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CELLULAR AND MOLECULAR BIOLOGY

Nuclear Factor-Kappa B-induced miRNA-518a-5p represses trophoblast cell migration and invasion by the Nuclear Factor-Kappa B pathway

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Abstract: Preeclampsia is associated with the insufficient invasion of trophoblasts. NF-κB is a transcription factor in almost all mammalian cells and has been validated to be upregulated in the maternal circulation and placenta of women with preeclampsia. MiR-518a-5p is also overexpressed in pre-eclamptic placenta. The present study was designed to explore whether NF-κB can transcriptionally activate miR-518a-5p and investigate the influences of miR-518a-5p on the viability, apoptosis, migration, and invasion of HTR8/SVneo trophoblast. *In situ* hybridization and real time polymerase chain reaction were used to reveal miR-518a-5p expression in placenta tissues and HTR8/SVneo cells, respectively. Cell migration and invasion were detected using Transwell inserts. Our findings indicated that NF-κB p52, p50, and p65 can bind to miR-518a-5p gene promoter. MiR-518a-5p further influences the levels of p50 and p65 but not p52. HTR8/SVneo cell viability and apoptosis were not influenced by miR-518a-5p. However, miR-518a-5p represses the migratory/invasive capacities of HTR8/SVneo cell and decreased gelatinolytic activity of MMP2 and MMP9, which was reversed by an NF-κB inhibitor. To sum up, miR-518a-5p is induced by NF-κB and represses trophoblast cell migration and invasion by the NF-κB pathway.

Key words: preeclampsia, HTR8/SVneo, invasion, NF-κB transcriptional factor, miR-518a-5p.

INTRODUCTION

Preeclampsia is a de-novo hypertension after gestation for 20 weeks combined with proteinuria, haematological complications, uteroplacental dysfunction, or fetal growth retardation (Kintiraki et al. 2015). According to the American College of Obstetrics and Gynecology, preeclampsia diagnosis is based on gestational hypertension, regardless of other diagnostic criteria that are complementary (ACOG 2019). Preeclampsia is associated with higher risk of disseminated intravascular coagulation, pulmonary oedema, hemolysis, elevated liver enzymes, and low platelets syndrome, and placental abruption in pregnant women (Kintiraki et al. 2015, Ahmed et al. 2017). There is no cure for preeclampsia at present, and the main treatment is placental and fetal delivery. Preeclampsia causes preterm birth, posing immediate and long-term health burdens to the fetus and mother in severe cases (Armistead et al. 2020). During preeclampsia, the placenta is exposed to excessive inflammation and oxidative stress, trophoblast differentiation is disrupted, and secretion of anti-angiogenic proteins is enhanced (Chiarello et al. 2020, Michalczyk et al. 2020). Impaired trophoblast migration/invasiveness leads to poor placental perfusion during early pregnancy and causes fetal injury and growth retardation, causing pre-eclampsia clinical manifestations (Lala & Chakraborty 2003).

The nuclear factor kappa B (NF-κB) protein family are a group of proteins and their subunits making up the Rel family (Torchinsky & Toder 2004), including NFκB-1 (p50 and p105), NFκB2 (p52 and p100), Rel-A (p65), c-Rel, and Rel-B (Schulze-Luehrmann & Ghosh 2006). Function of NF-κB as a transcription factor exists in almost all mammalian cells (Herrington et al. 2016). Activity of NF-κB can be increased by inflammation and oxidative stress conditions, and vice versa (Mitchell et al. 2016). Women with preeclampsia showed higher NF-κB expression in the maternal circulation and placenta than control pregnancies (Silva Carmona & Mendieta Zerón 2016, Litang et al. 2017).

MicroRNAs (miRNAs) are noncoding RNAs at the length of around 18-22 nucleotides. Differentially expressed miRNAs are characteristic of preeclampsia. MiRNAs target signaling pathway-related genes, altering the preeclampsia-involved biological processes in many conditions. Dysregulated miRNAs control trophoblast proliferation and invasion, angiogenesis, regulates the immunome system as well as other essential aspects of placentation, which makes them serve as promising diagnostic tool and therapeutic target for preeclampsia (Skalis et al. 2019). A previous study revealed that late-onset mild preeclampsia may have no placenta-specific causal factors but associated maternal factors with distinct regulators driving the different molecular pathways (Ren et al. 2021). Whereas other studies revealed that miRNAs are putative preeclampsia-specific biomarkers and can differentiate early onset preeclampsia and late onset preeclampsia from uncomplicated placentas (Lykoudi et al. 2018, Kolkova et al. 2021, Demirer et al. 2020). MiR-518a-5p is overexpressed in 11 placentas with early onset preeclampsia complicated compared with 8 healthy controls (Lykoudi et al. 2018). We made a hypothesis that miR-518a-5p can be activated by NF-κB in preeclampsia and leads to the dysregulated functions of trophoblast.

MATERIALS AND METHODS

Placental tissue collection

Term placentas were collected from healthy (n = 4) and pre-eclamptic women (n = 4) after cesarean birth in the Affiliated Huaian No.1 People's Hospital of Nanjing Medical University. The placentas are collected from decidua region far from the umbilical cord insertion. Preeclamptic women were at the age of 27-33 years old and delivered at 38-40 weeks. No participants had chronic hypertension, obesity, gestational diabetes, or eclampsia. The basic clinical characteristics of the participants in this study are provided in Table I. All participants had signed the written consents to donate placenta for the present study. All placental tissues were immediately kept in liquid nitrogen and then transferred to a laboratory refrigerator (−80°C). This study was granted by the Ethic Committee of the Affiliated Huaian No.1 People's Hospital of Nanjing Medical University.

In situ hybridization

MiR-518a-5p expression in pre-eclamptic placentas was assessed by *in situ* hybridization. Four placentas from each group were used for in situ hybridization. Each placenta was cut into 3-6 fragments, and about a third of the tissue is used for pathological analysis. Tissues were fixed on formalin, embedded in paraffin, cut into 5 μm sections, and deparaffinized with gradient concentration of ethanol (100%, 95%, 75%, 50%). miRCURY LNA miRNA Detection Probe specific to miR-518a-5p and the miRCURY LNA Optimization Kit (Qiagen, Germany) were used according to the manufacturer's instructions. The stained tissues were observed under a computer-connected light microscope.

Table I. Clinical characteristics of the selected pregnant women.

Subjects	Age (years)	Gestational age (weeks)	Systolic blood pressure (mm Hg)	Newborn weight (g)
P1	27	38	95	2034
P2	31	39	96	1846
P3	33	39	92	2184
P4	29	40	95	2378
N1	29	39	65	3853
N2	26	39	67	3475
N3	32	39	67	3221
N4	32	40	66	3385

P indicates pregnant women with preeclampsia; N indicates normal pregnant women.

Cell culture and treatment

HTR8/SVneo cell line (#CRL-3271, ATCC), derived from the invasive extravillous cytotrophoblast cells (EVT), was used in this study. HTR8/SVneo cells were cultured in DMEM supplemented with 10% FBS, 100 IU/ml penicillin, and 100 mg/ml streptomycin. The incubation atmosphere is 5% CO_2, 90% humidity, and 37°C. For gelatin zymography assay, HTR8/SVneo was cultured in serum-free media. To enhance or suppress the functions of endogenous miR-518a-5p, HTR8/SVneo cells were transfected with 50 nM miR-518a-5p inhibitor, 50 nM miR-518a-5p mimics, or their scrambled negative controls (NC) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) at room temperature for 12 h. All oligonucleotides were purchased from GenePharma (Shanghai, China). The transfection efficiency was more than 90%. Moreover, an NF-κB inhibitor, 4-N-[2-(4-phenoxyphenyl)ethyl] quinazoline-4,6-diamine (QNZ) (CAS: 545380-34-5; #ab141588, Abcam) was used to treat HTR8/SVneo for 12 h at the concentration of 2 nmol/L.

Chromatin immunoprecipitation (ChIP)

A ChIP assay kit (#P2078, Beyotime, Shanghai, China) was used according to the manufacturer's protocols. HTR8/SVneo cells were cross-linked with 1% formaldehyde for 12 min and sonicated into DNA fragments of 200 and 1000 bp. Cell lysates were incubated with the NF-κB antibodies (Abcam) that were coated with protein A/G beads at 4°C overnight. The goat-anti-rabbit IgG served as a negative control. The immunocomplexes that were bound to protein A/G beads were then eluted with elution buffer to remove the non-specific binding. Samples were treated with 5 M NaCl and heated at 65°C overnight to eliminate histone-DNA crosslinks. Next, proteinase K was added followed by incubation at 45°C for 2 h. A DNA Extraction Kit (BIO-RAD) was used to purify the bound DNA fragments. Products were finally analyzed by real-time PCR using the primers specific to miR-518a-5p promoter.

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was used to detect miR-518a-5p expression in HTR8/SVneo cell line after QNZ treatment. Primers for miR-518a-5p were purchased from Invitrogen (Carlsbad, CA). A TaqMan MicroRNA Reverse Transcription Kit and a TaqMan Universal Master Mix II (Applied Biosystems, CA, USA) were used for reverse transcription and miRNA amplification, respectively. qRT-PCR was conducted with an Applied Biosystems 7900HT Fast Real-Time PCR System. Expression of snRNA U6 was also assessed to serve as a loading control. Relative

miR-518a-5p expression was calculated using the $2^{-\Delta\Delta CT}$ method (Livak & Schmittgen 2001). CT refers to the number of fractional cycle when the signal passes a fixed threshold.

Cell apoptosis assay

HTR8/SVneo cells were stained with Annexin V/Prodium Iodide (PI) to measure cell apoptosis using a commercial kit (#40302ES20, YEASEN, Shanghai, China) according to the manufacturer's instructions. In brief, 5×10^5 cells were resuspended in 100 µL room temperature in the dark and added with 400 µL binding buffer on ice. Cell apoptosis was analyzed by flow cytometry using a BD FACSCalibur™ flow cytometer (BD Biosciences, Switzerland). Apoptotic cells (%) were defined as the percentage of cells in the third quadrant (late apoptosis) of total cells.

Cell viability assay

Cells were plated in a 96-well plate at the concentration of 5000 cells/well. After transfection of miR-518a-5p inhibitor/mimics for 12 h, cell viability was detected using a Cell Counting Kit-8 (Dojindo, Tokyo, Japan) according to the manufacturer's instructions. Optical density was detected by assessing cell absorbance of 450 nm using a microplate reader (SpectraMax i3x, Molecular Devices).

Migration and invasion assays

Cell invasion was assessed using Matrigel (Solarbio)-coated Transwell inserts (Costar) that contain polycarbonate filters (pore size: 8 mm). The inserts were pre-coated with Matrigel matrix (1 mg/ml; 50 µL) at 37°C for 4 h. Approximate 1×10^5 HTR8/SVneo cells in serum-free medium (200 µL) were transfected with miR-518a-5p inhibitor or mimics for 24 h and then placed in the upper chamber. The bottom chamber was added with medium containing 10% FBS. Cells on the Matrigel side of the Transwell insert were wiped by a cotton swab after 24 h of incubation. After fixation with methanol for 10 min, the remaining cells were stained with crystal violet (Beyotime, China) at room temperature. An Olympus IX51 light microscope was used to observe the cells in five random fields. To assess cell migration, similar methods were used except that Matrigel was not used.

Gelatin zymography

HTR8/SVneo was cultured in serum-free media and underwent transfection for 12 h. Culture media were collected for measuring the activities of gelatinases matrix metalloproteinase (MMP)-2 and MMP-9 using gelatin zymography. The 10% SDS-PAGE containing 0.1% gelatin (BIO-RAD) was used. After being diluted in NuPAGE™ LDS sample buffer (4×) containing LDS (pH 8.5), SERVA Blue G250, and phenolic red, the conditioned medium was incubated at 37°C for half an hour. The gel was washed with elution buffer twice, 40 min per washing, at room temperature after electrophoresis. Next, samples were incubated in calcium assay buffer (ab182458, Abcam) at 37°C for one day. Coomassie Brilliant Blue R250 (#20278, Thermo Scientific) was used to stain the gel for 3 h. Finally, gels were treated with the destaining solution 10% acetic acid (#984303, Thermo Scientific) for 1 h.

Western blotting

Proteins were extracted from HTR8/SVneo cells using RIPA lysis buffer (#89901, Thermo Scientific) containing a protease inhibitor cocktail (#5871, Cell Signaling Technology). A Microcon® centrifugal filter (Merck) was used to concentrate the proteins. The Bradford standard was used to quantitate protein concentration using a Beckman DU 530 UV/Vis Spectrophotometer with λ = 595 nm. Loaded proteins (30 µg/per well) were separated by 8% SDS-PAGE and then electrophoretically transferred onto a pure

nitrocellulose blotting membrane (0.45 μm, BIO-RAD). After blocking in normal goat serum for 20 min at room temperature, the membrane was incubated with primary antibodies against TIMP-1 (1/100, ab211926), TIMP-2 (1/500, ab180630), cleaved caspase 3 (1/500, ab32042), NF-κB p65 (1/1000, ab32536), NF-κB p65 (phospho S536) (1/2000, ab76302), NF-κB p105/p50 (1/1000, ab32360), NF-κB p105/p50 (phospho S337) (1/500, ab28849), NF-κB p100/NFKB2 (1/10000, ab175192), NF-κB p100/NFKB2 (phospho S865) (ab31474) and GAPDH (1/500, ab37187) overnight at 4°C, and washed with TBST thrice, 10 min per washing. Subsequently, samples were incubated with the HRP-labeled secondary antibody anti-IgG for 2 h at 37°C. All antibodies were commercially obtained from Abcam (Shanghai, China). Immunoreactive bands were detected using Bio-Rad Launches Clarity Max™ Western ECL Substrate.

Data analysis

GraphPad software v7.0 was used for statistical analysis and graph drawing. The significance of intergroup differences was calculated using the student's *t* test, or analysis of variance (ANOVA) as appropriate. All results are exhibited as the mean ± standard deviation from three independent biological and technical experiments. A probability level of < 0.05 indicates statistical significance.

RESULTS

NF-κB-induced miR-518a-5p is overexpressed in preeclamptic placenta and induces the NF-κB pathway

MiR-518a-5p is expressed in preeclamptic placentas, and its expression is higher in preeclamptic placentas than normal control placentas. MiR-518a-5p majorly exists in capsular decidua of preeclamptic placenta (Figure 1a). According to the prediction from Jaspar, three NF-κB subunits, p50, p52, and p65 can transcriptionally activate miR-518a-5p, and the potential binding sequences were provided in Supplementary material: Table II, III, and IV, respectively. A ChIP assay was conducted to confirm the binding of p50, p52, and p65 and miR-518a-5p promoter. The results revealed that NF-κB1, NF-κB2, RELA can all bind with miR-518a-5p promoter in preeclamptic placentas (Figure 1b) and in HTR8/SVneo cells (Figure 1c). After treatment with QNZ, miR-518a-5p expression was reduced (Figure 1d). Figure 1e showed that miR-518a-5p mimics increased the expression of p-p50, p50, p-p65, p65 while miR-518a-5p inhibitor suppressed these proteins. However, miR-518a-5p had no influences on p52 protein expression.

MiR-518a-5p inhibits the gelatinolytic activities of MMP-2/9, and enhances the protein expression of TIMP-1/2

Effects of miR-518a-5p on the apoptosis and viability of HTR8/SVneo cells were detected. MiR-518a-5p caused no significant effects on apoptotic cell rate (Figure 2a-b) nor cleaved caspase-3 protein expression (Figure 2c). The effect of miR-518a-5p on HTR8/SVneo cell viability was also negligible (Figure 2d). MMP-2 and -9 are implicated in remodeling extracellular matrix during the process of trophoblast invasion (Chen & Khalil 2017). The spent medium from the miR-518a-5p mimics treated HTR8/SVneo cells exhibited lower MMP-2/9 activities compared with NC mimics group (Figure 2e). Correspondingly, miR-518a-5p inhibitor increased MMP-2/9 activities. Furthermore, we observed that miR-518a-5p inhibitor decreased TIMP-1 and TIMP-2 proteins while miR-518a-5p mimics caused the opposite results (Figure 2e).

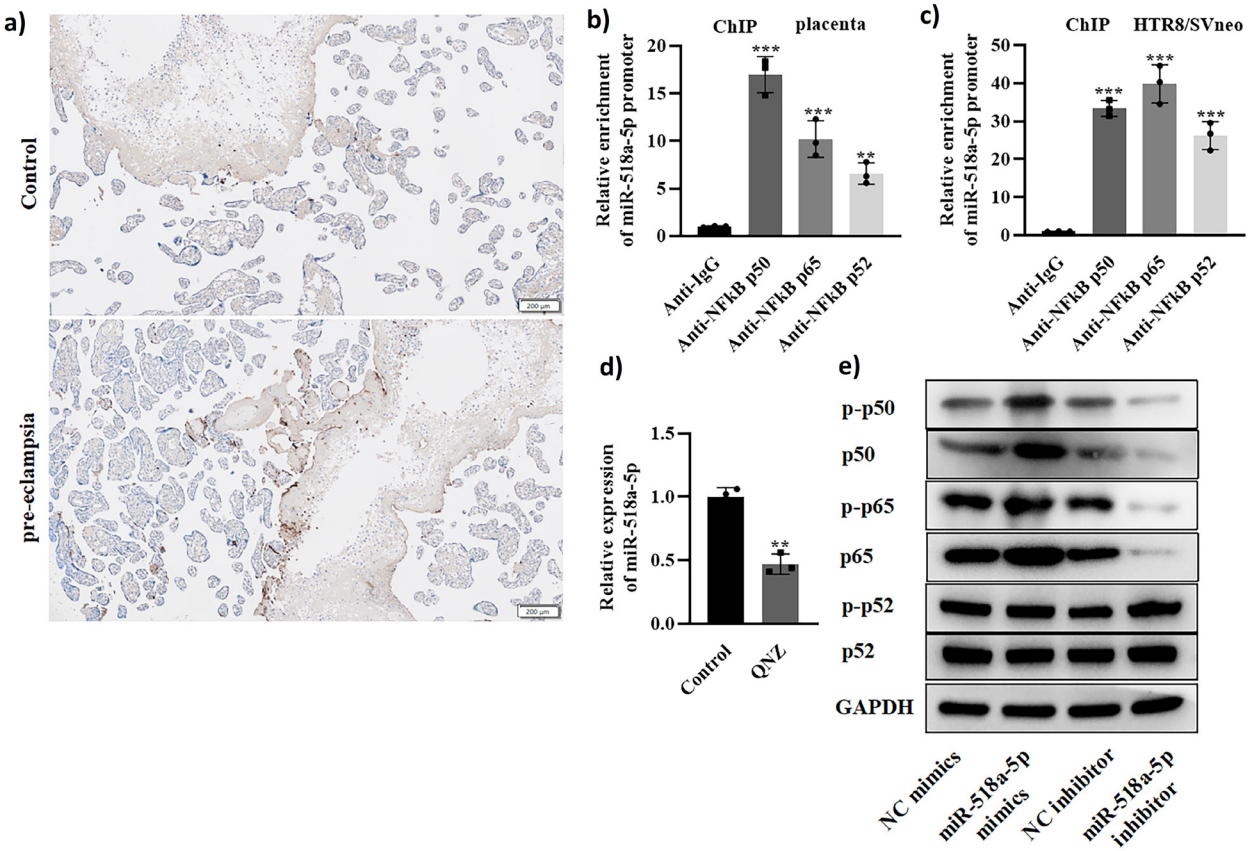

Figure 1. NF-κB-induced miR-518a-5p is overexpressed in preeclamptic placenta and induces the NF-κB pathway. **a)** MiR-518a-5p expression in normal (n = 4) and preeclamptic (n = 4) placenta was revealed by In Situ Hybridization. **b)** and **c)** Enrichment of miR-518a-5p promoter precipitated by anti-NF-kB p50, anti-NF-kB p65, and anti-NF-kB p52 in preeclamptic placenta (n = 4) and HTR8/SVneo cells (n = 3) was measured by ChIP assays followed by PCR. **p<0.01, ***p<0.001 vs anti-IgG. One way ANOVA followed by Dunnett's *post hoc* test was performed. **d)** MiR-518a-5p in HTR8/SVneo cells after treatment of QNZ was detected by PCR and normalized to U6. **p<0.01 vs Control. Student's *t* test was performed. **e)** NFkB p50, p65, p52 and their phosphorylated protein levels in HTR8/SVneo cells after treatment of miR-518a-5p mimics or inhibitor. N = 3 for the *in vitro* assays.

Table II. Binding of NFKB1 on miR-518a-5p promoter.

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence
MA0105.1	MA0105.1.NFKB1	10.111912	0.8804406115450805	seq1	1190	1199	+	gggattctcc
MA0105.1	MA0105.1.NFKB1	9.475084	0.8650183998769424	seq1	794	803	-	ggaattttcc
MA0105.1	MA0105.1.NFKB1	9.385446	0.8628475965947501	seq1	794	803	+	ggaaaattcc
MA0105.1	MA0105.1.NFKB1	8.369567	0.838245806066981	seq1	1190	1199	-	ggagaatccc
MA0105.1	MA0105.1.NFKB1	8.17706	0.833583820504899	seq1	639	648	-	ggggcaatcc
MA0105.1	MA0105.1.NFKB1	8.17706	0.833583820504899	seq1	1804	1813	-	ggggcaatcc
MA0105.1	MA0105.1.NFKB1	7.4274406	0.8154300961178544	seq1	124	133	+	gggagtttcg

Data were derived from Jaspar database. Relative profile score threshold is set as 80%.

MiR-518a-5p is a negative regulator of HTR8/SVneo cell migration and invasion

Number of migrated and invaded HTR8/SVneo cells was increased by miR-518a-5p inhibitor and decreased by miR-518a-5p mimics (Figure 3a-b), suggesting the role of miR-518a-5p in hindering trophoblast migration and invasion.

Table III. Binding of NFKB2 on miR-518a-5p promoter.

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence
MA0778.1	MA0778.1.NFKB2	6.661019	0.8316767346185034	seq1	1188	1200	+	aagggattctcct
MA0778.1	MA0778.1.NFKB2	4.274734	0.8037880592976412	seq1	123	135	+	agggagtttcgct
MA0778.1	MA0778.1.NFKB2	4.166825	0.8025269170068217	seq1	1188	1200	-	aggagaatccctt
MA0778.1	MA0778.1.NFKB2	4.0342913	0.8009779882667432	seq1	123	135	-	agcgaaactccct

Data were derived from Jaspar database. Relative profile score threshold is set as 80%.

Table IV. Binding of RELA on miR-518a-5p promoter.

Matrix ID	Name	Score	Relative score	Sequence ID	Start	End	Strand	Predicted sequence
MA0107.1	MA0107.1.RELA	9.973761	0.8614229851854865	seq1	124	133	+	gggagtttcg
MA0107.1	MA0107.1.RELA	9.531278	0.8498439531513128	seq1	795	804	-	tggaattttc
MA0107.1	MA0107.1.RELA	8.677542	0.8275031247692668	seq1	794	803	-	ggaattttcc
MA0107.1	MA0107.1.RELA	8.677542	0.8275031247692668	seq1	1190	1199	+	gggattctcc
MA0107.1	MA0107.1.RELA	8.623042	0.8260769615248453	seq1	794	803	+	ggaaaattcc
MA0107.1	MA0107.1.RELA	8.403238	0.8203250667493103	seq1	1136	1145	+	tggagtgtcc

Data were derived from Jaspar database. Relative profile score threshold is set as 80%.

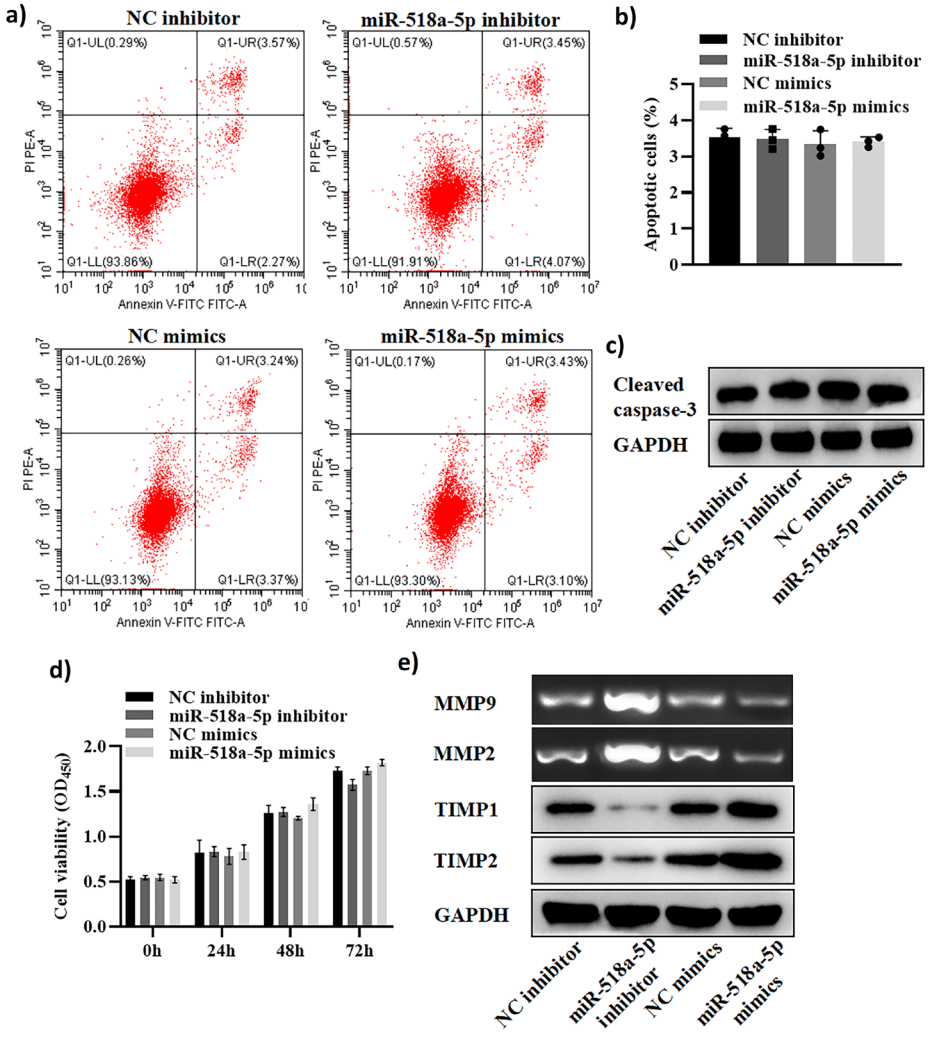

Figure 2. MiR-518a-5p inhibits the gelatinolytic activities of MMP-2/9, and enhances the protein expression of TIMP-1/2. **a)** and **b)** Flow cytometry apoptosis analysis of HTR8/SVneo cells after treatment of miR-518a-5p mimics or inhibitor. One way ANOVA followed by Tukey's *post hoc* test was performed. **c)** Cleaved caspase 3 protein expression in HTR8/SVneo cells after transfection with miR-518a-5p mimics or inhibitor. **d)** Cell viability of HTR8/SVneo cells was assessed by CCK-8. Two way ANOVA was performed. **e)** Gelatinolytic activities of MMP-2 and MMP-9 were measured by gelatin zymography; TIMP1 and TIMP1 protein levels were assessed by western blotting. N = 3 for each assay.

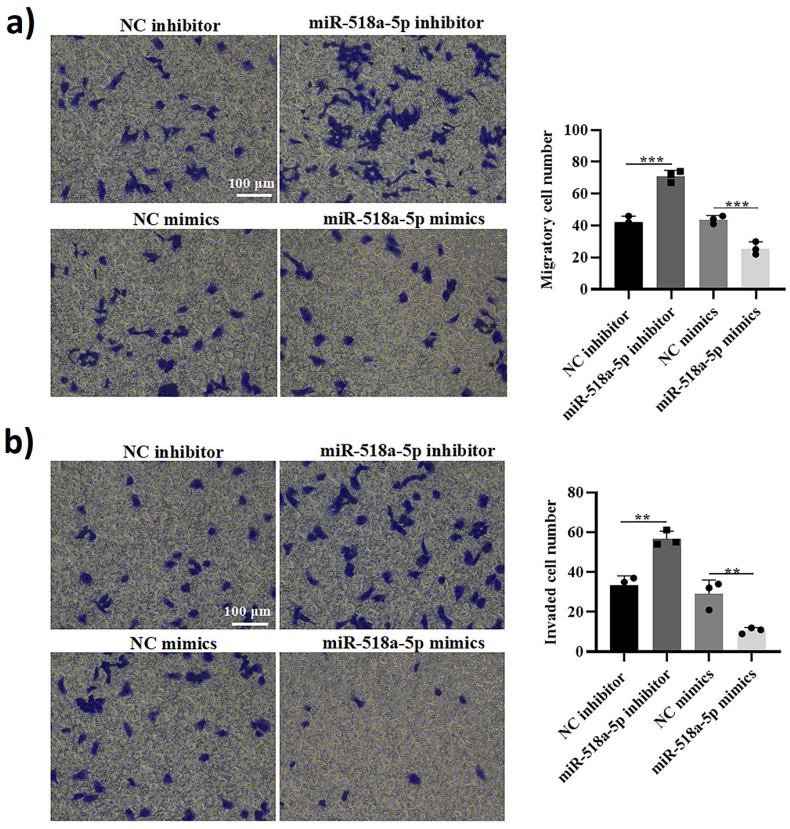

Figure 3. MiR-518a-5p is a negative regulator of HTR8/SVneo cell migration and invasion. a) and b) Migration and invasion of HTR8/SVneo cells after treatment of miR-518a-5p mimics or inhibitor were revealed using Transwell inserts that were pre-coated Matrigel or not. One way ANOVA followed by Tukey's *post hoc* test was performed. **p<0.01, ***p<0.001. N = 3 for each assay.

QNZ reverses the effects of miR-518a-5p on MMP-2/9 activities, migration, and invasion of HTR8/SVneo cells

The suppressive effects of miR-518a-5p mimics on MMP-2/9 activities and on TIMP-1/2 protein expression were rescued by QNZ (Figure 4a-b). Moreover, QNZ reverses the repressive effects of miR-518a-5p mimics on the migration and invasion of HTR8/SVneo cells (Figure 4c-d). These findings indicated that miR-518a-5p reduced HTR8/SVneo cell motility by the NF-κB pathway.

DISCUSSION

The miR-518 family is a special biomarker of the placenta (Yang et al. 2019). Hromadnikova et al. (2015) detected the decreased expression of miR-518f-5p in placentas of 36 fetal growth restriction pregnancies. A study revealed the downregulation of miR-518b in 30 fetal growth restriction placentas (Wang et al. 2014) while another study found the elevated miR-518b expression during early gestation in 7 pregnancies with later onset of preeclampsia (Hromadnikova et al. 2012). MiR-518a-5p is upregulated in preeclamptic placenta tissues (Inno et al. 2021, Lykoudi et al. 2018) or plasma (Yang et al. 2015), while its functions on trophoblasts were not studied. MiR-518a-5p has the potential to suppress diffuse large B cell lymphoma cell line proliferation and invasion (Huang et al. 2021), while miR-518a-5p induces the migration and invasion of cancer cancer cells (Qian et al. 2019). In the present study, we identified the negative influences of miR-518a-5p on the migration and invasion of HTR8/SVneo cells and revealed that miR-518a-5p has no significant effects on the apoptosis and viability of HTR8/SVneo cells.

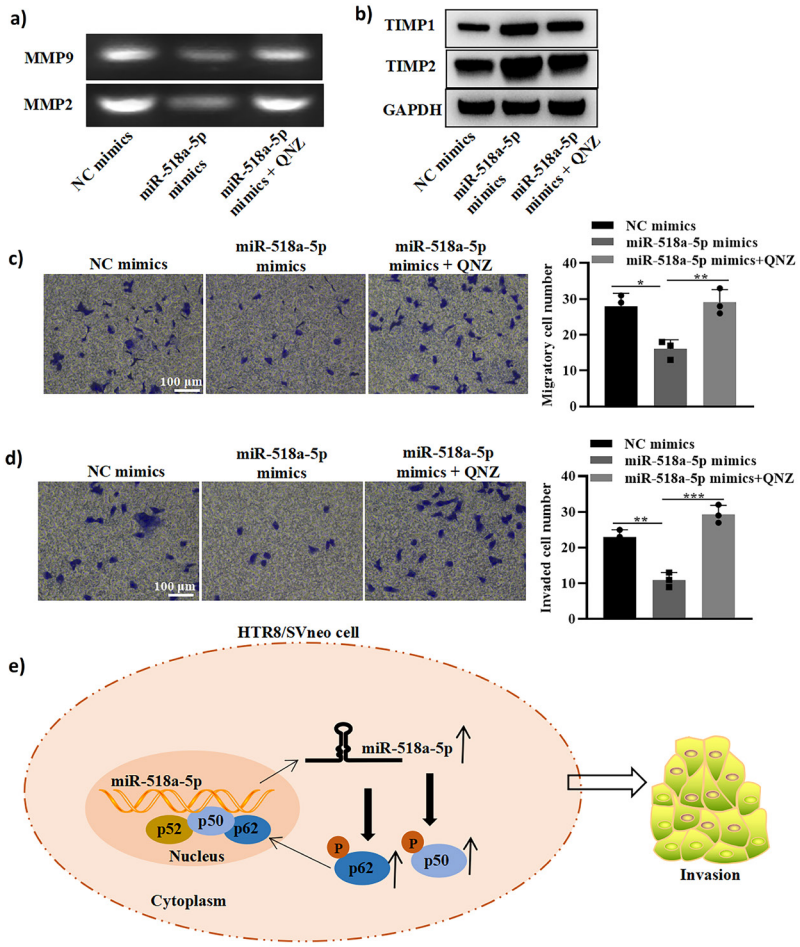

Figure 4. QNZ reverses the effects of miR-518a-5p on MMP-2/9 activities, migration, and invasion of HTR8/SVneo cells. **a)** Gelatinolytic activities of MMP-2 and MMP-9 under the influences of miR-518a-5p mimics + QNZ were measured by gelatin zymography. **b)** Western blotting of TIMP1 and TIMP1 proteins. **c)** and **d)** Migration and invasion of HTR8/SVneo cells after treatment of miR-518a-5p mimics or miR-518a-5p mimics + QNZ were revealed using Transwell inserts. **e)** A graphical abstract: miR-518a-5p is transcriptionally induced by NF-κB and represses trophoblast cell invasion by the NF-κB pathway. One way ANOVA followed by Tukey's *post hoc* test was performed. *p<0.05, **p<0.01, ***p<0.001. N = 3 for each assay.

Degradation of extra-cellular matrix promotes EVT invasion in human placenta. MMPs are secreted from the cell and can degrade the extra-cellular matrix (Hiden et al. 2018). TIMPs inhibit MMPs activities in the extracellular space (Librach et al. 1991). Migratory trophoblasts express MMPs (Lala & Chakraborty 2003), while decidua produces TIMPs (Schatz & Lockwood 1993) to restrict invasiveness. In this study, miR-518a-5p decreased the gelatinolytic activities of MMP-2 and MMP-9 in the culture medium of HTR8/SVneo. Secretion of TIMP-1/2 was increased in HTR8/SVneo cells by overexpressing miR-518a-5p. However, whether miR-518a-5p directly targets these MMPs and TIMPs remains unknown, and the underling mechanisms need further investigation.

We also found that NF-κB p50, p52, and p65 can bind to miR-518a-5p promoter, which indicates that miR-518a-5p is transcriptionally activated by NF-κB, explaining that the upregulation of miR-518a-5p in preeclamptic tissues is caused by NF-κB. Furthermore, miR-518a-5p has a positive effect on the NF-κB pathway by regulating p50, p52, and their phosphorylated levels. NFκB induces the secretion of cytokines including interleukin-6 and interleukin-8 from cells to partially regulate EVT invasion in an autocrine and paracrine manner (Pollheimer et al. 2018). NF-κB increases the expression of MMP-2 and -9 during early pregnancy (Tabruyn & Griffioen 2008, Liu et al. 2018). A study revealed that the invasion and migration of HTR-8/SVneo can be induced by activation of NF-κB, which upregulates MMP-9 expression (Liu et al. 2018). Tumor necrosis factor

induces MMP9 secretion and activates the NF-κB pathway in human chorionic trophoblast cells (Li et al. 2010). In our study, the NF-κB inhibitor QNZ rescued the influences of miR-518a-5p on MMP-2/9 and TIMP-1/2 expression and on the migration/invasion of HTR8/SVneo cells, indicating that miR-518a-5p decreased HTR8/SVneo cell invasion by the NF-κB pathway.

In conclusion, this study confirms the upregulation of miR-518a-5p in human preeclamptic placentas, reveals a vital role for miR-518a-5p in suppressing the migration and invasion of HTR8/SVneo trophoblast, and supports the NF-κB/miR-518a-5p feedback as a possible mechanism of preeclampsia.

REFERENCES

ACOG – AMERICAN COLLEGE OF OBSTETRICS AND GYNECOLOGY. 2019. ACOG Practice Bulletin No. 202 Summary: Gestational Hypertension and Preeclampsia. Obstet Gynecol 133: 1.

AHMED A, REZAI H & BROADWAY-STRINGER S. 2017. Evidence-Based Revised View of the Pathophysiology of Preeclampsia. Adv Exp Med Biol 956: 355-374.

ARMISTEAD B, KADAM L, DREWLO S & KOHAN-GHADR HR. 2020. The Role of NFκB in Healthy and Preeclamptic Placenta: Trophoblasts in the Spotlight. Int J Mol Sci 21(5): 1775.

CHEN J & KHALIL RA. 2017. Matrix Metalloproteinases in Normal Pregnancy and Preeclampsia. Prog Mol Biol Transl Sci 148: 87-165.

CHIARELLO DI, ABAD C, ROJAS D, TOLEDO F, VÁZQUEZ CM, MATE A, SOBREVIA L & MARÍN R. 2020. Oxidative stress: Normal pregnancy versus preeclampsia. Biochim Biophys Acta Mol Basis Dis 1866: 165354.

DEMIRER S, HOCAOGLU M, TURGUT A, KARATEKE A & KOMURCU-BAYRAK E. 2020. Expression profiles of candidate microRNAs in the peripheral blood leukocytes of patients with early- and late-onset preeclampsia versus normal pregnancies. Pregnancy Hypertens 19: 239-245.

HERRINGTON FD, CARMODY RJ & GOODYEAR CS. 2016. Modulation of NF-κB Signaling as a Therapeutic Target in Autoimmunity. J Biomol Screen 21: 223-242.

HIDEN U, EYTH CP, MAJALI-MARTINEZ A, DESOYE G, TAM-AMERSDORFER C, HUPPERTZ B & GHAFFARI TABRIZI-WIZSY N. 2018. Expression of matrix metalloproteinase 12 is highly specific for non-proliferating invasive trophoblasts in the first trimester and temporally regulated by oxygen-dependent mechanisms including HIF-1A. Histochem Cell Biol 149: 31-42.

HROMADNIKOVA I, KOTLABOVA K, DOUCHA J, DLOUHA K & KROFTA L. 2012. Absolute and relative quantification of placenta-specific microrna s in maternal circulation with placental insufficiency-related complications. J Mol Diagn 14: 160-167.

HROMADNIKOVA I, KOTLABOVA K, ONDRACKOVA M, PIRKOVA P, KESTLEROVA A, NOVOTNA V, HYMPANOVA L & KROFTA L. 2015. Expression profile of C19MC microRNAs in placental tissue in pregnancy-related complications. DNA Cell Biol 34: 437-457.

HUANG Q, ZHANG F, FU H & SHEN J. 2021. Epigenetic regulation of miR-518a-5p-CCR6 feedback loop promotes both proliferation and invasion in diffuse large B cell lymphoma. Epigenetics 16: 28-44.

INNO R, KIKAS T, LILLEPEA K & LAAN M. 2021. Coordinated Expressional Landscape of the Human Placental miRNome and Transcriptome. Front Cell Dev Biol 9: 697947.

KINTIRAKI E, PAPAKATSIKA S, KOTRONIS G, GOULIS DG & KOTSIS V. 2015. Pregnancy-Induced hypertension. Hormones (Athens) 14: 211-223.

KOLKOVA Z, HOLUBEKOVA V, GRENDAR M, NACHAJOVA M, ZUBOR P, PRIBULOVA T, LODERER D, ZIGO I, BIRINGER K & HORNAKOVA A. 2021. Association of Circulating miRNA Expression with Preeclampsia, Its Onset, and Severity. Diagnostics (Basel) 11(3): 476.

LALA PK & CHAKRABORTY C. 2003. Factors regulating trophoblast migration and invasiveness: possible derangements contributing to pre-eclampsia and fetal injury. Placenta 24: 575-587.

LI W, LI H, BOCKING AD & CHALLIS JR. 2010. Tumor necrosis factor stimulates matrix metalloproteinase 9 secretion from cultured human chorionic trophoblast cells through TNF receptor 1 signaling to IKBKB-NFKB and MAPK1/3 pathway. Biol Reprod 83: 481-487.

LIBRACH CL, WERB Z, FITZGERALD ML, CHIU K, CORWIN NM, ESTEVES RA, GROBELNY D, GALARDY R, DAMSKY CH & FISHER SJ. 1991. 92-kD type IV collagenase mediates invasion of human cytotrophoblasts. J Cell Biol 113: 437-449.

LITANG Z, HONG W, WEIMIN Z, XIAOHUI T & QIAN S. 2017. Serum NF-κBp65, TLR4 as Biomarker for Diagnosis of Preeclampsia. Open Med (Wars) 12: 399-402.

LIU J, LV SS, FU ZY & HOU LL. 2018. Baicalein Enhances Migration and Invasion of Extravillous Trophoblasts

via Activation of the NF-κB Pathway. Med Sci Monit 24: 2983-2991.

LIVAK KJ & SCHMITTGEN TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408.

LYKOUDI A, KOLIALEXI A, LAMBROU GI, BRAOUDAKI M, SIRISTATIDIS C, PAPAIOANOU GK, TZETIS M, MAVROU A & PAPANTONIOU N. 2018. Dysregulated placental microRNAs in Early and Late onset Preeclampsia. Placenta 61: 24-32.

MICHALCZYK M, CELEWICZ A, CELEWICZ M, WOŹNIAKOWSKA-GONDEK P & RZEPKA R. 2020. The Role of Inflammation in the Pathogenesis of Preeclampsia. Mediators Inflamm 2020: 3864941.

MITCHELL S, VARGAS J & HOFFMANN A. 2016. Signaling via the NFκB system. Wiley Interdiscip Rev Syst Biol Med 8: 227-241.

POLLHEIMER J, VONDRA S, BALTAYEVA J, BERISTAIN AG & KNÖFLER M. 2018. Regulation of Placental Extravillous Trophoblasts by the Maternal Uterine Environment. Front Immunol 9: 2597.

QIAN L, GUAN J, WU Y & WANG Q. 2019. Upregulated circular RNA circ_0074027 promotes glioblastoma cell growth and invasion by regulating miR-518a-5p/IL17RD signaling pathway. Biochem Biophys Res Commun 510: 515-519.

REN Z ET AL. 2021. Distinct placental molecular processes associated with early-onset and late-onset preeclampsia. Theranostics 11: 5028-5044.

SCHATZ F & LOCKWOOD CJ. 1993. Progestin regulation of plasminogen activator inhibitor type 1 in primary cultures of endometrial stromal and decidual cells. J Clin Endocrinol Metab 77: 621-625.

SCHULZE-LUEHRMANN J & GHOSH S. 2006. Antigen-receptor signaling to nuclear factor kappa B. Immunity 25: 701-715.

SILVA CARMONA A & MENDIETA ZERÓN H. 2016. NF-κB and SOD expression in preeclamptic placentas. Turk J Med Sci 46: 783-788.

SKALIS G, KATSI V, MILIOU A, GEORGIOPOULOS G, PAPAZACHOU O, VAMVAKOU G, NIHOYANNOPOULOS P, TOUSOULIS D & MAKRIS T. 2019. MicroRNAs in Preeclampsia. Microrna 8: 28-35.

TABRUYN SP & GRIFFIOEN AW. 2008. NF-kappa B: a new player in angiostatic therapy. Angiogenesis 11: 101-106.

TORCHINSKY A & TODER V. 2004. To die or not to die: the function of the transcription factor NF-kappaB in embryos exposed to stress. Am J Reprod Immunol 51: 138-143.

WANG D, NA Q, SONG WW & SONG GY. 2014. Altered Expression of miR-518b and miR-519a in the placenta is associated with low fetal birth weight. Am J Perinatol 31: 729-734.

YANG S, LI H, GE Q, GUO L & CHEN F. 2015. Deregulated microRNA species in the plasma and placenta of patients with preeclampsia. Mol Med Rep 12: 527-534.

YANG W, LU Z, ZHI Z, LIU L, DENG L, JIANG X & PANG L. 2019. Increased miRNA-518b inhibits trophoblast migration and angiogenesis by targeting EGR1 in early embryonic arrest†. Biol Reprod 101: 664-674.

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