

miR-342-3p suppresses cell migration and invasion in preeclampsia by targeting platelet-derived growth factor receptor α

XIUHUA YANG¹ and FENG GUO²

¹Department of Obstetrics, The First Hospital of China Medical University, Shenyang, Liaoning 110001;

²Department of Emergency, Shengjing Hospital of China Medical University, Shenyang, Liaoning 110004, P.R. China

Received February 4, 2018; Accepted May 17, 2019

DOI: 10.3892/mmr.2019.10372

Abstract. miR-342-3p expression was increased in the placentas of women with preeclampsia (PE) according to previous examinations; the mechanism underlying the development and progression of PE requires further investigation. The present study aimed to explore the mechanism and functionality of microRNA (miR)-342-3p in trophoblastic cells. The expression of miR-342-3p and platelet-derived growth factor receptor α (PDGFRA) in the placentas of 30 patients with PE and 30 normal controls was detected. In addition, HTR8/SVneo cells were transfected with miR-342-3p mimics, small interfering RNA (siR)-PDGFRA or their corresponding negative controls; then the proliferation, migration, invasion and the distribution of the cell cycle of these cells were analyzed. Additionally, a dual-luciferase reporter assay was performed. According to these analyses, the expression of miR-342-3p was significantly increased, while that of PDGFRA was significantly lower in the PE group compared with the normal group. Transfection with miR-342-3p mimics led to a significant decrease in cell proliferation, migration and invasion, and also affected the cell cycle. Furthermore, miR-342-3p mimics reduced the expression of PDGFRA; miR-342-3p overexpression also reduced the mRNA and protein levels of BCL-2 and Caspase-3. In addition, transfection of siR-PDGFRA exhibited similar effects to those of miR-342-3p mimics. Finally, PDGFRA was reported to be a direct target of miR-342-3p.

In conclusion, miR-342-3p was proposed to inhibit the proliferation, migration, invasion and G1/S phase transition of HTR8/SVneo cells by suppressing PDGFRA. Our findings suggest that miR-342-3p may be a novel clinical indicator or prognostic marker for PE.

Introduction

Preeclampsia (PE) is a serious obstetric complication, and poses a serious threat to pregnant women and fetuses (1). The incidence of PE around the world is 2-8% (2). At present, PE is one of the main causes of maternal mortality. The pathogenesis of PE is not fully understood; thus, as an effective treatment for PE, pregnancies may be terminated. At present, PE is considered to be associated with dysfunction of the placenta (3), endothelial dysfunction (4) and abnormal increases in the apoptosis of trophoblasts (5). Of note, increased migration and invasion of trophoblastic cells, and decreased apoptosis have been hypothesized as potential therapeutic strategies for the treatment of PE; however, further investigation is required.

MicroRNAs (miRNAs/miRs) regulate the expression of their target genes in a negative manner. Numerous studies have shown that miRNAs are associated with cell migration, apoptosis, differentiation and proliferation (6-9). It was reported that the expression profiles of miRNAs in PE were markedly diverse in umbilical cord blood, maternal serum, placental samples and mesenchymal stem cells (MSCs) (10-14). miR-342-3p is one of the most highly expressed miRNAs in placentas from patients with PE (15), but its biological mechanism in PE remains unknown. miR-342-3p has been proposed to inhibit cell migration in cervical cancer (16), and suppress cell invasion and metastasis in lung cancer (17). Additionally, miR-342-3p may inhibit the progression of hepatocellular carcinoma through the nuclear factor- κ B pathway (18). These findings suggest that miR-342-3p may serve vital roles in trophoblastic cell proliferation, migration and invasion in PE.

Gene expression profiling revealed that the expression levels of platelet-derived growth factor receptor α (PDGFRA) were decreased in PE patients compared with the normal group (19), yet the mechanism of PDGFRA in PE remains

Correspondence to: Dr Xiuhua Yang, Department of Obstetrics, The First Hospital of China Medical University, 155 Nanjing North Street, Shenyang, Liaoning 110001, P.R. China
E-mail: 57470040@qq.com

Abbreviations: PE, preeclampsia; miRNA, microRNA; MSCs, mesenchymal stem cells; PDGFRA, platelet-derived growth factor receptor α ; EVT, extravillous trophoblast; MAPK, mitogen-activated protein kinase

Key words: miRNA-342-3p, PDGFRA, PE

unclear. TargetScan, microcosm and miRanda analyses identified PDGFRA as one of the putative target genes for miR-342-3p (20). Further study is needed to determine the association between miR-342-3p and PDGFRA in PE.

In the present study, we investigated the roles of miR-342-3p and PDGFRA in the placental tissues of patients with PE and in HTR8/SVneo cells. In our research, miR-342-3p was reported to reduce the migration and invasion of trophoblastic cells by suppressing PDGFRA. This suggested that miR-342-3p may be associated with the pathology of PE.

Materials and methods

Tissue collection. A total of 30 placentas were obtained from patients with PE and healthy controls who underwent cesarean section from January 2013 to September 2017. The age range of the patients was 23-39 years (mean age, 28.63 ± 2.24 years). The diagnosis of PE was conducted as previously reported (21). The clinical data of patients was present in Table I. Patients with PE were characterized by high blood pressure and a high protein content in the urine. Tissues were quickly snap-frozen in liquid nitrogen during surgery and stored at -80°C until use. Written informed consent was obtained from each patient. The exclusion criteria for the two groups included: Patients with essential hypertension or kidney disease, a history of drug or alcohol abuse half year prior to providing written informed consent. In addition, the patients with PE were pathologically analyzed by two specialists.

Cell culture. HTR8/SVneo cells were acquired from the American Type Culture Collection (ATCC). Cells were cultured in RPMI-1640 (Labdel Comércio de Produtos para Laboratório) and 10% fetal bovine serum (Labdel Comércio de Produtos para Laboratório) and 100 streptomycin mg/ml, 100 penicillin U/ml, in a humidified chamber at 37°C with 5% CO_2 .

Cell transfection. The cell line was transfected with miR-342-3p mimics and the negative control (Thermo Fisher Scientific, Inc., cat. no. 4464058.) at a concentration of 100 nmol/l. The cells were transfected using LipofectamineTM 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). The duration between transfection and subsequent analysis was 48 h. The sequence of the miR-342-3p mimics was 5'-GAAACUGGGCUCAAGGUGAGGGGUGCUAUCUGUGAUUGAGGGACAUGGUUAAUGGAAUUGUCUCACACAGAAUUCGCACCCGUCACCUUGGCCUACUUA-3'.

Knockdown of PDGFRA in the cell line. Small interfering RNAs (siRs) against PDGFRA (siRPDGFRA) and the control (scramble) were purchased from Santa Cruz Biotechnology, Inc. HTR8/SVneo cells were transfected with 20 μM siRPDGFRA or scramble siRNA utilizing Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocols.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from tissues and cells utilizing TRIzol reagent (Thermo Fisher Scientific, Inc.). For detecting mRNAs, a PrimeScriptTM RT reagent kit (Takara Bio, Inc., cat. no. RR047A) was used for RT; the experiment was performed

as follows: Three times at 37°C , 15 min for each time; and inactivation at 85°C for 5 sec. RT was performed for miRNAs using an Mir-XTM miRNA First-Strand Synthesis kit (Takara Bio, Inc, cat. no. 638313) according to the manufacturer's protocols. qPCR was performed using an SYBR[®] qRT-PCR kit (Clontech Laboratories, Inc.) for mRNAs and miRNAs under the following thermocycling conditions: 94°C for 4 min, followed by 40 cycles of 94°C for 30 sec and 60°C for 60 sec. The sequences of primers employed were presented in Table II. Relative miRNA expression levels were standardized to that of small nucleolar RNA U6, whereas that for relative mRNA expression was normalized to the expression levels of β -actin using the $2^{-\Delta\Delta\text{Cq}}$ method (22).

Western blot analysis. Cells were collected 48 h after transfection and prepared for lysis in radioimmunoprecipitation assay buffer (BioVision, Inc.). Protein concentration was determined via a bicinchoninic acid assay. Protein (50 μg /lane) was separated via 8% SDS-PAGE. Separated proteins were transferred to polyvinylidene fluoride membranes (EMD Millipore). The membranes were blocked with 5% fat-free milk powder for 2 h at room temperature, and then incubated with primary antibodies against PDGFRA (1:1,000; Santa Cruz Biotechnology, Inc., cat. no. sc-338), anti-BCL-2 (1:1,000; Abcam, cat. no. ab59348), anti-Caspase-3 (1:500; Abcam, cat. no. ab13847) and β -actin antibody (1:1,000; Santa Cruz Biotechnology, Inc., cat. no. sc-47778) at 4°C overnight. Subsequently, the membranes were incubated with horseradish peroxidase-conjugated antimouse IgG (H+L) (1:10,000; Invitrogen; Thermo Fisher Scientific, Inc., cat. no. 62-6520) or anti-rabbit IgG (1:5,000; Invitrogen; Thermo Fisher Scientific, Inc., cat. no. 65-6122) antibody for 1 h at room temperature. The membranes were developed utilizing an ECL kit (Pierce; Thermo Fisher Scientific, Inc.) and visualized with X-ray film. Protein expression levels were standardized to those of β -actin. Data was analyzed by Quantity One version 4.62 software (Bio-Rad Laboratories, Inc.).

Cell proliferation analysis. To analyze cell proliferation, a Cell Counting Kit-8 (CCK-8) assay was conducted using a CCK-8 proliferation assay kit (Dojindo Molecular Technologies, Inc.) at 0, 12, 24 and 48 h following transfection, according to the manufacturer's protocols. Cells were transfected with mimics or negative control (ctrl group), or treated with Lipofectamine 2000 without miRNA molecules in the mock group. Cells (1×10^5) were seeded in 96-well plates for analysis. Then, 100 μl CCK-8 reagent was added to the well; at 2 h later, the absorbance was measured at 450 nm with a microplate reader.

Transwell invasion and migration assay. Cell invasion was investigated based on the capacity of the cells invade through the 8 mm pores of polycarbonate membranes. Briefly, HTR8/SVneo cells (1.0×10^5 cells/well) transfected with siRPDGFRA or scramble were placed in the upper chamber, and 600 μl of RPMI1640 medium with 10% fetal bovine serum was placed in the lower chamber. After incubating for 1 day under standard conditions at 37°C , the cells on the upper surface that had not migrated were removed with a sterile cotton swab. Migrated cells were stained with 0.1% crystal violet for 5 min at room temperature and analyzed under a light microscope in 5 random fields (magnification, x200). The techniques used in

Table I. Clinical information of patients enrolled in the present study.

Variable	Control (n=30)	PE (n=30)	P-value
Age (years)	28.83±2.42	28.63±2.24	0.741
Gestational age (weeks)	38.67±0.55	36.07±0.78	<0.0001
Systolic pressure (mmHg)	113.03±9.02	156.67±5.80	<0.0001
Diastolic pressure (mmHg)	67.70±7.26	95.80±3.21	<0.0001
Proteinuria (g/24 h)	N/A	3.55±0.86	N/A

PE, preeclampsia.

Table II. Primers for reverse transcription-quantitative polymerase chain reaction.

Gene	Primer sequence (5'→3')
Hsa-miR-342-3p stem-loop primer	GCGCGTGAGCAGGCTGGAGAAATTAACCGCGCACGGGT
Hsa-miR-342-3p forward primer	TCTCACACAGAAATCGC
Hsa-miR-342-3p reverse primer	GAGCAGGCTGGAGAA
U6 forward primer	GCTTCGGCAGCACATATACTAAAT
U6 reverse primer	CGCTTCACGAATTTGCGTGTCTAT
PDGFRA forward primer	TTGAAGGCAGGCACATTTACA
PDGFRA reverse primer	GCGACAAGGTATAATGGCAGAAT
BCL-2 forward primer	CCGTTGGCCCCCGTTGCTTT
BCL-2 reverse primer	CTGGCGGAGGGTCAGGTGGA
Caspase-3 forward primer	CTCGGTCTGGTACAGATGTCGATG
Caspase-3 reverse primer	GGTTAACCCGGGTAAGAATGTGCA
β-actin forward primer	TGGCACCCAGCACAAATGAA
β-actin reverse primer	CTAAGTCATAGTCCGCCTAGAAGCA

Hsa, homo sapiens; miR, microRNA; BCL-2, B-cell lymphoma 2; PDGFRA, platelet-derived growth factor receptor α .

the Transwell invasion assay were based on the cell migration assay; however, Matrigel was used.

Cell cycle assay. The cell cycle was analyzed via flow cytometry. After 48 h post-transfection, HTR8/SVneo cells were trypsinized in chilled PBS, fixed in 70% ethanol for 24 h at -20°C, and then stained with propidium iodide for 10 min on ice. The samples were calculated using a flow cytometer and CellQuest Pro version 5.1 software (BD Biosciences).

miRNA target prediction and dual-luciferase reporter assay. TargetScan (version 6.0; http://www.targetscan.org/vert_60/), microcosm (version 1.1; https://tools4mirs.org/software/mirna_databases/microcosm-targets/) and miRanda (version August 2010; <http://www.microrna.org/microrna/home.do>) were used to predict miRNAs that could potentially target PDGFRA and identify possible binding regions. The fragment of the human PDGFRA with [wild type (wt)] or without [mutant (mut)] the miR-342-3p binding site at the 3'-untranslated region (3'-UTR) was cloned and inserted into the pGL3-basic luciferase report plasmid (Promega Corporation) to generate the luciferase reporter vectors, PDGFRA 3'-UTR-wt and PDGFRA 3'-UTR-mut. 293T cells (American Type Culture Collection) were treated in 96-well plates at 5,000 cells per well and

incubated for 24 h at 37°C with 5% CO₂ prior to transfection. Then, miR-342-3p mimics or miR-negative control was transfected into 293T cells using Lipofectamine 2000 with 100 ng of PDGFRA 3'-UTR-wt or PDGFRA 3'-UTR-mut, or 10 ng of pRL-TK *Renilla* plasmid (Promega Corporation). Following incubation for 48 h, the luciferase activities were determined with a Dual Luciferase Reporter System (Promega Corporation); *Renilla* luciferase was used for normalization.

Statistical analysis. SPSS 19.0 (IBM Corp.) was employed for statistical analysis. Data were presented as the mean ± standard deviation, and an independent samples t-test was conducted for comparisons between two groups; analysis was performed in a two-tailed manner. Cell viability was analyzed by one-way analysis of variance with a Student-Newman-Keuls post hoc test. P<0.05 was considered to indicate a statistically significant manner.

Results

Analysis of tissue samples. The clinical information of patients was presented in Table I. Compared with the control group, patients with PE exhibited significantly higher systolic pressure and diastolic pressure, notable

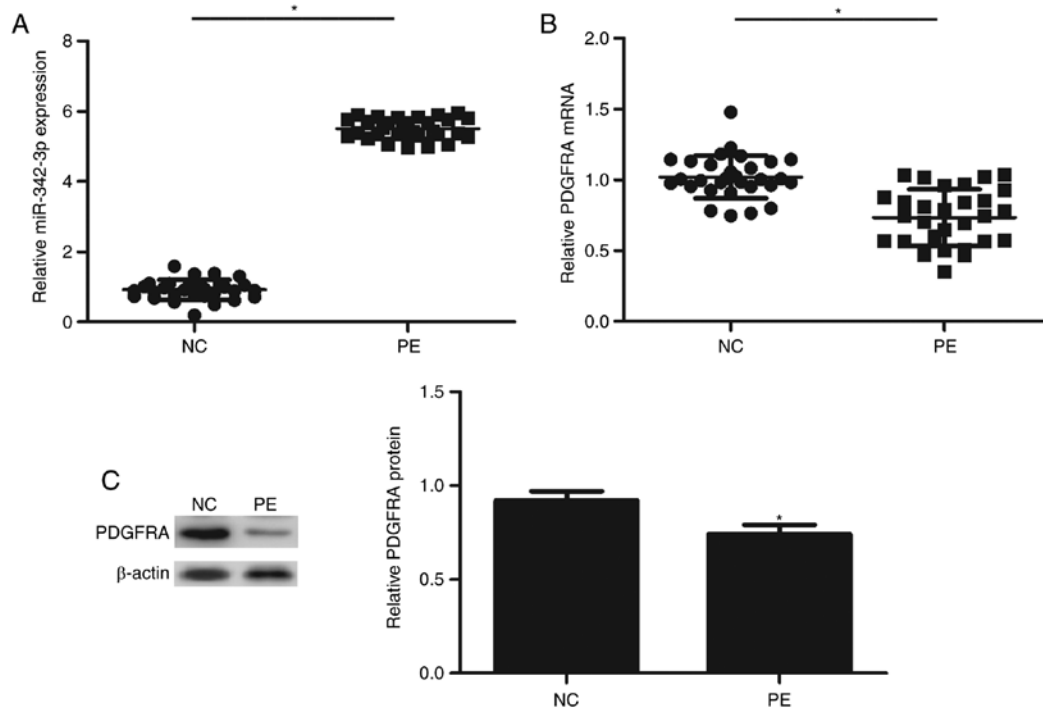


Figure 1. Expression of miR-342-3p and PDGFRA in the placental tissues from patients with PE and healthy controls. (A) miR-342-3p expression was upregulated in the PE group. (B) PDGFRA was significantly downregulated in the PE group at the (B) mRNA and (C) protein levels. * $P < 0.05$ vs. NC. miR, microRNA; NC, NC, negative control; PE, preeclampsia; PDGFRA, platelet-derived growth factor receptor α .

proteinuria and a significantly shorter duration of gestation ($P < 0.0001$).

Expression levels of miR-342-3p and PDGFRA in the two patient groups. The expression of miR-342-3p and PDGFRA was analyzed in tissues via RT-qPCR. As presented in Fig. 1, miR-342-3p was significantly upregulated in the PE group compared with the control group ($P < 0.01$; Fig. 1A). On the contrary, the expression of PDGFRA was significantly decreased in patients with PE compared with the control ($P < 0.01$; Fig. 1B and C).

miR-342-3p may affect cell proliferation and the cell cycle. Additionally, to explore the role of miR-342-3p, we performed a CCK-8 assay to determine the effects of miR-342-3p on cell proliferation. After 48 h, we confirmed that transfection of miR-342-3p mimics into cells significantly increased miR-342-3p expression compared with the control group ($P < 0.01$; Fig. 2A). Furthermore, RT-qPCR and western blotting were conducted to investigate the effects of miR-342-3p on PDGFRA in a cell line. Overexpression of miR-342-3p resulted in a significant decrease in the expression of PDGFRA at the mRNA ($P = 0.001$; Fig. 2B) and protein levels ($P = 0.006$; Fig. 2D) compared with the control. Cell proliferation was significantly inhibited ($P = 0.006$; Fig. 2E) after transfection with miR-342-3p mimics compared with the control. In addition, the effects of miR-342-3p on the cell cycle of HTR-8/SVneo cells were analyzed via flow cytometry. The results demonstrated that the percentage of HTR-8/SVneo cells in G1 phase significantly increased from $59.66 \pm 3.75\%$ prior transfection to $66.94 \pm 2.39\%$ at 48 h post-transfection ($P = 0.047$; Fig. 3A). The percentage of cells in S phase was

significantly reduced from $24.79 \pm 1.87\%$ prior transfection to $21.07 \pm 1.36\%$ following transfection ($P = 0.049$; Fig. 3A).

miR-342-3p suppresses cell migration and invasion. Additionally, Transwell migration and invasion assays were conducted. As presented in Fig. 4, transfection with miR-342-3p mimics significantly inhibited cell migration ($P = 0.027$; Fig. 4A) and invasion ($P = 0.022$; Fig. 4B) compared with the control group.

miR-342-3p may affect the expression of BCL-2 and Caspase-3 in cells. Transfection with miR-342-3p mimics significantly reduced the mRNA ($P = 0.017$; Fig. 5A) and protein expression ($P = 0.035$; Fig. 5C and E) levels of BCL-2. On the contrary, overexpression of miR-342-3p significantly increased the mRNA ($P = 0.001$; Fig. 5B) and protein expression ($P = 0.001$; Fig. 5D and E) levels of Caspase-3.

siR-PDGFR α exhibits similar effects to miR-342-3p mimics. PDGFRA knockdown was performed in cells to its effects in PDGFRA in HTR-8/SVneo cells. After 48 h post-transfection, the mRNA and protein expression levels of PDGFRA were significantly decreased by $43.7 \pm 3.2\%$ ($P = 1.56 \times 10^{-4}$; Fig. 2C) and $47.0 \pm 5.0\%$ ($P = 3.08 \times 10^{-4}$; Fig. 2F), respectively, compared with the control. Additionally, PDGFRA knockdown significantly increased the percentage of HTR-8/SVneo cells in G1 phase from $56.63 \pm 1.40\%$ prior to knockdown to $66.30 \pm 4.07\%$ at 48 h following siR-PDGFR α transfection ($P = 0.018$; Fig. 3B) compared with the control. Furthermore, cell proliferation ($P = 0.023$; Fig. 2G), migration ($P = 0.01$; Fig. 4C) and invasion ($P = 0.003$; Fig. 4D) were significantly inhibited following PDGFRA knockdown compared with the control.

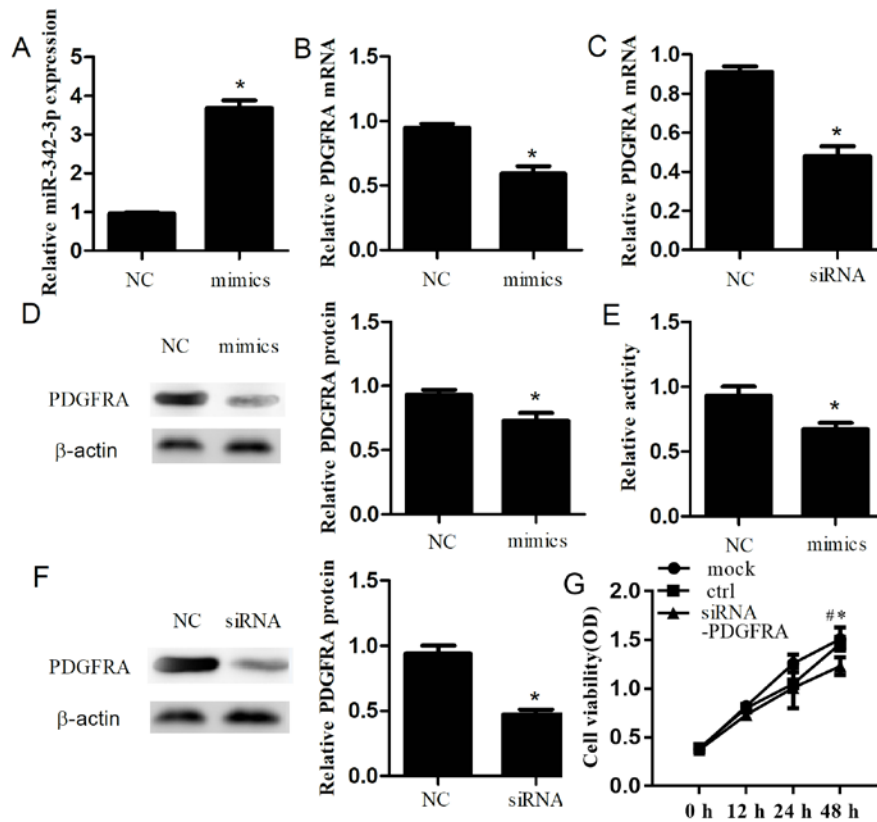


Figure 2. miR-342-3p regulates the proliferation of HTR-8/SVneo cells via PDGFRA. (A) Fold change in miR-342-3p expression in HTR-8/SVneo cells transfected with miR-342-3p mimics. (B) PDGFRA mRNA expression in HTR-8/SVneo cells decreased following transfection with miR-342-3p mimics. (C) Alterations in the mRNA expression levels of PDGFRA in HTR-8/SVneo cells 48 h after transfection with siRNA-PDGFR and siRNA-NC. (D) Alterations in the protein expression levels of PDGFRA in HTR-8/SVneo cells transfected with miR-342-3p mimics or NC. (E) miR-342-3p mimics transfection reduced the proliferation of HTR-8/SVneo cells. (F) Alterations in the protein expression levels of PDGFRA in HTR-8/SVneo cells 48 h post-transfection with siRNA-PDGFR or siRNA-NC. * $P < 0.05$ vs. NC. (G) siRNA-PDGFR suppressed the proliferation of HTR-8/SVneo cells. * $P < 0.05$ vs. mock; # $P < 0.05$ vs. ctrl group. Ctrl, control; miR, microRNA; NC, NC, negative control; OD, optical density; PDGFRA, platelet-derived growth factor receptor α ; siRNA, small interfering RNA.

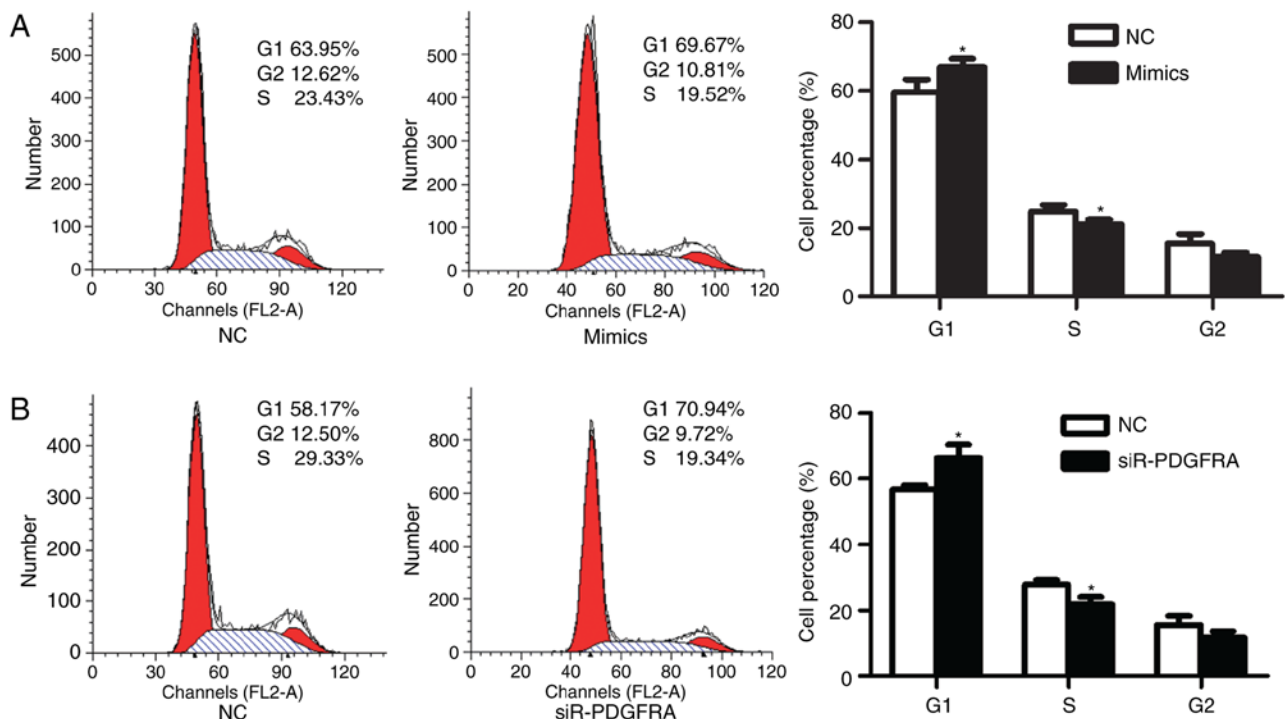


Figure 3. Effects of miR-342-3p mimics and siR-PDGFR on the cell cycle of HTR8/SVneo cells. (A) Overexpression of miR342-3p inhibited G1/S phase transition. (B) Knockdown of PDGFRA promoted G1 phase arrest. * $P < 0.05$ vs. NC. NC, negative control; PDGFRA, platelet-derived growth factor receptor α .

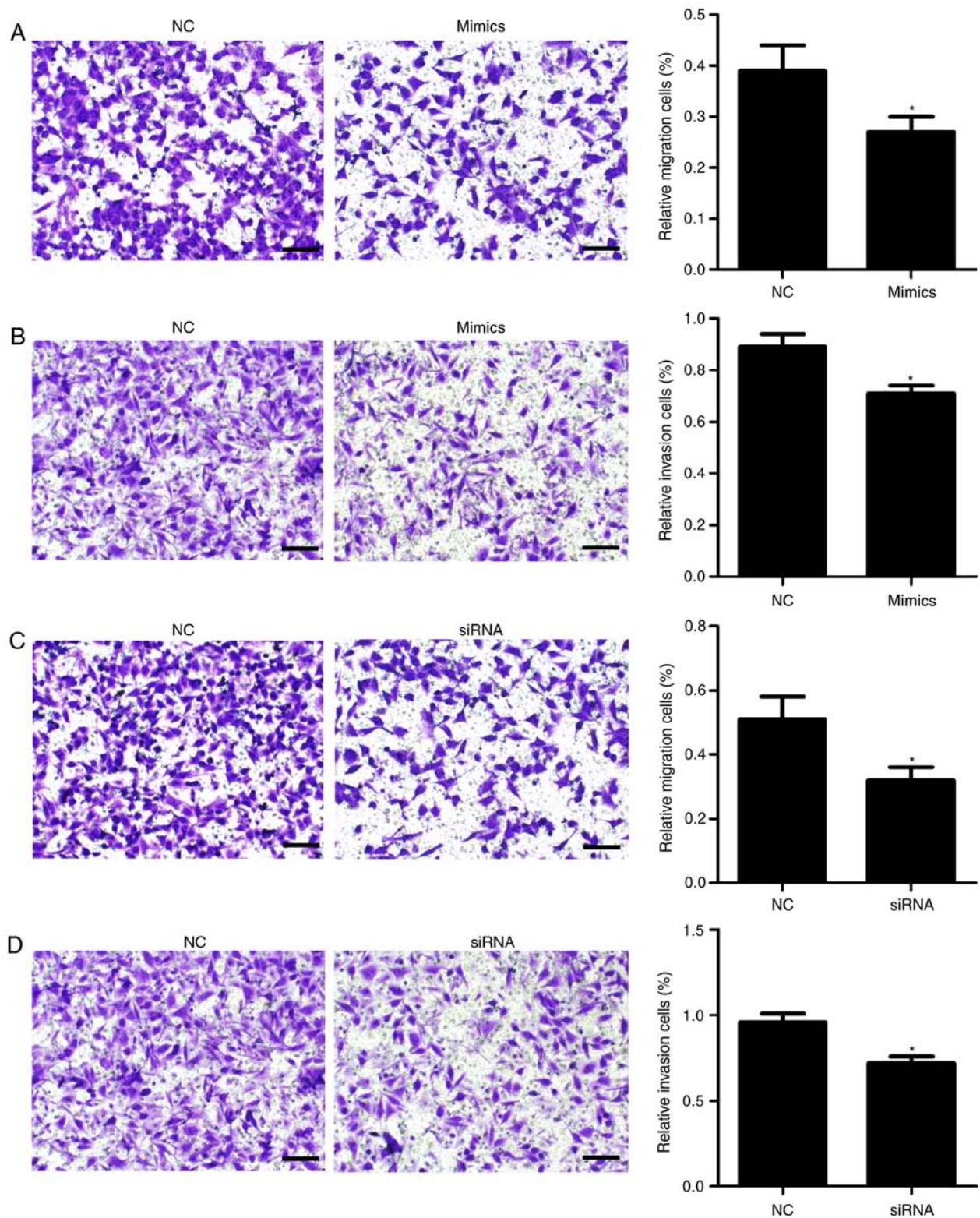


Figure 4. Effects of miR-342-3p mimics and siR-PDGFR α on the migration and invasive abilities of HTR8/SVneo cells. (A) Overexpression of miR-342-3p inhibited cell migration. Scale bar, 100 μ m. (B) Overexpression of miR-342-3p suppressed cell invasion. Scale bar, 100 μ m. (C) Downregulation of PDGFR α inhibited cell migration. Scale bar, 100 μ m. (D) Downregulation of PDGFR α suppressed cell invasion. Scale bar, 100 μ m. * P <0.05 vs. NC. NC, negative control; PE, preeclampsia; PDGFR α , platelet-derived growth factor receptor α ; siRNA, small interfering RNA.

PDGFR α is a direct target of miR-342-3p. As presented in Fig. 6A, bioinformatics analysis indicated that the 3'-UTR of PDGFR α has a target sequence for miR-342-3p. Transfection with miR-342-3p mimics significantly decreased the luciferase activity of cells possessing PDGFR α 3'-UTR-wt compared

with the control (P <0.01), while transfection with miR-342-3p mimics had no significant effects on the luciferase activity of cells possessing PDGFR α 3'-UTR-mut (Fig. 6B). These findings indicated that PDGFR α is likely to be a target of miR-342-3p.

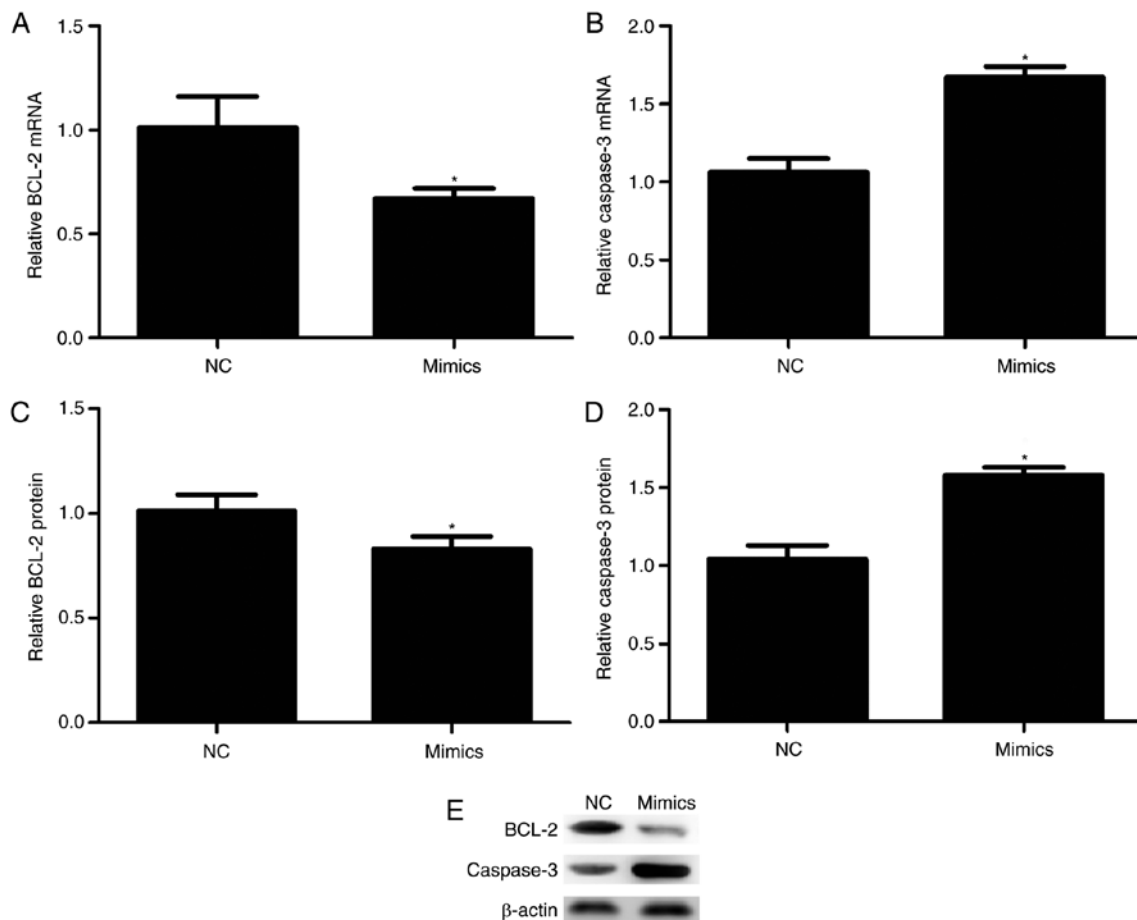


Figure 5. Effects of transient overexpression of miR-342-3p on the expression of BCL-2 and Caspase-3 in HTR-8/SVneo cells. (A) mRNA expression levels of BCL-2 in the two transfection groups. (B) mRNA expression levels of Caspase-3 in the two transfection groups. (C) The protein expression levels of BCL-2 in the two transfection groups. (D) The protein expression levels of Caspase-3 in the two transfection groups. (E) Western blot gel of BCL-2, Caspase-3 and β -actin expression. *P<0.05 vs. NC. BCL-2, B-cell lymphoma 2; NC, negative control; PDGFRA, platelet-derived growth factor receptor α .

Discussion

In the first trimester of pregnancy, extravillous trophoblasts (EVTs) with an invasive phenotype serve an important role in the formation of the maternal-fetal interface (23). At present, the importance of incomplete EVT invasion and subsequent abnormal remodeling of the spiral arteries was highlighted in the etiology of PE; these processes are modulated by a variety of inflammatory and immune cells (23). The migration ability of trophoblasts is important in deep placentation for the normal and healthy development of the fetus (23). Incomplete trophoblast migration and invasion could lead to insufficient deep placentation, which has been associated with PE (23). Reduced trophoblast proliferation has been reported to serve an important role in the development of PE (24).

The importance of miRNAs in PE has been reported. The transient upregulation of miR-136 could induce the apoptosis of MSCs, and affect the formation and development of capillaries (25). miR-125b may be associated with the occurrence of PE by targeting sphingosine-1-phosphate lyase 1 (26). In addition, miR-128a could be involved in the pathogenesis of PE by means of initiating apoptosis (27). miR-195 was associated with the pathogenesis of PE by targeting the activin receptor type-2B (28). In the present study, the expression of miR-342-3p was upregulated in the placentas of patients with

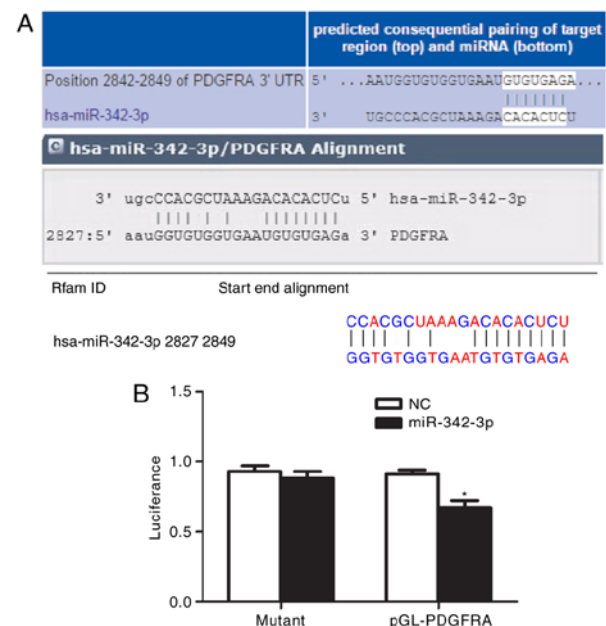


Figure 6. PDGFRA is a direct target of miR-342-3p. (A) Binding site for miR-342-3p within the 3' UTR of PDGFRA. Lines represent the targeting sites between the seed sequence of miR-342-3p and the UTR of PDGFRA. (B) Luciferase activities of the different groups. *P<0.05 vs. NC. hsa, *homo sapiens*; PDGFRA, platelet-derived growth factor receptor α ; miR, microRNA; NC, negative control; UTR, untranslated region.

PE; the transient overexpression of miR-342-3p decreased cell proliferation, migration and invasion. Overexpression of miR-342-3p also inhibited G1/S phase transition; the expression of BCL-2 was decreased while that of Caspase-3 had increased, which suggested miR-342-3p may serve a role in the proliferation, migration, invasion and the cell cycle of trophoblastic cells. miR-342-3p may play a key role in the pathogenesis of PE.

In the field of obstetrics, miR-342-3p was downregulated in intrauterine growth restriction pregnancies if the gestational weeks was <34 weeks (29). In addition, miR-342-3p was reported to be increased in the plasma in pregnancies with severe PE (30), which was in line with our outcomes. miR-342-3p has been suggested to target certain genes, including Forkhead box protein M1, RAP2B, member of RAS oncogene family, I κ B kinase, transforming growth factor- β activated kinase 1 binding protein (TAB)2, TAB3, anterior gradient 2, astrocyte elevated gene-1, T-lymphoma invasion and metastasis-inducing protein 1, E2F transcription factor 1 and C-terminal binding protein 2 (16-18,31-36). We revealed PDGFRA to be a novel direct target of miR-342-3p in trophoblastic cells. In our study, PDGFRA expression in the placental tissues of patients with PE was decreased, whereas that of miR-342-3p was increased. In addition, the expression of PDGFRA decreased following transfection with miR-342-3p mimics in cells; siR-PDGFRA transfection appeared to exhibit similar effects to those of miR-342-3p mimics. Furthermore, the results of the dual-luciferase assay indicated a direct connection between miR-342-3p and PDGFRA. Collectively, these findings suggest PDGFRA as a direct target of miR-342-3p in PE.

PDGFRA is a type III receptor tyrosine kinase that regulates cell proliferation, differentiation, adhesion and survival. Ligand binding activates the kinase, stimulating cellular signaling proteins, including mitogen-activated protein kinases (MAPKs), signal transducers and activators of transcription and phosphatidylinositol-3 kinases (37). High expression of PDGFRA has been detected in various cancers, such as breast cancer (38), ovarian cancer (37), gastrointestinal stromal tumors (39) and melanoma (40). These findings indicate that PDGFRA is associated with various physiological and pathological processes, such as cell survival, migration and wound healing. In our study, PDGFRA was determined to promote cell proliferation, migration and invasion, and affected the cell cycle in trophoblastic cells. Increases in PDGFRA expression could induce cell proliferation, and activate the AKT and MAPK signal pathways. On the contrary, these pathways were suppressed in response to reductions in PDGFRA expression (41); further investigation into the mechanism underlying the roles of PDGFRA in PE is required.

Our study has certain limitations. Only 30 cases of PE placentas and 30 normal placentas were employed for histological analysis. In the future, a larger sample size is required to validate our findings. In addition, *in vitro* experiments were performed; however, further insight from investigations may be obtained by conducted *in vivo* research in rats or mice as models. The present study aimed to determine the mechanism underlying incomplete trophoblastic invasion in PE. However, endothelial dysfunction is also one of the major causes of

PE (4), yet this was not explored in our study this paper; thus, this should be investigated in the future.

In conclusion, our research demonstrated that miR-342-3p was upregulated and PDGFRA expression was decreased in PE. Functional experiments showed that miR-342-3p could affect the proliferation, migration, invasion and cell cycle of trophoblastic cells by targeting PDGFRA. The present study proposed that miR-342-3p may be a novel clinical indicator or prognostic marker for PE.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

The datasets used during the present study are available from the corresponding author on reasonable request.

Authors' contributions

XY analyzed and interpreted the patient data, and was a major contributor in writing the manuscript. FG performed the statistical analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The Ethics Committee in the First Hospital of China Medical University agreed our research. Written informed consent for participation in the study or use of their tissues was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Cheung HC, Leung KY and Choi CH: Diagnostic accuracy of spot urine protein-to-creatinine ratio for proteinuria and its association with adverse pregnancy outcomes in Chinese pregnant patients with pre-eclampsia. *Hong Kong Med J* 22: 249-255, 2016.
2. Askie LM, Duley L, Henderson-Smart DJ and Stewart LA; PARIS Collaborative Group: Antiplatelet agents for prevention of pre-eclampsia: A meta-analysis of individual patient data. *Lancet* 369: 1791-1798, 2007.
3. Turner RJ, Bloemenkamp KW, Bruijn JA and Baelde HJ: Loss of thrombomodulin in placental dysfunction in preeclampsia. *Arterioscler Thromb Vasc Biol* 36: 728-735, 2016.
4. Hastie R, Brownfoot FC, Pritchard N, Hannan NJ, Cannon P, Nguyen V, Palmer K, Beard S, Tong S and Kaitu'u-Lino TJ: EGFR (epidermal growth factor receptor) signaling and the mitochondria regulate sFlt-1 (soluble FMS-like tyrosine kinase-1) secretion. *Hypertension* 73: 659-670, 2019.

5. Yang X and Meng T: MicroRNA-431 affects trophoblast migration and invasion by targeting ZEB1 in preeclampsia. *Gene* 683: 225-232, 2019.
6. Liang T, Hu XY, Li YH, Tian BQ, Li ZW and Fu Q: MicroRNA-21 regulates the proliferation, differentiation, and apoptosis of human renal cell carcinoma cells by the mTOR-STAT3 signaling pathway. *Oncol Res* 24: 371-380, 2016.
7. Liu M, Chen Y, Song G, Chen B, Wang L, Li X, Kong X, Shen Y and Qian L: MicroRNA-29c overexpression inhibits proliferation and promotes apoptosis and differentiation in P19 embryonal carcinoma cells. *Gene* 576: 304-311, 2016.
8. Zhou N, Fei D, Zong S, Zhang M and Yue Y: MicroRNA-138 inhibits proliferation, migration and invasion through targeting hTERT in cervical cancer. *Oncol Lett* 12: 3633-3639, 2016.
9. Yang X, Lei P, Huang Y, Zhang Z and Zhang Y: MicroRNA-133b inhibits the migration and invasion of non-small cell lung cancer cells via targeting FSCN1. *Oncol Lett* 12: 3619-3625, 2016.
10. Zhao G, Zhou X, Chen S, Miao H, Fan H, Wang Z, Hu Y and Hou Y: Differential expression of microRNAs in decidua-derived mesenchymal stem cells from patients with pre-eclampsia. *J Biomed Sci* 21: 81, 2014.
11. Biró O, Nagy B and Rigó J Jr: Identifying miRNA regulatory mechanisms in preeclampsia by systems biology approaches. *Hypertens Pregnancy* 36: 90-99, 2017.
12. Yang S, Li H, Ge Q, Guo L and Chen F: Deregulated microRNA species in the plasma and placenta of patients with preeclampsia. *Mol Med Rep* 12: 527-534, 2015.
13. Li H, Ge Q, Guo L and Lu Z: Maternal plasma miRNAs expression in preeclamptic pregnancies. *Biomed Res Int* 2013: 970265, 2013.
14. Hromadnikova I, Kotlabova K, Ivankova K, Vedmet'skaya Y and Krofta L: Profiling of cardiovascular and cerebrovascular disease associated microRNA expression in umbilical cord blood in gestational hypertension, preeclampsia and fetal growth restriction. *Int J Cardiol* 249: 402-409, 2017.
15. Choi SY, Yun J, Lee OJ, Han HS, Yeo MK, Lee MA and Suh KS: MicroRNA expression profiles in placenta with severe preeclampsia using a PNA-based microarray. *Placenta* 34: 799-804, 2013.
16. Li XR, Chu HJ, Lv T, Wang L, Kong SF and Dai SZ: miR-342-3p suppresses proliferation, migration and invasion by targeting FOXM1 in human cervical cancer. *FEBS Lett* 588: 3298-3307, 2014.
17. Xie X, Liu H, Wang M, Ding F, Xiao H, Hu F, Hu R and Mei J: miR-342-3p targets RAP2B to suppress proliferation and invasion of non-small cell lung cancer cells. *Tumour Biol* 36: 5031-5038, 2015.
18. Zhao L and Zhang Y: miR-342-3p affects hepatocellular carcinoma cell proliferation via regulating NF- κ B pathway. *Biochem Biophys Res Commun* 457: 370-377, 2015.
19. Jarvenpää J, Vuoristo JT, Savolainen ER, Ukkola O, Vaskivuo T and Ryyanen M: Altered expression of angiogenesis-related placental genes in pre-eclampsia associated with intrauterine growth restriction. *Gynecol Endocrinol* 23: 351-355, 2007.
20. Saito T and Saetrom P: MicroRNAs-targeting and target prediction. *N Biotechnol* 27: 243-249, 2010.
21. Zhang C, Li Q, Ren N, Li C, Wang X, Xie M, Gao Z, Pan Z, Zhao C, Ren C and Yang W: Placental miR-106a~363 cluster is dysregulated in preeclamptic placenta. *Placenta* 36: 250-252, 2015.
22. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
23. Schatz F, Guzeloglu-Kayisli O, Arlier S, Kayisli UA and Lockwood CJ: The role of decidual cells in uterine hemostasis, menstruation, inflammation, adverse pregnancy outcomes and abnormal uterine bleeding. *Hum Reprod Update* 22: 497-515, 2016.
24. Redline RW and Patterson P: Pre-eclampsia is associated with an excess of proliferative immature intermediate trophoblast. *Hum Pathol* 26: 594-600, 1995.
25. Ji L, Zhang L, Li Y, Guo L, Cao N, Bai Z, Song Y, Xu Z, Zhang J, Liu C and Ma X: MiR-136 contributes to pre-eclampsia through its effects on apoptosis and angiogenesis of mesenchymal stem cells. *Placenta* 50: 102-109, 2017.
26. Yang W, Wang A, Zhao C, Li Q, Pan Z, Han X, Zhang C, Wang G, Ji C, Wang G, *et al*: miR-125b enhances IL-8 production in early-onset severe preeclampsia by targeting sphingosine-1-phosphate lyase 1. *PLoS One* 11: e0166940, 2016.
27. Ding GC, Chen M, Wang YX, Rui C, Xu W, Ding HJ and Shi ZH: MicroRNA-128a-induced apoptosis in HTR-8/SVneo trophoblast cells contributes to pre-eclampsia. *Biomed Pharmacother* 81: 63-70, 2016.
28. Wu H, Wang H, Liu M, Bai Y, Li YX, Ji L, Peng C, Yu Y and Wang YL: MiR-195 participates in the placental disorder of preeclampsia via targeting activin receptor type-2B in trophoblastic cell. *J Hypertens* 34: 1371-1379, 2016.
29. Hromadnikova I, Kotlabova K, Hympanova L and Krofta L: Cardiovascular and cerebrovascular disease associated microRNAs are dysregulated in placental tissues affected with gestational hypertension, preeclampsia and intrauterine growth restriction. *PLoS One* 10: e0138383, 2015.
30. Wu L, Zhou H, Lin H, Qi J, Zhu C, Gao Z and Wang H: Circulating microRNAs are elevated in plasma from severe preeclamptic pregnancies. *Reproduction* 143: 389-397, 2012.
31. Tao K, Yang J, Guo Z, Hu Y, Sheng H, Gao H and Yu H: Prognostic value of miR-221-3p, miR-342-3p and miR-491-5p expression in colon cancer. *Am J Transl Res* 6: 391-401, 2014.
32. Xue X, Fei X, Hou W, Zhang Y, Liu L and Hu R: miR-342-3p suppresses cell proliferation and migration by targeting AGR2 in non-small cell lung cancer. *Cancer Lett* 412: 170-178, 2017.
33. Zhang S, Liu L, Lv Z, Li Q, Gong W and Wu H: MicroRNA-342-3p inhibits the proliferation, migration, and invasion of osteosarcoma cells by targeting astrocyte-elevated gene-1 (AEG-1). *Oncol Res* 25: 1505-1515, 2017.
34. Huang H, Fan L, Zhan R, Wu S and Niu W: Expression of microRNA-10a, microRNA-342-3p and their predicted target gene TIAM1 in extranodal NK/T-cell lymphoma, nasal type. *Oncol Lett* 11: 345-351, 2016.
35. Tai MC, Kajino T, Nakatomi M, Arima C, Shimada Y, Suzuki M, Miyoshi H, Yatabe Y, Yanagisawa K and Takahashi T: miR-342-3p regulates MYC transcriptional activity via direct repression of E2F1 in human lung cancer. *Carcinogenesis* 36: 1464-1473, 2015.
36. Wang L, Xu L, Xu M, Liu G, Xing J, Sun C and Ding H: Obesity-Associated miR-342-3p promotes adipogenesis of mesenchymal stem cells by suppressing CtBP2 and releasing C/EBP α from CtBP2 binding. *Cell Physiol Biochem* 35: 2285-2298, 2015.
37. Lan H, Chen W, He G and Yang S: miR-140-5p inhibits ovarian cancer growth partially by repression of PDGFRA. *Biomed Pharmacother* 75: 117-122, 2015.
38. Xu H, Han Y, Lou J, Zhang H, Zhao Y, Györfy B and Li R: PDGFRA, HSD17B4 and HMGB2 are potential therapeutic targets in polycystic ovarian syndrome and breast cancer. *Oncotarget* 8: 69520-69526, 2017.
39. Kalfusová A and Kodet R: Molecular mechanisms of primary and secondary resistance, molecular-genetic features and characteristics of KIT/PDGFRA non-mutated GISTs. *Cesk Patol Winter* 53: 167-173, 2017 (In Czech).
40. Bai X, Kong Y, Chi Z, Sheng X, Cui C, Wang X, Mao L, Tang B, Li S, Lian B, *et al*: MAPK pathway and TERT promoter gene mutation pattern and its prognostic value in melanoma patients: A retrospective study of 2,793 cases. *Clin Cancer Res* 23: 6120-6127, 2017.
41. Chen W, Kuang Y, Qiu HB, Cao Z, Tu Y, Sheng Q, Eilers G, He Q, Li HL, Zhu M, *et al*: Dual targeting of insulin receptor and KIT in imatinib-resistant gastrointestinal stromal tumors. *Cancer Res* 77: 5107-5117, 2017.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.