Abstract. Insufficient invasion of trophoblasts is correlated with the development of preeclampsia (PE). MicroRNA (miR)-491-5p has been reported to be implicated in human cancer cell invasion; however, whether miR-491-5p is involved in the development of PE remains largely unclear. The aim of the present study was to investigate the role of miR-491-5p in trophoblastic invasion in vitro and to determine its underlying mechanism of action. The expression levels of miR-491-5p were validated using reverse transcription-quantitative PCR. The effects of miR-491-5p on trophoblast cell invasion were evaluated in vitro. Then, the association between miR-491-5p and its downstream target was investigated in both cell lines and clinical specimens. miR-491-5p expression levels were observed to be significantly increased in the placental tissues from patients with PE. The invasive capacity of HTR-8/SVneo trophoblast cells was suppressed following the upregulation of miR-491-5p and increased following the inhibition of miR-491-5p. Matrix metalloproteinase-9 (MMP-9), a well-known regulator of trophoblast cell invasion, was discovered to be a direct target of miR-491-5p in HTR-8/SVneo trophoblast cells. Moreover, miR-491-5p expression levels were found to be inversely correlated with MMP-9 expression levels in placental tissues from patients with PE. The overexpression of MMP-9 partly attenuated the inhibitory effects of miR-491-5p on HTR-8/SVneo trophoblast cells invasion. Collectively, these findings suggested that the aberrant expression of miR-491-5p may contribute to PE through suppressing trophoblast invasion, thus highlighting the novel roles of miR-491-5p in the molecular pathogenesis of PE. The present study also showed that the miR-491-5p/MMP-9 axis may be an effective biomarker or a viable drug target for therapeutic intervention in PE.

Introduction

Preeclampsia (PE) is a systemic disease that results from placental defects; this pregnancy-associated disease causes complications in 5-8% of all pregnancies and increases both maternal and neonatal morbidity and mortality rates worldwide (1-3). It is characterized by elevated maternal blood pressure and proteinuria (4). It is has been established that trophoblastic dysfunction, such as inadequate migration and invasion, is one of the primary pathological manifestations of PE (5); however, the molecular mechanisms associated with insufficient trophoblastic invasion remain largely unknown.

MicroRNAs (miRNAs) are a group of small non-coding RNAs ~22 nucleotides in length, which regulate target gene expression through either inducing transcript degradation or inhibiting translation (6). Several previous studies have demonstrated that various miRNAs are aberrantly expressed in preeclamptic placentas (7,8), of which some are involved in trophoblastic invasion, such as miRNA (miR)-218, miR-520c-3p, miR-520g and miR-203 (9-12). Notably, miR-491-5p has previously been reported to be upregulated in hypoxic primary human trophoblasts (13); however, this study did not investigate the effects of miR-491 on trophoblastic invasion. In addition, miR-491-5p has been observed to serve as a tumor suppressor in several types of cancer (14,15); for example, miR-491-5p expression levels were found to be decreased in gastric cancer tissues and its upregulation suppressed gastric cancer metastasis through targeting SNAIL and fibroblast growth factor receptor 4 (15). Xu et al (15) demonstrated that miR-491-5p suppressed cellular invasion through targeting platelet-derived growth factor receptor α in prostate cancer. Based on the evidence from these previous studies, it is hypothesized that miR-491-5p may affect the invasion of trophoblasts. Thus, in the present study the relative expression levels of miR-491-5p in placental tissues between patients with PE and normal healthy pregnant women were investigated, and the role of miR-491-5p in trophoblast invasion and migration, and its underlying molecular mechanisms were determined.

Materials and methods

Patient studies. The present study was approved by the Research Ethics Committee of Hebei Medical
University (Tangshan, China) and informed consent was obtained from all patients. A total of 30 human placental tissues were collected from pregnancies with PE (mean age, 41.28±4.01 years) who underwent cesarean sections at the Department of Obstetrics and Gynecology, Tangshan Gongren Hospital, Hebei Medical University between June 2016 and February 2017. A total of 30 samples from normal pregnancies (mean age, 39.18±3.02 years) were recruited and used as the control group. All samples were immediately frozen in liquid nitrogen and stored at -80˚C for subsequent analysis. PE was defined according to the International Society for the Study of Hypertension in Pregnancy (17). Briefly, patients must have had a newly onset systolic blood pressure of ≥160 mmHg or a diastolic blood pressure of ≥110 mmHg on two or more occasions, in addition to accompanying severe proteinuria (≥3+ or ≥2 g/24 h) at ≥20 week of gestation. The blood pressure of all patients must have returned to normal and symptoms of proteinuria must have disappeared 6 weeks postpartum.

For the control group, women with renal disease, cardiovascular disease, transient hypertension in pregnancy, gestational diabetes mellitus, hepatitis, any evidence of spontaneous abortion, intrauterine fetal death, fetal chromosomal or other pregnancy complications were excluded from this study.

**Reverse transcription-quantitative PCR (RT-qPCR).** Total RNA was extracted from 20 mg tissues using TRIzol® (Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol. Total RNA was reverse transcribed into cDNA using the PrimeScript™ RT reagent kit (Takara Biotechnology Co., Ltd.) at 42˚C for 30 min and 85˚C for 5 sec. qPCR for miRNA and mRNA was subsequently performed using an ABI PRISM 7300 sequence detection system and the SYBR®-Green I Real-Time PCR kit (Applied Biosystems; Thermo Fisher Scientific, Inc.). The reaction mixtures were denatured at 95˚C for 30 sec, followed by 40 cycles of 95˚C for 5 sec and 60˚C for 30 sec, then final extension at 72˚C for 5 min. The MMP-9 primer sequences were as follows: Sense, 5'-CGGCTGACACCACTAAACATCAC-3' and antisense, 5'-GGGCTGACACCACTAATCTGATC-3'. Transfection of HTR-8/SVneo cells with miRNAs (50 nM) and pcDNA-MMP9 (2 µg) was performed using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). Cells were harvested 48 h after transfection for further experiments.

**Cell invasion assays.** The invasive abilities of HTR-8/SVneo cells were analyzed using 8-µm pore Transwell plates, containing membranes precoated with Matrigel (Becton-Dickinson and Company). Briefly, 48 h after transfection, a total of 1x10⁴ HTR-8/SVneo cells/well were plated in the upper chambers of Transwell plates in 200 µl serum-free RPMI-1640 medium. The lower chambers were plated with 500 µl RPMI-1640 medium, supplemented with 10% FBS. Following incubation at 37˚C for 24 h, non-invasive cells in the upper chamber were removed and the invasive cells on the lower surface of the chamber were fixed with 4% paraformaldehyde at room temperature for 30 min and subsequently stained with 0.1% crystal violet (Sigma-Aldrich; Merck KGaA) for 10 min at room temperature. Stained cells were counted and photographed using a fluorescent microscope (Nikon Corporation; x200 magnification).

**Wound healing assay.** Upon reaching 70-80% confluence, HTR-8/SVneo cells were transfected with pcDNA-MMP9, miR-491-5p mimics, miR-491-5p inhibitor and their respective negative controls for 24 h, and the wound was subsequently created in the middle of the well using a 200 µl pipette tip. Then, cells were washed twice with PBS and incubated in serum-free RPMI-1640 medium. The micrographs were captured using a BX51 Olympus confocal microscope (Olympus Corporation) at 0 and 24 h (x200 magnification). Image analysis and quantification was performed using ImageJ version 1.46 software (National Institutes of Health).

**Prediction of the putative targets of miR-491-3p.** The putative targets of miR-491-3p were predicted by the online software Targetscan 7.0 (targetscan.org) and miRanda (microRNA.org).

**Dual-luciferase reporter assay.** The wild-type (wt) and mutant (mut) 3'-untranslated region (UTR) of human MMP-9 was
inserted into the pGL3 control vector (Promega Corporation). HTR-8/SVneo cells (8x10^4) were transfected with the wild-type or mutant vector (200 ng/well), the pRL-TK Renilla luciferase reporter (10 ng/well), miR-491-5p mimic or mimic NC (50 nmol/l) using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.). Following transfection for 48 h, the luciferase activity was measured using the Dual-Luciferase Reporter Assay system according to the manufacturer’s instructions (Promega Corporation). The pRL-TK plasmid (Promega Corporation) was used as a normalizing control. All experiments were performed in triplicate.

Western blotting. Total protein was extracted from HTR-8/SVneo cells using RIPA lysis buffer (Beyotime Institute of Biotechnology). Total protein was quantified using a bicinchoninic acid assay kit (Pierce; Thermo Fisher Scientific, Inc.) and 30 µg protein/lane was separated via 12% SDS-PAGE. The separated proteins were subsequently transferred onto a PVDF membrane (GE Healthcare) and blocked for 1 h at room temperature with 5% non-fat dry milk. The membranes were incubated with the following primary antibodies overnight at 4°C: Anti-MMP-9 (1:4,000; cat. no. 13667; Cell Signaling Technology, Inc.) and anti-β-actin (1:2,000; cat. no. 4970; Cell Signaling Technology, Inc.). Following the primary antibody incubation, membranes were incubated with HRP-conjugated Mouse Anti-Rabbit IgG (Light-Chain Specific) (1:10,000; cat. no. 93702; Cell Signaling Technology, Inc.) for 1 h at 37°C. Protein bands were visualized using an enhanced chemiluminescence kit (GE Healthcare). Protein expression was quantified using ImageJ software (National Institutes of Health).

Statistical analysis. Statistical analysis was performed using GraphPad Prism version 5.01 software (GraphPad Software, Inc.) and data are presented as the mean ± standard deviation of 3 independent repeats. Differences between sample groups were analyzed using a one-way ANOVA and multiple comparisons were performed using a Tukey’s post hoc test.

miR-491-5p expression levels are increased in PE clinical samples. To investigate the role of miR-491-5p in the development of PE, miR-491-5p expression levels were detected using RT-qPCR in 30 placental tissues from women with PE and 30 placental tissues from women who had a healthy pregnancy. The expression levels of miR-491-5p were significantly increased in placental tissues from women with PE compared with women in the control group (Fig. 1A). This indicated that miR-491-5p may be a novel factor associated with the pathogenesis of PE. To further establish the role of miR-491-5p in trophoblast cell invasion, miR-491-5p mimics or miR-491-5p inhibitors were transfected into HTR-8/SVneo cells. RT-qPCR analysis demonstrated the significant increase or decrease in cellular miR-491-5p expression levels in the miR-491-5p mimic- or miR-491-5p inhibitor-transfected cells, respectively, compared with their respective NC-transfected cells (Fig. 1B).

miR-491-5p suppresses the invasive capacity of trophoblast cells. The invasion of normal trophoblasts is closely associated with PE (5). Using a Matrigel invasion assay, it was demonstrated that the overexpression of miR-491-5p in miR-491-5p mimic-transfected cells significantly suppressed the invasive ability of HTR-8/SVneo cells compared with cells transfected with the NC mimic, whereas the inhibition of miR-491-5p in miR-491-5p inhibitor-transfected cells significantly increased the invasive ability of cells compared with NC inhibitor-transfected cells (Fig. 2A). In contrast, it was demonstrated that the upregulation of miR-491-5p expression levels significantly decreased the migratory ability of HTR8/SVneo cells compared with cells transfected with the NC mimic (Fig. 2B).
miR-491-5p INHIBITS TROPHOBLAST CELL MIGRATION AND INVASION

The migratory ability of HTR-8/SVneo cells compared with the NC inhibitor-transfected cells (Fig. 2B). These data suggest that the upregulation of miR-491-5p may inhibit the invasion and migration of trophoblast cells.

**MMP-9 is a direct target of miR-491-5p.** To further elucidate the underlying molecular mechanisms involved in the miR-491-5p-mediated inhibitory effects on the invasion of trophoblasts, TargetScan version 7.0 (targetscan.org) (19) and miRanda algorithm v3.0 (microrna.org/microrna/) (20) was used to predict the target genes of miR-491-5p. It was discovered that the wt 3’-UTR of the MMP-9 mRNA contained the complementary sequence for miR-491-5p (Fig. 3A). Previous studies have reported that MMP-9 is critical for regulating

Figure 2. Overexpression of miR-491-5p suppresses the invasion and migration of trophoblast cells. HTR-8/SVneo cells were transfected with miR-491-5p mimic, miR-491-5p inhibitor or their respective NCs. (A) Cell invasion was measured using a Transwell assay (x200 magnification). (B) Wound healing assay was used to determine cell migration. Representative micrographs of cells in each group at 0 and 24 h are shown (x200 magnification). All data represent the mean ± standard deviation of three independent experimental repeats. *P<0.01 vs. mimic NC; †P<0.05; ‡P<0.01 vs. inhibitor NC. miR, microRNA; NC, negative control.
trophoblastic invasion (21,22). To verify whether miR-491-5p directly bound to MMP-9, a dual-luciferase reporter assay was performed in HTR8/SVneo cells. The relative luciferase activity of the wt MMP-9 3'-UTR was significantly reduced in HTR8/SVneo cells transfected with the miR-491-5p mimic compared with the NC mimic, whereas the relative luciferase activity of the wt MMP-9 3'-UTR was significantly increased in the NC mimic-transfected cells, whereas miR-491-5p inhibition significantly increased the expression levels of MMP-9 in HTR8/SVneo cells compared with NC inhibitor-transfected cells (Fig. 3).

To further examine the expression of MMP-9 and its correlation with miR-491-5p expression in PE, the expression levels of MMP-9 mRNA were analyzed in all placenta samples used in the present study. It was observed that the expression levels of MMP-9 were significantly decreased in the placental tissues from women with PE compared with the women in the control group (Fig. 3D) and miR-491-5p expression was found to be inversely correlated with the expression levels of MMP-9 in placental tissues from women with PE (r=−0.6455; P<0.01; Fig. 3E). These findings suggested that MMP-9 may be regulated by miR-491-5p in PE placenta tissues and trophoblast cells.
miR-491-5p suppresses the invasive and migratory ability of trophoblast cells through targeting MMP-9. To determine whether miR-491-5p suppressed the invasion of trophoblast cells through regulating MMP-9 expression, MMP-9 was overexpressed in HTR8/SVneo cells using pcDNA-MMP-9 plasmids. Western blotting demonstrated that the expression levels of MMP-9 were increased in HTR8/SVneo cells following pcDNA-MMP-9 transfection compared with cells transfected with the control vector (Fig. 4A). Subsequently, the invasive and migratory ability of HTR8/SVneo cells co-transfected with miR-491-5p mimics and pcDNA-MMP-9 or pcDNA empty for 48 h (C) migration were investigated using a Transwell and wound healing assay (x200 magnification), respectively. All data represent the mean ± standard deviation of three independent experimental repeats. *P<0.05, **P<0.01 vs. control; ##P<0.01 vs. miR-491-5p mimic + pcDNA empty. miR, microRNA; NC, negative control; MMP-9, matrix metalloproteinase-9.

**Figure 4. Overexpression of miR-491-5p inhibits the invasion and migration of trophoblast cells through targeting MMP-9. (A) Expression levels of MMP-9 were analyzed using western blotting in HTR-8/SVneo cells following transfection with the pcDNA-MMP-9 overexpression plasmid. **P<0.01 vs. pcDNA empty. (B and C) HTR-8/SVneo cells were co-transfected with miR-491-5p mimic and pcDNA-MMP-9 or pcDNA empty for 48 h and (B) cell invasion and (C) migration were investigated using a Transwell and wound healing assay (x200 magnification), respectively. All data represent the mean ± standard deviation of three independent experimental repeats. *P<0.05, **P<0.01 vs. control; ##P<0.01 vs. miR-491-5p mimic + pcDNA empty. miR, microRNA; NC, negative control; MMP-9, matrix metalloproteinase-9.

Discussion

The present study demonstrated that miR-491-5p expression levels were increased in the placental tissue from women with PE, with in vitro analysis further indicating that miR-491-5p could suppress the invasion and migration of trophoblast cells through targeting of MMP-9. Collectively, these findings suggested that the miR-491-5p/MMP9 axis may represent a potential therapeutic target for the development of novel treatment strategies for patients with PE.

Previous studies have reported that miRNAs are aberrantly expressed in PE placenta tissues and are involved in several pathophysiological processes, including the invasion and migration of trophoblast cells (8,23-27). For example, miR-136 was shown to be increased in PE placenta tissue, where it inhibited the invasion of trophoblast cells (28); Xue et al (29) found that miR-34a-5p expression levels were increased in patients with PE, and these upregulated levels suppressed the invasion and migration of trophoblast cells through directly targeting SMAD4; Guo et al (30) revealed that miR-423-5p expression levels were increased in the blood plasma of pregnant women with PE, which effectively inhibited migration and invasion through targeting IGF2BP1 in HTR-8/SVneo cells; and Gao et al (31) found that increased expression levels of miR-299 suppressed the invasion and migration of HTR-8/SVneo trophoblast cells through targeting HDAC2 in PE. These previous findings suggested that miRNAs may be an attractive therapeutic target against PE. In the present study, it was discovered that miR-491-5p expression levels were increased in the placental tissue of patients with PE compared with normal placentas, suggesting that miR-491-5p may be associated with the pathogenesis of PE. miR-491-5p has been reported to be served as a novel regulator of tumor cell function, including cell invasion and migration (16). Therefore, it was hypothesized that miR-491-5p may affect the progression of PE through the regulation of trophoblastic invasion and migration,
which, to the best of our knowledge, had not been previously investigated. In the present study, it was shown that the invasive capabilities of HTR8/SVneo cells were suppressed following miR-491-5p overexpression, whereas they were increased following miR-491-5p inhibition. Taken together, these results indicated that the increased expression of miR-491-5p may contribute to PE development through suppressing invasion and migration in trophoblasts.

To investigate the potential mechanisms underlying the miR-491-5p-mediated inhibition of migration and invasion in trophoblasts, bioinformatics analysis was performed to predict the putative targets of miR-491-5p; MMP-9 was identified as a potential target of miR-491-5p. MMP-9, a member of the MMP family, has been previously demonstrated to mediate trophoblast invasion and migration (32-34); for example, Rasstrigina et al (35) reported that the decreased expression levels of MMP-9 in the placenta contributed to the reduced invasiveness of trophoblasts. Notably, a previous study also observed that miR-491-5p suppressed the invasive ability of gastric, breast and lung cancer cells through targeting MMP-9 (36). Nonetheless, whether MMP-9 was implicated in the inhibitory effects of miR-491-5p on trophoblastic invasion remained relatively unknown. In the present study, MMP-9 was validated as a target gene of miR-491-5p in HTR8/SVneo cells. In addition, MMP-9 expression levels were found to be decreased in the placental tissue of patients with PE compared with normal placentas, and an inverse relationship was observed between MMP-9 and miR-491-5p expression levels. Furthermore, MMP-9 overexpression reversed the inhibitory effects of the miR-491-5p mimic on the invasion and wound healing ability of trophoblast cells. These results suggest that miR-491-5p may suppress the trophoblastic invasion and migration, at least partly, through decreasing the expression levels of MMP-9.

In conclusion, the results from the present study showed that the expression levels of miR-491-5p were increased in the placental tissues from women with PE and the upregulation of miR-491-5p could inhibit the invasion and migration of trophoblast cells, which was partly through targeting MMP-9. These data suggested that miR-491-5p may be an effective drug target for therapeutic intervention in PE; however, the present study may be limited by the fact that the expression levels of miR-491-5p were only investigated in placental tissues collected from pregnant women undergoing cesarean sections, which suggested that its expression levels may not be suitable for the prenatal screening of patients with PE. In future studies, plasma samples from pregnant patients should be collected and processed to detect the expression levels of miR-491-5p in a multi-center study design.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors’ contributions

EL, YZ, JL and DZ all performed the experiments, analyzed the data and wrote the manuscript. EL conceptualized the experimental design. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by Research Ethics Committee of Hebei Medical University. All individuals provided informed consent for the use of their samples for clinical research.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Redman CW and Sargent IL: Latest advances in understanding preeclampsia. Science 308: 1592-1594, 2005.
2. Kanasaki K and Kalluri R: The biology of preeclampsia. Kidney Int 76: 831-837, 2009.
3. Al-Jameil N, Aziz Khan F, Fareed Khan M and Tabassum H: A brief overview of preeclampsia. J Clin Med Res 6: 1-7, 2014.
4. Vitoratos N, Economou E, Iavazzo C, Panoulis K and Creatsas G: Maternal serum levels of TNF-alpha and IL-6 long after delivery in preeclamptic and normotensive pregnant women. Mediators Inflamm 2010: 908649, 2010.
5. Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR and van Assche A: A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. Br J Obstet Gynaecol 101: 669-674, 1994.
6. Bartel DP: MicroRNAs: Genomics, biogenesis, mechanism, and function. Cell 116: 281-297, 2004.
7. Xu P, Zhao Y, Liu M, Wang Y, Wang H, Li YX, Zhu X, Yao Y, Wang H, Qiao J, et al: Variations of microRNAs in human placentas and plasma from preeclamptic pregnancy. Hypertension 63: 1276-1284, 2014.
8. Pineles BL, Romero R, Montenegro D, Tarca AL, Han YM, Kim YM, Draghici S, Espinoza J, Kusanovic JP, Mittal P, et al: Distinct subsets of microRNAs are expressed differentially in the human placentas of patients with preeclampsia. Am J Obstet Gynecol 196: 261 e261-266, 2007.
9. Takahashi H, Ohkuchi A, Kuwata T, Usui R, Baba Y, Suzuki H, Chaw Kyi TT, Matsubara S, Saito S and Takizawa T: Endogenous and exogenous miR-520c-3p modulates CD44-mediated extravilous trophoblast invasion. Placenta 50: 25-31, 2017.
10. Fang M, Du H, Han B, Xia G, Shi X, Zhang F,Fu Q and Zhang T: Hypoxia-inducible microRNA-218 inhibits trophoblast invasion by targeting LASP1: Implications for preeclampsia development. Int J Biochem Cell Biol 87: 95-103, 2017.
11. Jiang L, Long A, Tan L, Hong M, Wu J, Cai L and Li Q: Elevated microRNA-520g in pre-eclampsia inhibits migration and invasion of trophoblasts. Placenta 51: 70-75, 2017.
12. Liu F, Fu K, Wu W, Chen Y, Wu H, Wang H and Zhang W: miR203 contributes to preeclampsia via inhibition of VEGFA expression. Mol Med Rep 17: 5627-5634, 2018.
13. Mouillet JF, Chu T, Nelson DM, Mishima T and Sadovsky Y: MiR-205 silences MED1 in hypoxic primary human trophoblasts. FASEB J 24: 2030-2039, 2010.
14. Zhang J, Ren J, Hao S, Ma F, Xin Y, Jia W, Sun Y, Liu Z, Yu H, Jia J and Li W: MiRNA-491-5p inhibits cell proliferation, invasion and migration via targeting JMJ2B and serves as a potential biomarker in gastric cancer. Am J Transl Res 10: 525-534, 2018.
15. Xu Y, Hou R, Lu Q, Zhang Y, Chen L, Zheng Y and Hu B: MiR-491-5p negatively regulates cell proliferation and motility by targeting PDGFRA in prostate cancer. Am J Cancer Res 7: 2545-2553, 2017.
16. Yu T, Wang LN, Li W, Zuo QF, Li MM, Zou QM and Xiao B: Downregulation of miR-491-5p promotes gastric cancer metastasis by regulating SNAIL and FGFR4. Cancer Sci 109: 1393-1403, 2018.

17. Chaiworapongsa T, Chaemsaithong P, Korzeniewski SJ, Yeo L and Romero R: Pre-eclampsia part 2: Prediction, prevention and management. Nat Rev Nephrol 10: 531-540, 2014.

18. 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.

19. Lewis BP, Burge CB and Bartel DP: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell 120: 15-20, 2005.

20. Betel D, Wilson M, Gabow A, Marks DS and Sander C: The microRNA.org resource: Targets and expression. Nucleic Acids Res 36 (Database Issue): D149-D153, 2008.

21. Yu Y, Wang L, Liu T and Guan H: MicroRNA-204 suppresses trophoblast-like cell invasion by targeting matrix metalloproteinase-9. Biochem Biophys Res Commun 463: 285-291, 2015.

22. Liang Y, Lin Q, Luo F, Wu W, Yang T and Wan S: Requirement of miR-144 in CsA induced proliferation and invasion of human trophoblast cells by targeting titin. J Cell Biochem 115: 690-696, 2014.

23. Wang L, Song G, Liu M, Chen B, Chen Y, Shen Y, Zhu J and Zhou X: MicroRNA-375 overexpression influences P19 cell proliferation, apoptosis and differentiation through the Notch signaling pathway. Int J Mol Med 37: 47-55, 2016.

24. Zhang S, Lai N, Liao K, Sun J and Lin Y: MicroRNA-210 regulates cell proliferation and apoptosis by targeting regulator of differentiation 1 in glioblastoma cells. Folia neuropathol 53: 236-244, 2015.

25. Wang H, Chao K, Ng SC, Bai AH, Yu Q, Yu J, Li M, Cui Y, Chen M, Hu JF and Zhang S: Pro-inflammatory miR-223 mediates the cross-talk between the IL23 pathway and the intestinal barrier in inflammatory bowel disease. Genome Biol 17: 58, 2016.

26. 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.

27. Jairajpuri DS and Almawi WY: MicroRNA expression pattern in pre-eclampsia (Review). Mol Med Rep 13: 2351-2358, 2016.

28. 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.

29. Xue F, Yang J, Li Q and Zhou H: Down-regulation of microRNA-34a-5p promotes trophoblast cell migration and invasion via targeting Smad4. Bioscience Reports 39: BSR20181631, 2019.

30. Guo L, Liu Y, Guo Y, Yang Y and Chen B: MicroRNA-423-5p inhibits the progression of trophoblast cells via targeting IGF2BP1. Placenta 74: 1-8, 2018.

31. Gao Y, She R, Wang Q, Li Y and Zhang H: Up-regulation of miR-299 suppressed the invasion and migration of HTR-8/SVneo trophoblast cells partly via targeting HDAC2 in pre-eclampsia. Biomed Pharmacother 97: 1222-1228, 2018.

32. Luo J, Qiao F and Yin X: Hypoxia induces FGF2 production by vascular endothelial cells and alters MMP9 and TIMP1 expression in extravillous trophoblasts and their invasiveness in a cocultured model. J Reprod Dev 57: 84-91, 2011.

33. Singh M, Kindelberger D, Nagymanyoki Z, Ng SW, Quick CM, Elias KM, Yamamoto H, Fichorova R, Fulop V and Berkowitz RS: Matrix metalloproteinases and their inhibitors and inducer in gestational trophoblastic diseases and normal placenta. Gynecol Oncol 122: 178-182, 2011.

34. Karthikeyan VJ, Lane DA, Beevers DG, Lip GY and Blann AD: Matrix metalloproteinases and their tissue inhibitors in hypertension-related pregnancy complications. J Hum Hypertens 27: 72-78, 2013.

35. Rasstrigina IM, Milovanov AP, Fokin TV and Kadyrov M: The intensity of expression of matrix metalloproteinases type 2 and type 9 by invasive trophoblast cells in uncomplicated pregnancy and preclampsia. Arkh Patol 76: 24-29, 2014 (In Russian).

36. Pirooz HJ, Jafari N, Rastegari M, Fathi-Roudsari M, Tasharrofi N, Shokri G, Tamadon M, Szegar H and Kouhkan F: Functional SNP in microRNA-491-5p binding site of MMP9 3'-UTR affects cancer susceptibility. J Cell Biochem 119: 5126-5134, 2018.