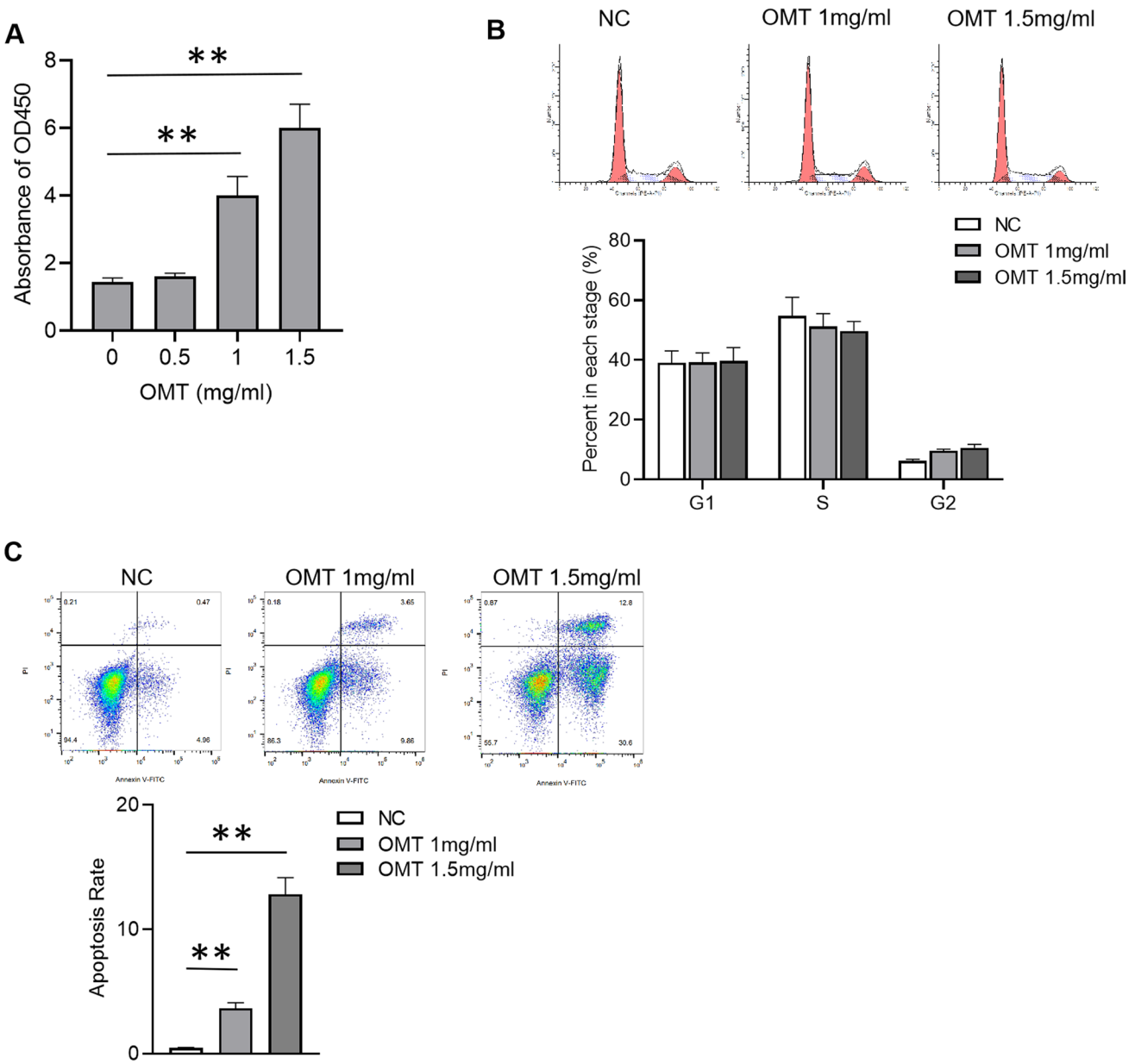

Fig. 2 Effects of OMT on proliferation and apoptosis of hemangioma endothelial cells. **A** Percentage of the effect of different concentrations of matrine on the viability of hemangioma cells relative to control. **B** Effects of different concentrations of matrine on the cell cycle of hemangioma cells after treatment with different concentrations of matrine. **C** Flow cytometry analysis of matrine treated hemangioma cells, showing the distribution of cells at different stages of apoptosis. And the quantitative analysis of apoptotic cells (early and late apoptosis) induced by matrine treatment in hemangioma cells

behaviors of HemECs such as proliferation, migration and apoptosis. This may be one of the mechanisms by which OMT exerts therapeutic effects on hemangioma cells.

3.4 Inhibition of miR-494 slowed the proliferation, migration and invasion of HemECs, and promoted apoptosis

Furthermore, the effects of miR-494 inhibitor on the biological behaviors of HemECs were investigated. First, the expression level of miR-494 in HemECs was quantified using qRT-PCR (Fig. 5A). The results showed that compared with the NC group, the miR-494 inhibitor group significantly reduced the expression level of miR-494, proving that the inhibitor was effective in down-regulating the expression of miR-494. Next, the effect of miR-494 inhibition on HemECs proliferation

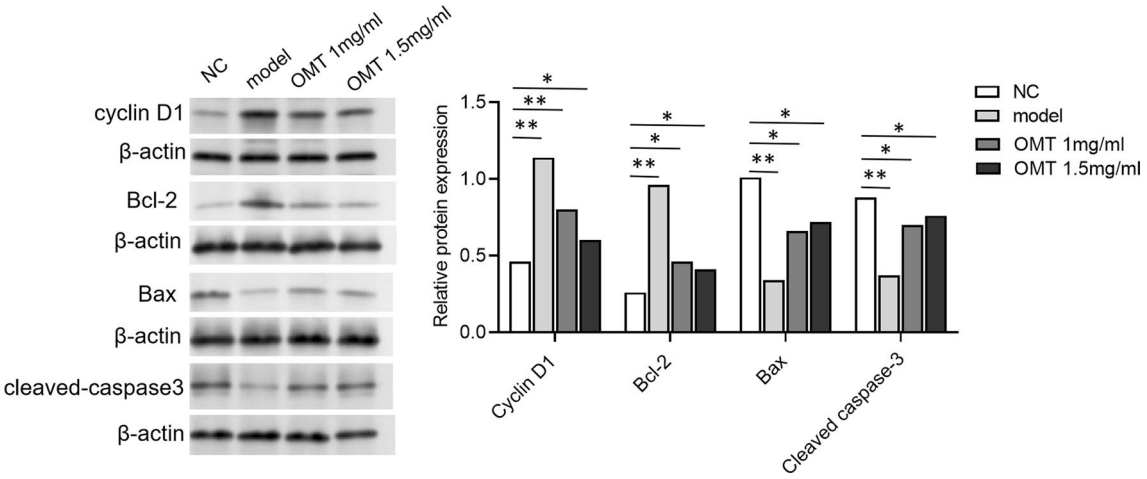

Fig. 3 Effect of OMT on the expression of proteins associated with proliferation and apoptosis. Western blot was used to detect the protein expression of Cyclin D1, Bcl-2, Bax and Cleaved caspase-3. Gray value analysis of the target protein relative to β-actin. Data were expressed as mean ± standard error, $P < 0.05$, $P < 0.01$

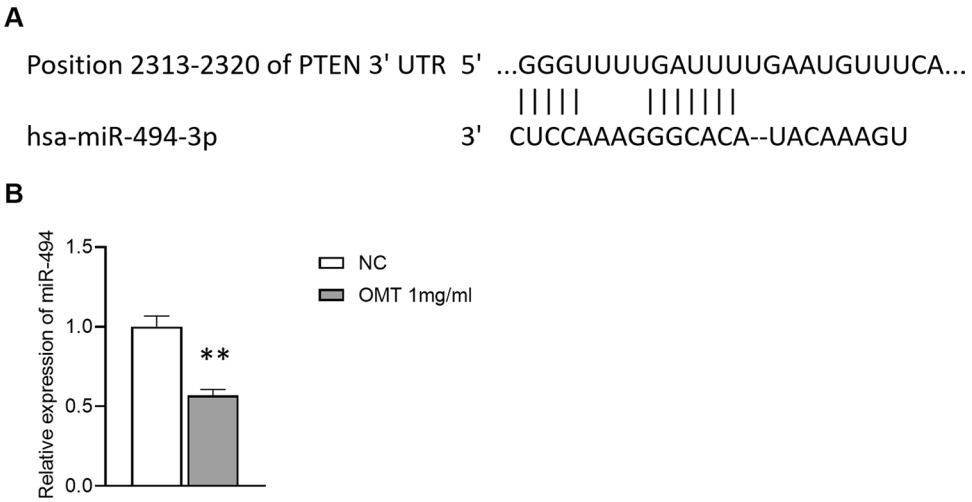

Fig. 4 Effect of OMT on miR-494 expression and prediction analysis of miR-494 targeting PTEN. **A** The binding sites of miR-494 and PTEN predicted by miRDB and TargetScanHuman 7.1 databases showed that miR-494 could target the 3'utr region of PTEN. **B** qRT-PCR analysis of miR-494 expression levels in HemECs after OMT treatment (1 mg/mL, 24 h). Data are presented as mean ± SE ($P < 0.01$)

was evaluated by CCK-8 assay (Fig. 5B). At different time points (0 h, 24 h, 48 h), the cell proliferation activity of NC group and miR-494 inhibitor treated group was recorded. The results showed that inhibition of miR-494 significantly reduced the proliferation activity of HemECs, and this inhibitory effect was more obvious with the extension of time, especially at 48 h, the cell proliferation activity of the inhibitor treatment group was significantly lower than that of the control group. To assess the effect of miR-494 inhibition on the migration and invasion abilities of HemECs, Transwell migration and invasion assays were performed (Fig. 5C). The results showed that the number of migrating and invading cells in the miR-494 inhibition group was significantly reduced compared with the control group ($P < 0.01$). Finally, the effect of miR-494 inhibition on HemECs apoptosis was investigated by Annexin V-FITC/PI double staining and flow cytometry analysis (Fig. 5D). The results showed that miR-494 inhibition group could significantly increase the early and late apoptosis rate of HemECs, and the difference was statistically significant compared with the control group ($P < 0.01$). This suggests that miR-494 inhibitors may inhibit hemangioma growth and development by promoting apoptosis.

3.5 OMT combined with miR-494 regulated PTEN and ERK signaling pathways

We evaluated the effect of different treatments on the expression levels of key genes in HemECs by RT-PCR. The results showed that different combinations of OMT and miR-494 inhibitors had different effects on PTEN, VEGFR-2 and its phosphorylated form (p-VEGFR-2), and ERK signaling pathways. In particular, PTEN expression was significantly increased in the miR-494 inhibitor combined with OMT treatment group, whereas phosphorylated ERK (p-ERK) expression was

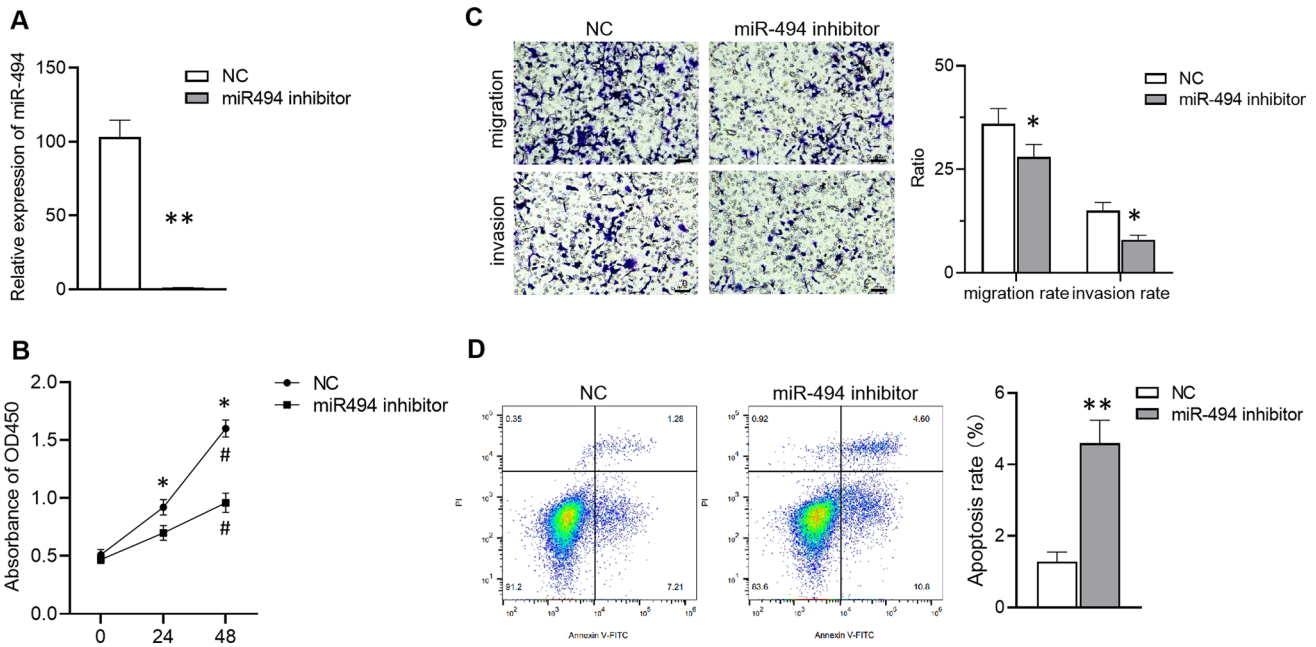

Fig. 5 Inhibition of miR-494 slowed the proliferation, migration and invasion of HemECs, and promoted apoptosis. **A** qRT-PCR was used to detect the effect of miR-494 inhibitor on the expression level of miR-494 in HemECs. **B** CCK-8 assay was used to evaluate the effect of miR-494 inhibitor on HemECs proliferation. Cell proliferative activity was recorded at 0, 24, and 48 h. **C** Transwell migration and invasion assay was used to analyze the effect of miR-494 inhibitor on the migration and invasion ability of HemECs. The left panel shows the migration and invasion of NC group and miR-494 inhibition group, respectively. The right panel presents the migration and invasion rates of the two groups of cells. **D** An examination using flow cytometry and Annexin V/PI double labeling to see how a miR-494 inhibitor affects HemEC apoptosis. The left dot plot shows the percentage of apoptotic cells in the miR-494 inhibitor treatment group versus control (NC), the right bar graph represents the apoptosis rate for each group, which was measured and presented

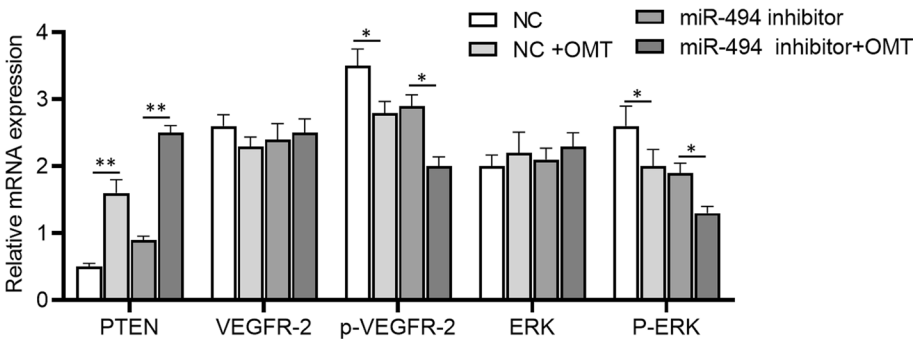

Fig. 6 OMT combined with miR-494 regulated PTEN and ERK signaling pathways. The mRNA expression levels of PTEN, VEGFR-2, phosphorylated VEGFR-2 (p-VEGFR-2), ERK and phosphorylated ERK (P-ERK) in different treatment groups were detected by RT-PCR

significantly decreased in the same group (Fig. 6). This suggests that OMT and miR-494 inhibitors may have a synergistic effect in regulating these signaling pathways. These results suggest that OMT may relieve the inhibition of PTEN by down-regulating the expression of miR-494, and then inhibit the activities of VEGFR-2 and ERK signaling pathways, and finally inhibit the proliferation and migration of HemECs.

3.6 Effects of OMT on proliferation, migration and cell cycle of hemangioma endothelial cells through miR-494

In order to evaluate the effect of OMT combined with miR-494 inhibitor on the proliferation of HemECs, CCK-8 assay was performed to detect the cell proliferation activity of each treatment group at different time points (0 h, 24 h, 48 h). The results showed that the cell proliferation activity of the OMT treatment group (NC + OMT), the miR-494 inhibitor treatment group, and the OMT combined with miR-494 inhibitor group was significantly decreased at 24 and 48 h compared with the NC group (Fig. 7A). Among them, the miR-494 inhibition combined with OMT

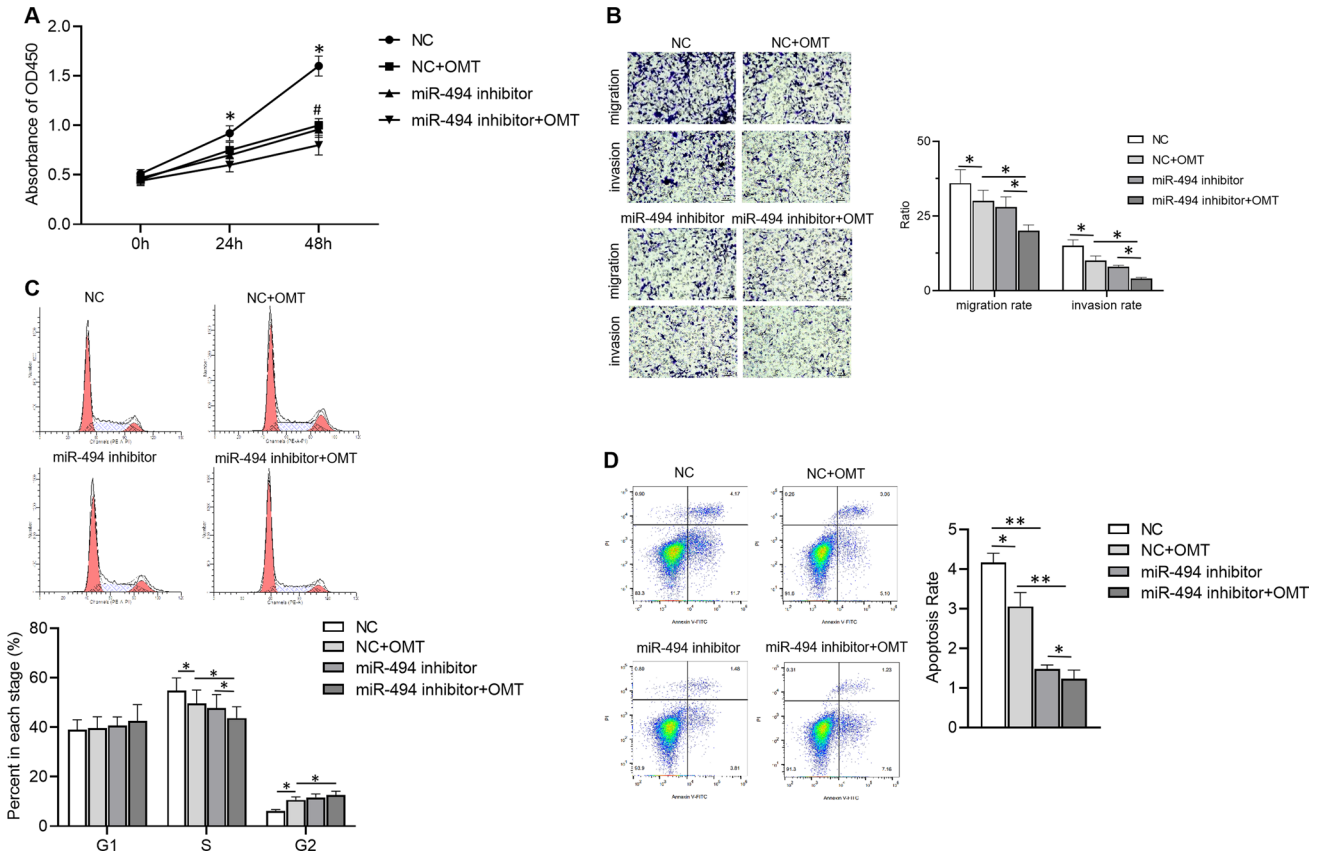

Fig. 7 Effects of OMT on proliferation, migration and cell cycle of hemangioma endothelial cells through miR-494. **A** CCK-8 assay was used to detect the cell proliferation activity of each group. **B** Transwell migration and invasion assay was used to analyze the migration and invasion ability of cells in each group. **C** Flow cytometry analysis of cell cycle distribution in each group. **D** Annexin V-FITC/PI double staining was used to detect the apoptosis rate of cells in each group. Data are expressed as mean ± standard error (SEM), $P < 0.05$, $P < 0.01$, compared with the control group, the difference was significant

treatment group had the most significant inhibitory effect on cell proliferation, especially at 48 h, the proliferation activity was the lowest ($P < 0.05$). The effect of OMT combined with miR-494 inhibition on HemECs migration and invasion ability was evaluated using Transwell assay. The results showed that, compared with the control group, the OMT treatment group, the miR-494 inhibition group, and the OMT combined with miR-494 inhibition group had a significant reduction in the number of migrating and invading cells (Fig. 7B), and the miR-494 inhibition combined with OMT treatment group had the most significant inhibitory effect on migration and invasion ($P < 0.05$). These results indicated that the combined effect of OMT and miR-494 inhibition could effectively inhibit the migration and invasion of HemECs. The effect of each treatment group on the cell cycle distribution of HemECs was analyzed by flow cytometry. The results showed that OMT and miR-494 inhibition treatment resulted in an increase in the proportion of cells in G1 phase and a decrease in the proportion of cells in S phase, indicating that cell cycle progression was inhibited. In addition, the proportion of cells in G2 phase was significantly increased in the combined treatment group, suggesting that the combined treatment of OMT and miR-494 inhibitor may cause G2/M phase arrest, thereby inhibiting cell proliferation (Fig. 7C). Annexin V-FITC/PI double staining combined with flow cytometry was used to analyze the effect of each treatment group on HemECs apoptosis. The results showed that compared with the control group, the OMT treatment group, the miR-494 inhibition group, and the OMT combined with miR-494 inhibition group all significantly increased the apoptosis rate of cells (Fig. 7D), and the miR-494 inhibition combined with OMT group had the highest apoptosis rate ($P < 0.05$). These results indicate that combined treatments have a synergistic effect in promoting apoptosis of HemECs.

4 Discussion

This study demonstrates that Oxymatrine (OMT) promotes the proliferation of Hemangioma Endothelial Cells (HemECs) at higher doses, consistent with findings by Lee et al. [25], who reported that OMT activates intracellular signaling pathways to enhance the proliferation of various cell types. A key molecular player in this process is Cyclin D1, a crucial regulator of the cell cycle. Our data show that OMT increases the expression of Cyclin D1, which is associated with cell cycle progression and the entry of cells into the division cycle [26]. Additionally, we observed an increase in the proportion of cells in the G2 phase, which suggests that OMT may act at this specific phase of the cell cycle, potentially delaying the onset of mitosis. This finding is consistent with Kim et al. [27], who reported that certain natural compounds regulate cell proliferation by modulating the cell cycle.

Although OMT promoted HemEC proliferation, it also significantly increased the early apoptosis rate, indicating a dual role in regulating both cell proliferation and cell death. This dual effect suggests that OMT exerts a complex regulatory mechanism on cell fate. A similar phenomenon was observed by Johnson et al. [28], who found that certain anticancer agents can simultaneously promote proliferation and induce apoptosis, highlighting the context-dependent nature of drug effects in different cell types and microenvironments. The present study further supports the idea that OMT may exert a dual regulatory role by balancing cell survival and apoptosis, which could be influenced by its interaction with key molecular pathways.

Our findings also suggest that OMT regulates HemEC proliferation and apoptosis through the modulation of miR-494 expression. Specifically, OMT upregulates miR-494, which in turn downregulates the expression of PTEN, a well-known tumor suppressor. The mechanism by which miR-494 regulates cell survival and proliferation by targeting PTEN has been widely documented in various cell types. For example, Wang et al. [29] reported that miR-494 negatively regulates PTEN expression, thereby promoting cell survival and proliferation. In the context of our study, this suggests that OMT indirectly regulates the PTEN/PI3K/Akt signaling axis through miR-494, which in turn influences HemEC proliferation and apoptosis. The PTEN/PI3K/Akt pathway is critical for regulating cell growth, survival, and metabolism, and its dysregulation is often associated with cancer development and progression [14].

Moreover, our study found that the combination of OMT and miR-494 inhibitors had a stronger inhibitory effect on HemEC proliferation and migration compared to either treatment alone. This synergistic effect may be attributed to the combined regulation of key signaling pathways, including PTEN and VEGF/VEGFR, which play vital roles in cell proliferation, migration, and apoptosis [30]. The VEGF/VEGFR signaling pathway, in particular, is crucial for angiogenesis, and its dysregulation is often linked to tumor progression and metastasis. By targeting both the PTEN/PI3K/Akt and VEGF/VEGFR pathways, OMT and miR-494 inhibitors may work together to block key steps in hemangioma cell proliferation and migration.

This study significantly advances our understanding of the molecular mechanisms by which OMT influences the biological behavior of hemangioma cells, particularly in terms of proliferation and apoptosis. Our findings suggest that OMT may have therapeutic potential in the treatment of hemangiomas by modulating critical signaling pathways, including PTEN/PI3K/Akt and VEGF/VEGFR. However, further studies are needed to validate these results, especially regarding the therapeutic efficacy and safety of OMT in animal models. Such studies are crucial for translating the potential of OMT into clinical practice. Additionally, future research should investigate the broader implications of OMT in other tumor types and pathological conditions to better understand its full clinical potential.

5 Conclusion

OMT has significant anti-proliferation, anti-migration and pro-apoptotic effects on hemangioma endothelial cells, and its mechanism may involve the regulation of the key regulatory proteins of cell cycle and apoptosis-related proteins Bcl-2, Cyclin D1, Bax and Cleaved caspase-3. The combination of miR-494 inhibitor further enhanced the therapeutic effect of OMT, providing a potential new strategy for the treatment of hemangioma. These findings support the further development of OMT-based strategies for the treatment of hemangiomas and may expand its application in cancer therapy.

Core tips This work elucidated the method by which oxymatrine (OMT) modulates the miR-494/PTEN axis and VEGFA/VEGFR-2 autocrine loop to suppress the proliferation, migration, and promote death of HemECs. The trials demonstrated

that OMT might cause apoptosis and markedly reduce HemEC migration and proliferation. OMT changed the distribution of the cell cycle and raised the proportion of cells entering the G2 phase by altering Bax and Cleaved Caspase-3 levels, while boosting Bcl-2 and Cyclin D1 expression subsequent research revealed that whereas miR-494 mimic decreased OMT's effectiveness, miR-494 inhibitor may increase its therapeutic effect. Furthermore, studies using Western Blot and RT-PCR verified that OMT controls the VEGFA/VEGFR-2 signaling pathway. The findings from this research suggest that OMT could potentially offer additional uses in cancer therapy in addition to being a promising medication for the treatment of hemangiomas by controlling the miR-494/PTEN axis.

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Data availability All data has been stored in the Figshare database, https://figshare.com/s/5350c767cff818cd06bf.

Declarations

Competing interests The authors declare no competing interests.

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