

MicroRNA-524-5p regulates the proliferation and invasion of HTR-8/SVneo trophoblasts by targeting NUMB in the Notch signaling pathway

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Abstract. Preeclampsia is a pregnancy disorder that is primarily associated with maternal and neonatal or fetal morbidity and mortality. The discovery of dysregulated microRNAs (miRs) and their roles in preeclampsia has provided new insight into the mechanisms involved in pregnancy-related disorders. In the present study, quantitative PCR demonstrated that the expression levels of miR-524-5p were lower in patients with preeclampsia than those in normal pregnant women. Cell Counting Kit-8 and Transwell assays indicated that overexpression of miR-524-5p promoted the proliferation and invasion of HTR-8/SVneo cells, whereas inhibition of miR-524-5p suppressed HTR-8/SVneo cell proliferation and invasion. Furthermore, NUMB endocytic adaptor protein (NUMB), a negative regulator of the Notch signaling pathway and a target gene of miR-524-5p, limited the effects of miR-524-5p on HTR-8/SVneo cell invasion and migration. The present study demonstrated that miR-524-5p regulated the proliferation and invasion of HTR-8/SVneo cells at least partly by targeting NUMB to regulate the Notch signaling pathway.

Introduction

Preeclampsia is a common serious disorder of obstetrics and is a hypertensive complication that occurs after the 20th week of gestation, characterized by new-onset hypertension (1).

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Abbreviations: NUMB, NUMB endocytic adaptor protein; PCNA, proliferating cell nuclear antigen

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The primary cause of maternal mortality in preeclampsia has not yet been elucidated. At present, it is hypothesized that placental maternal surface spiral arterial remodeling disorder and insufficient extravillous trophoblast invasion are key factors leading to preeclampsia (2,3).

MicroRNAs (miRNAs/miRs) are a type of small non-coding RNAs that have been reported to be post-transcriptional gene regulators and tumor suppressors (4,5). Previous studies have investigated the role of miR-524-5p in numerous types of cancer. For example, Chen *et al* (6) demonstrated that high/low miR-524-5p expression is associated with improved/worse survival rate and pathological grade of patients with glioma, and Liu *et al* (7) detected lower levels of miR-524-5p expression in human melanoma, whereas overexpression of miR-524-5p effectively inhibited melanoma cell proliferation and migration. Furthermore, Liu *et al* (7) demonstrated that tumors overexpressing miR-524-5p were significantly smaller than those in negative control (NC) mice. Additionally, a previous study has demonstrated that miR-524-5p expression is down-regulated in preeclampsia (8). However, the role of miR-524-5p in preeclampsia has not been fully elucidated.

NUMB endocytic adaptor protein (NUMB), a cell fate determinant, serves an important role in asymmetric cell division (9). NUMB is a key negative regulator of the Notch signaling pathway (10) and is involved in numerous physiological processes, such as differentiation/proliferation balance, apoptosis regulation, cell migration and tissue regeneration (11-13). To the best of our knowledge, NUMB expression in placental tissues with extremely active growth and differentiation has not yet been identified. Therefore, the present study investigated the potential roles of miR-524-5p and NUMB in trophoblast proliferation and invasion.

Materials and methods

Patients. Patients at Hainan Provincial People's Hospital (Haikou, China) were enrolled in the present study between September 2017 and January 2019. A total of 40 patients with preeclampsia and 40 healthy pregnant women were admitted in the present study. The risk factors for the development of pre-eclampsia were recorded according to a previous study (14). According to the American College of Obstetricians

and Gynecologists practice bulletin for diagnosis and management of preeclampsia and eclampsia, severe preeclampsia was defined as either sustained systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 110 mmHg (measured twice, ≥ 6 h apart) or severe proteinuria (>5 g/24 h specimen or ≥ 3 g/l in ≥ 2 random samples collected 4 h apart) (15). Normal systolic blood pressure is <140 mmHg and diastolic blood pressure is <110 mmHg. Age, BMI and smoking history were also recorded but were not used as exclusion criteria. Patients with non-severe preeclampsia according to the criterion listed in the International Society for the Study of Hypertension in Pregnancy were excluded. Clinical information was recorded up to the expected date of delivery and the placenta was obtained for follow-up study. The present study was approved by the Ethics Committee of Hainan General Hospital and all patients provided written informed consent.

Cell culture. The human HTR-8/SVneo trophoblast cell line was purchased from Shanghai Enzyme Research Biotechnology Co., Ltd. HTR-8/SVneo cells were seeded in a 10-cm cell culture dish with DMEM medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin/streptomycin at 37°C in a humidified atmosphere with 5% CO₂.

miR inhibitor and mimic transfection. miR-524-5p inhibitor, mimic and negative control (NC) oligonucleotides (20 μ M; Guangzhou RiboBio Co., Ltd.) were transfected into cells using 5 μ l Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) in 6-well plates at 37°C for 24 h. The cells were divided into four groups: Scrambled Inhibitor NC (5'-GAGAAAGUG CUUCGGUUUUUUG-3'), miR-524-5p inhibitor (5'-GAG AAAGUGCUUCCCUUUGUAG-3'), scrambled mimic NC (5'-CAAAAACCGAAGCACUUUCUC-3') and miR-524-5p mimic (5'-CUACAAAGGGAAGCACUUUCUC-3').

Cell counting Kit-8 (CCK-8) assay. Cells were seeded into 6-well plates at a density of 1x10⁴ cells/ml for 0, 24, 48 or 72 h. Cells were incubated with 10 μ l CCK-8 solution (Beijing Solarbio Science & Technology Co., Ltd.) at 37°C for 2 h, and cell viability was measured. Absorbance values were measured using a microplate reader at a wavelength of 450 nm.

Bioinformatics analysis. The target genes of miR-524-5p were predicted using TargetScan (http://www.targetscan.org/mamm_31/) and microRNA.org databases (<http://www.mirbase.org/>) (16,17). Binding sites between miR-524-5p and the target genes were searched to identify potential interactions between them.

Luciferase reporter assay. A luciferase reporter assay was conducted to explore the association between miR-524-5p and NUMB, as described previously (18). HTR-8/SVneo cells were divided into two groups, wild-type and mutant NUMB with 3'-untranslated region (UTR) reporters. HTR-8/SVneo cells at 80% confluence were co-transfected at 37°C for 24 h with wild-type or mutant NUMB 3'-UTR reporters (16 μ g/ml; Generay Biotech Co., Ltd.) together with miR-524-5p mimic (50 pmol/ml) or NC using Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, Dual-Luciferase

Reporter Assay System (Promega Corporation) was performed according to the manufacturer's protocol. Cells were harvested and lysed for the assay 24 h after transfection. *Renilla* luciferase was used to normalize the data.

Transwell invasion assay. Transwell assays were carried out as described previously (19). Cells (2x10⁴ cells/well) were cultured at 37°C for 24 h in 24-well plates with Transwell inserts precoated with Matrigel[®] (37°C for 30 min; BD Pharmingen; BD Biosciences). DMEM medium was added to the upper chamber, and DMEM containing 10% FBS in the lower chamber. Subsequently, cells were stained with 0.1% crystal violet (Sigma-Aldrich; Merck KGaA) at 20°C for 10 min and the number of cells that had invaded through the Matrigel membrane was counted using a light microscope (magnification, x100; Leica Microsystems GmbH).

Reverse transcription-quantitative (RT-q)PCR analysis. Total RNA was extracted from cells using TRIzol[®] reagent and quantified using NanoDrop 2000c (both Thermo Fisher Scientific, Inc). For miR-524-5p detection, RNA was reverse transcribed to cDNA using the RevertAid First Strand cDNA kit (Thermo Fisher Scientific, Inc), according to the manufacturer's protocol. qPCR was performed in 96-well plates in the ABI Step-One plus Real-Time PCR system (Thermo Fisher Scientific, Inc.) using SYBR Green Master Mix (Takara Biotechnology Co., Ltd.). Expression levels of miR-524-5p were normalized to those of small nuclear RNA U6, while GAPDH was used as an endogenous control for NUMB. Primers were obtained from Invitrogen (Thermo Fisher Scientific, Inc.). The primer sequences were as follows: miR-524-5p forward, 5'-CTACAAAGGGAAGCACTTTTCTCAA-3' and reverse, 5'-GTGCAGGGTCCGAGGT-3'; U6 forward, 5'-CGCTTC GGCAGCACATATAC-3' and reverse, 5'-CAGGGGCCATGC TAATCTT-3'; NUMB forward, 5'-AAGGCTTCTTTGGAA AACTGG-3' and reverse, 5'-CATGGCTCAACCTTTCAC CT-3'; and GAPDH forward, 5'-TGTTTCGTCATGGGTGTGA AC-3' and reverse, 5'-ATGGCATGGACTGTGGTCAT-3'. Each well contained 1 μ l template, 10 μ l Master Mix, 0.5 μ l forward primer (10 μ M), 0.5 μ l reverse primer (10 μ M) and 8 μ l diethyl pyrocarbonate H₂O in a 20- μ l reaction system. qPCR thermocycling conditions were as follows: Initial denaturation at 95°C for 2 min, followed by 40 cycles of denaturation at 95°C for 20 sec, annealing at 60°C for 45 sec and extension at 72°C for 30 sec. Finally, gene expression levels were calculated using the 2^{- $\Delta\Delta$ C_q} method (20).

Western blotting. Proteins were extracted from cells using RIPA buffer (EMD Millipore) and quantified using a BCA kit (Beijing Solarbio Science & Technology Co., Ltd.). Target proteins (30 μ g) were separated according to their mass via 10% SDS-PAGE, then transferred to a PVDF membrane. Membranes were incubated with primary antibodies against proliferating cell nuclear antigen (PCNA; 1:1,000; cat. no. ab92552), Ki67 (1:2,000; cat. no. ab92742), NUMB (1:1,000; cat. no. ab220362), Bcl-2 (1:500; cat. no. ab196495), Notch1 (1:1,000; cat. no. ab52627), cyclin D1 (1:500; cat. no. ab40754), CDK6 (1:2,000; cat. no. ab151247) and GAPDH (1:10,000; cat. no. ab181602) (all Abcam) at 4°C overnight, then incubated with horseradish peroxidase-conjugated goat

Table I. Clinical information of patients with PE and normal pregnant females.

Parameter	Control (n=40)	PE (n=40)	P-value
Maternal age at delivery, years	29.53±2.90	30.43±3.15	0.1878
Gestational age, weeks	38.18±2.24	36.70±3.15	<0.05
Proteinuria, g/24 h	-	4.57±1.40	<0.001
Systolic blood pressure, mmHg	105.81±13.41	168.43±5.43	<0.001
Diastolic blood pressure, mmHg	77.38±4.33	116.06±3.75	<0.001
Fetal birth weight, g	3,556.88±309.88	2,689.85±428.90	<0.001
BMI	23.42±3.28	23.01±3.66	0.594
Number of individuals who smoked, n	3.00	4.00	0.694

Data are expressed as the mean ± standard deviation, unless otherwise stated. Significance was calculated between control and PE groups. PE, preeclampsia.

anti-rabbit IgG secondary antibody (1:5,000; cat. no. A0208; Beyotime Institute of Biotechnology) at 25°C for 1 h. Bands were visualized using the ECL reagent (Merck KGaA) and density was measured via ImageJ software (v 2.1.4.7).

Statistical analysis. Data are presented as the mean ± standard deviation of three independent experiments. Statistical comparisons were performed using SPSS 17.0 software (SPSS, Inc.) Differences between two groups were compared using an unpaired Student's t-test. One-way ANOVA followed by Tukey's multiple comparison test was used for comparisons among three groups. Pearson's correlation analysis was used to examine the correlation between NUMB and miR-524-5p expression. The χ^2 test was used to analyze the number of volunteers who smoked. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

miR-524-5p is downregulated in preeclamptic placenta compared with in normal placenta. A total of 40 patients with preeclampsia and 40 normal pregnant females (controls) were enrolled in the present study. Clinical characteristics of patients and normal pregnant females were recorded, including age, BMI, smoking history and systolic/diastolic blood pressure (Table I). Maternal age, BMI and smoking history exhibited no significant difference between the two groups. Patients with preeclampsia had a significantly shorter gestational age and lower fetal birth weight, while systolic and diastolic blood pressure in patients with preeclampsia were significantly higher than in normal controls. qPCR demonstrated that expression levels of miR-524-5p were significantly lower in patients with preeclampsia than in normal controls (Fig. 1), which indicated that downregulation of miR-524-5p expression may be a biomarker of preeclampsia.

miR-524-5p regulates proliferation and invasion of human HTR-8/SVneo trophoblasts. In order to investigate the effect of miR-524-5p on the proliferative and invasive abilities of human HTR-8/SVneo trophoblasts, miR-524-5p mimic and inhibitor were transfected into cells. Expression levels of miR-524-5p were significantly higher in the miR-524-5p

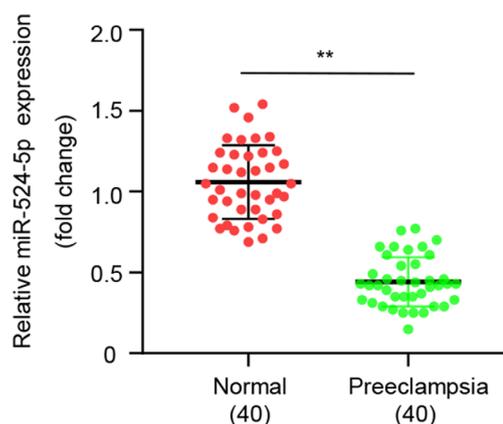


Figure 1. Relative miR-524-5p expression levels in controls and patients with preeclampsia. ** $P < 0.001$. miR, microRNA.

mimic group than in the mimic NC group (Fig. 2A) and significantly lower in the miR-524-5p inhibitor group than in the inhibitor NC group (Fig. 2B), which indicated that mimic and inhibitor had been transfected successfully. The CCK-8 assay demonstrated that cell viability of the miR-524-5p mimic group was significantly higher than that of the mimic NC group at 72 h (Fig. 2C), while cell viability of the miR-524-5p inhibitor group was significantly lower compared with the inhibitor NC group (Fig. 2D). Expression levels of PCNA and Ki67 were determined by western blotting to investigate the underlying mechanism of cell proliferation. Overexpression of miR-524-5p increased PCNA and Ki67 protein expression, while PCNA and Ki67 expression in the miR-524-5p inhibitor group was lower than in the inhibitor NC group, which demonstrated that inhibition of miR-524-5p inhibited cell proliferation (Fig. 2E).

In order to investigate the influence of miR-524-5p on the invasive ability of human HTR-8/SVneo trophoblasts, the degree of cell invasion was determined by Transwell invasion assay. Overexpression and inhibition of miR-524-5p in HTR-8/SVneo cells regulated cell invasion. After 24 h, the invasive ability of cells in the miR-524-5p mimic group was significantly increased compared with that in the mimic NC group (Fig. 2F), while the invasive ability of cells in the

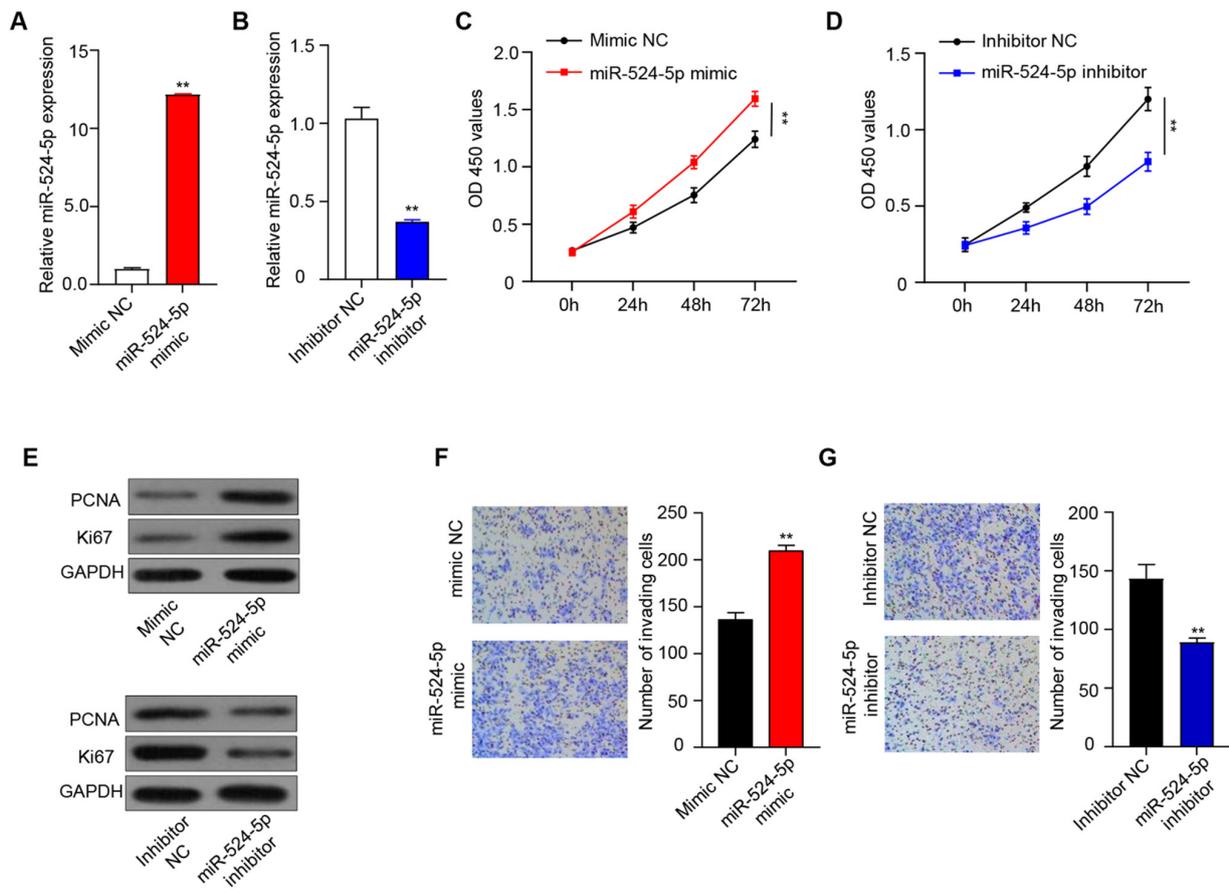


Figure 2. Low expression levels of miR-524-5p impede trophoblast cell proliferation and invasion. Relative miR-524-5p expression levels in HTR-8/SVneo cells following transfection with (A) miR-524-5p mimic and (B) inhibitor. Viability of HTR-8/SVneo cells transfected with (C) miR-524-5p mimic and (D) inhibitor for 0, 24, 48 and 72 h. (E) Western blot analysis of PCNA and Ki67 protein levels in HTR-8/SVneo cells transfected with miR-145-5p mimic or NC, and miR-145-5p inhibitor or NC. Transwell invasion assay was performed to assess the invasiveness of HTR-8/SVneo cells transfected with (F) miR-145-5p mimic or NC or (G) miR-145-5p inhibitor or NC. Scale bar, 100 μ m. * P <0.01; miR, microRNA; PCNA, proliferating cell nuclear antigen; NC, negative control; OD, optical density.

miR-524-5p inhibitor group was significantly decreased compared with that in the inhibitor NC group (Fig. 2G).

miR-524-5p regulates the expression levels of NUMB via binding to the 3'-UTR of NUMB mRNA. In order to determine the target genes of miR-524-5p, TargetScan and microRNA.org databases were used. Results from the databases indicated that NUMB was a candidate target gene regulated by miR-524-5p, and microRNA.org database was used to predict the binding sites (Fig. 3A).

In order to determine whether inhibition of NUMB by miR-524-5p occurred via these predicted miR-524-5p binding sites, the binding site was mutated. The luciferase reporter assay indicated that transfection with the NUMB mutant 3'-UTR did not lead to a reduction in the relative luciferase activity in the presence of miR-524-5p, compared with miR NC (Fig. 3B). Subsequently, the expression levels of NUMB were determined following miR-524-5p mimic or inhibitor treatment. Inhibition of miR-524-5p increased the expression levels of NUMB mRNA and protein (Fig. 3C and E); conversely, when miR-524-5p was overexpressed, NUMB mRNA and protein expression levels were decreased (Fig. 3D and F). Additionally, Pearson's correlation analysis showed that NUMB exhibited a negative correlation with miR-524-5p (Fig. 3G), which

suggested that the highly conserved sequence of the NUMB 3'-UTR may be the primary binding site of miR-524-5p, and further confirmed that miR-524-5p directly inhibited NUMB.

miR-524-5p regulates proliferation and invasion of HTR-8/SVneo cells by targeting NUMB to regulate the Notch signaling pathway. pcDNA3.1-NUMB and miR-524-5p mimic were transfected into human HTR-8/SVneo trophoblasts. Cells were divided into 4 groups, including mimic NC+ pcDNA 3.1, miR-524-5p mimic + pcDNA 3.1, mimic NC + pcDNA 3.1-NUMB and miR-524-5p mimic + pcDNA 3.1-NUMB. Cell viability was determined by CCK-8 assay, and the results revealed that cell viability was highest in the miR-524-5p mimic + pcDNA 3.1 group and lowest in mimic NC + pcDNA 3.1-NUMB, which indicated that activation of miR-524-5p may inhibit the expression levels of NUMB and cell viability was increased due to elevated miR-524-5p expression at 72 h (Fig. 4A).

Apoptosis rate is significantly increased in the syncytiotrophoblast in preeclampsia (21). The effect of miR-524-5p and NUMB on cell proliferation was investigated. PCND and Ki67 are common proliferation markers in apoptosis (22), and therefore western blot analysis was performed to detect

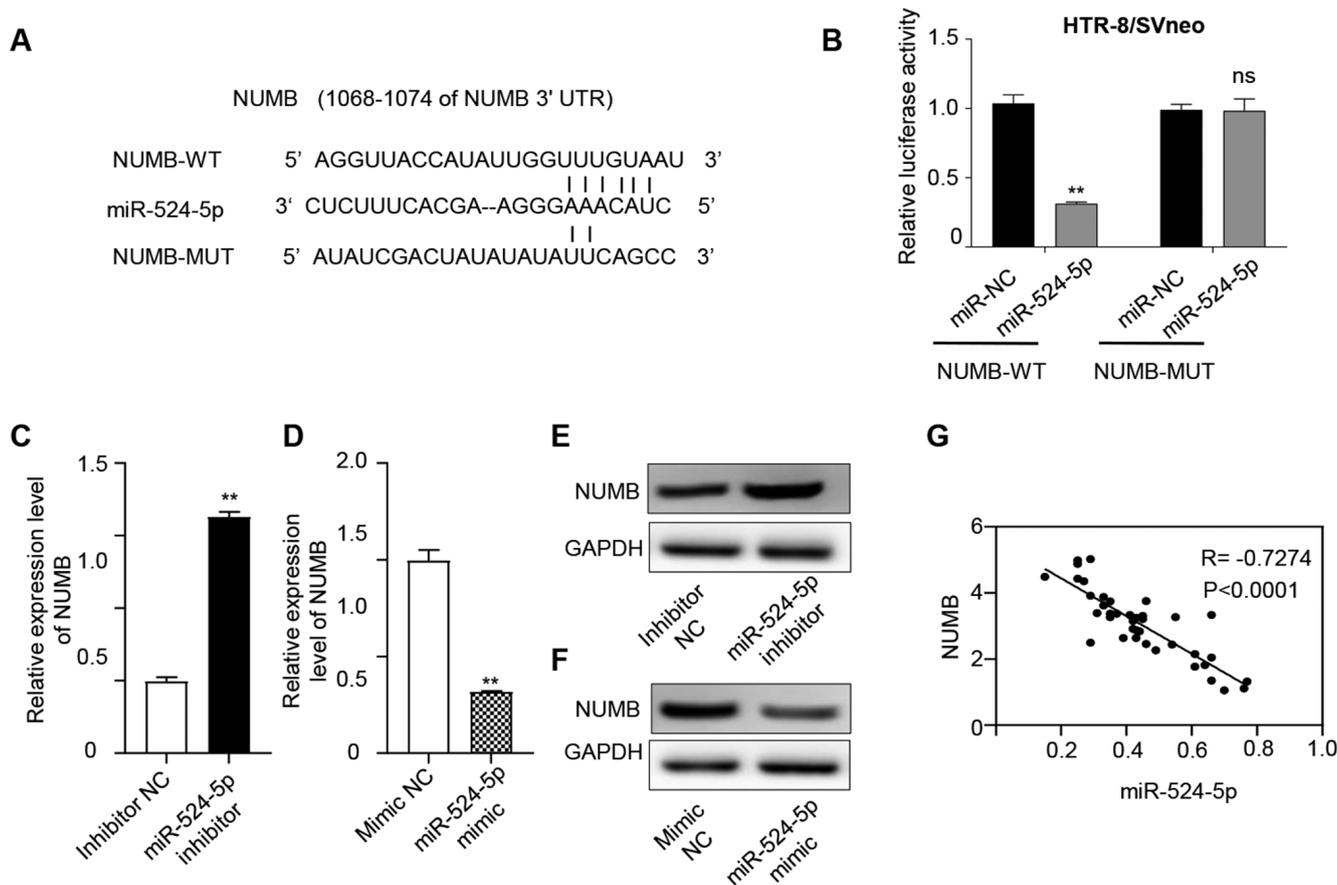


Figure 3. miR-524-5p regulates the expression levels of NUMB. (A) microRNA.org database was used to predict binding sites between miR-524-5p and NUMB. (B) Fluorescence intensity was measured using a dual luciferase assay. Overexpressed miR-524-5p bound to NUMB-WT and fluorescence intensity was weakened. NUMB-MUT exhibited no significant difference in fluorescence intensity. RT-qPCR was used to detect NUMB expression in the (C) inhibitor and (D) mimic groups. Compared with the inhibitor NC group, NUMB expression was significantly upregulated in the miR-524-5p inhibitor group, while compared with the mimic NC group, NUMB expression in the miR-524-5p mimic group was significantly downregulated. Western blotting was used to detect NUMB expression in the (E) inhibitor and (F) mimic groups. Compared with the inhibitor NC group, NUMB expression was significantly upregulated in the miR-524-5p inhibitor group, while it was significantly downregulated in the miR-524-5p mimic group compared with the mimic NC group. (G) RT-qPCR was used to detect the expression levels of NUMB in 40 preeclampsia tissues and Pearson's correlation coefficient was used to analyze the correlation between NUMB and miR-524-5p expression. There was a significant negative association between NUMB and miR-524-5p expression. ** $P < 0.01$. miR, microRNA; NUMB, NUMB endocytic adaptor protein; WT, wild-type; MUT, mutant; RT-q, reverse transcription-quantitative; NC, negative control; UTR, untranslated region; ns, not significant.

the expression levels of these two proteins. Expression levels of PCNA and Ki67 were highest in the miR-524-5p mimic + pcDNA 3.1 group and lowest in the mimic NC + pcDNA 3.1-NUMB group, which indicated that overexpression of miR-524-5p decreased NUMB and increased the cell proliferative ability (Fig. 4B). Additionally, the effect of miR-524-5p mimic on cell invasion was investigated. The results revealed that the miR-524-5p mimic + pcDNA3.1 group had the highest number of invading cells and the mimic NC + pcDNA3.1-NUMB group exhibited the lowest number. The number of invading cells in the miR-524-5p mimic + pcDNA3.1-NUMB was between the two aforementioned groups (Fig. 4C and D).

The present results suggested that miR-524-5p mimic inhibited NUMB expression and that high expression levels of NUMB inhibited cell proliferation and invasion. As the mechanism remains unknown, the present study investigated the specific mechanism by which miR-524-5p stimulates proliferation and invasion of HTR-8/SVneo cells by detecting the expression levels of signaling molecules

associated with the Notch signaling pathway. Protein expression levels of Bcl-2, Notch, cyclin D1 and CDK6 were determined by western blotting. Protein expression level trends of Bcl-2, Notch, cyclin D1 and CDK6 in each group were downregulated when pcDNA 3.1-NUMB were transformed simultaneously with NC or mimic (Fig. 4E and S1). This indicated that miR-524-5p stimulated proliferation and invasion of HTR-8/SVneo cells by targeting NUMB to regulate the Notch signaling pathway.

Discussion

In recent years, numerous studies have continued to investigate preeclampsia (1,23), but the exact cause has not yet been elucidated. There is still no ideal animal model that replicates all pathological conditions of preeclampsia that are produced. Preeclampsia is hypothesized to be associated with insufficient cell invasion and endothelial cell dysfunction (24). In the present study, patients with severe PE symptoms exhibited higher systolic and diastolic blood

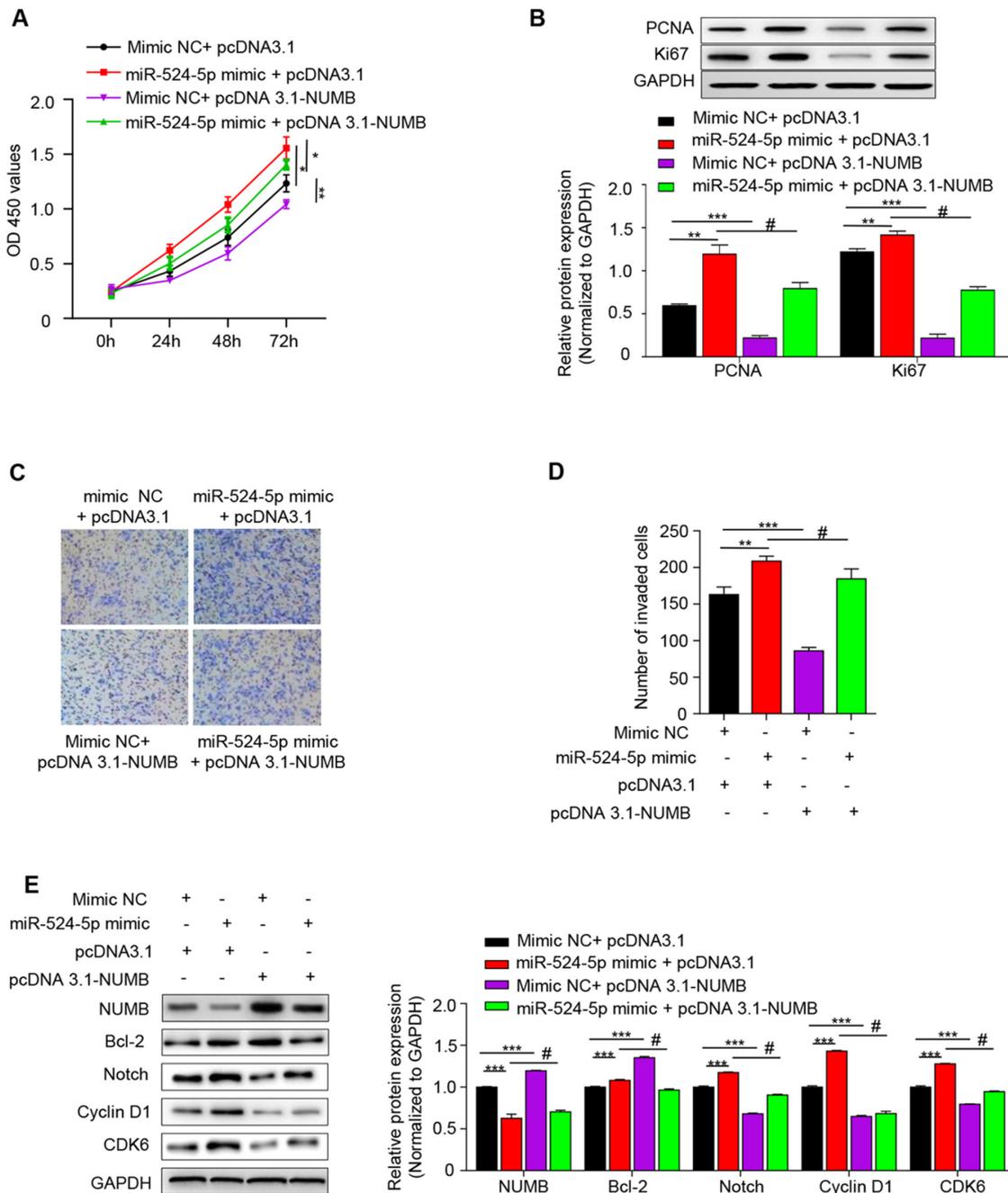


Figure 4. miR-524-5p regulates the Notch signaling pathway via NUMB. (A) Cell Counting Kit-8 was used to detect the proliferation ability of each group for 0, 24, 48 and 72 h. (B) Western blot analysis of PCNA and Ki67 protein expression. (C) Invasive ability was detected using Transwell assay (Scale bar, 100 μ m) and (D) quantified. (E) Western blot was used to detect the expression levels of NUMB, Notch1, Bcl-2, Cyclin D1 and CDK6 in the Notch signaling pathway. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. mimic NC+ pcDNA3.1 group; # $P < 0.001$ vs. miR-524-5p mimic + pcDNA3.1 group. miR, microRNA; NUMB, NUMB endocytic adaptor protein; PCNA, proliferating cell nuclear antigen; NC, negative control; OD, optical density; EV, empty vector.

pressure and proteinuria levels, whereas in normal controls, proteinuria was not detected.

miRNAs serve roles in a number of diseases, such as sclerosis, lung cancer and neurodegenerative diseases like Alzheimer's disease (25). The discovery of dysregulated miRNAs and their gene-regulatory roles in placental development has provided a novel approach for elucidating the underlying mechanisms of pregnancy-specific diseases (26). In the present study, bioinformatics analysis was used to predict the target genes of a specific miRNA, revealing that miR-524-5p was expressed at lower levels in patients with

preeclampsia compared with normal controls. miR-524-5p expression in patients with preeclampsia should continue to be monitored in future studies. The predicted target gene of miR-524-5p was NUMB (confirmed by luciferase reporter assay); expression levels of NUMB, a negative regulator of the Notch signaling pathway, were regulated by miR-524-5p (10). Notch is a type of receptor that mediates transmembrane communication and cell fate (27). Bcl-2 is a factor that regulates apoptosis in the intrinsic mitochondrial apoptosis pathway (28). Cyclin D1 is one member of the Cyclin-D family; it is commonly overexpressed and known

to be a direct target of Jagged1-mediated Notch signaling in breast cancer (29). Inactive CDK6 kinase is reported to disrupt Notch-dependent survival and proliferation by altering the expression levels of Notch target gene (30).

NUMB facilitates the migration and invasion of trophoblastic cells (31). A number of mechanisms have been suggested to explain the dysregulation of NUMB in trophoblastic cells. NUMB serves a key role in asymmetric division (32), as well as in regulating cell recognition, differentiation, tissue renewal and stabilization of the differentiation environment (33). In addition, NUMB acts as an important negative regulator of the Notch signaling pathway (10). The present study demonstrated that miR-524-5p negatively regulated both mRNA and protein levels of NUMB. Viability of HTR-8/SVneo cells was decreased when miR-524-5p expression was inhibited, while cell viability was increased when miR-524-5p was activated using a miR mimic. Subsequently, the effect of miR-524-5p on the proliferative and invasive abilities of HTR-8/SVneo cells were investigated. While low expression levels of miR-524-5p significantly inhibited cell proliferation and migration compared with the control group, activation of miR-524-5p promoted cell proliferation and migration. Next, western blotting was performed to detect the association between miR-524-5p and NUMB, revealing that inhibition of miR-524-5p resulted in upregulation of NUMB. Finally, to investigate the mechanism underlying the miR-524-5p-mediated upregulation of cell proliferation and invasion, Notch signaling pathway-associated proteins were detected via western blotting, including Bcl-2, Notch, cyclin D1 and CDK6. Larger patient cohorts and animal studies are required to validate the results, as these were the two primary limitations of the present research.

In conclusion, the present results demonstrated that miR-524-5p regulated proliferation and invasion of HTR-8/SVneo cells by targeting NUMB to regulate the Notch signaling pathway.

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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

LS conceptualized the study and gave final approval of the version to be published. LZ and JS designed and performed the experiments, and wrote and revised the manuscript. LW, RT, XC, DW and HC performed the experiments, analyzed the data and prepared figures and tables. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Hainan General Hospital. All patients provided written informed consent prior to participation in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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