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Up-regulation of miR-517-5p inhibits ERK/MMP-2 pathway: potential role in preeclampsia

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Abstract. – OBJECTIVE: To investigate the potential role of miRNA-517-5p in preeclampsia and its underlying mechanism.

MATERIALS AND METHODS: Placenta samples were obtained from 20 women with preeclampsia and 20 women with normal pregnancies. Expression level of miR-517-5p in placenta samples and JAR cells was detected. MiRNA-517-5p mimics or inhibitor was transfected in JAR cells, followed by detection of proliferative and invasive abilities of JAR cells. In addition, the expressions of extracellular regulated protein kinases (ERK), phospho-extracellular regulated protein kinases (p-ERK) and matrix metalloproteinase-2 (MMP-2) in JAR cells were evaluated by Western blot. Meanwhile, the mRNA level of MMP-2 was evaluated by Real-time polymerase chain reaction (PCR). The luciferase assay was applied to identify the target gene of miRNA-517-5p.

RESULTS: Increased level of miR-517-5p was detected in placenta samples of preeclampsia patients compared with normal pregnancies. MiRNA-517-5p could regulate proliferative and invasive abilities of JAR cells. Furthermore, miRNA-517-5p could regulate ERK/MMP-2 pathway in JAR cells, which would contribute to the pathophysiology of preeclampsia. The luciferase assay showed MMP-2 was the target gene of miR-517-5p. Further studies showed that MMP-2 was dysregulated in preeclampsia.

CONCLUSIONS: MiR-517-5p is highly expressed in placenta samples of preeclampsia pregnancies, which could promote proliferative and invasive abilities of JAR cells by inhibiting ERK/MMP-2 pathway.

Key Words:
Preeclampsia, MiR-517-5p, ERK/MMP-2 pathway, Proliferation, Invasion.

Introduction

Preeclampsia (PE) is a pregnancy-specific systemic complication with high blood pressure and proteinuria after 20 weeks after of pregnancy[1]. The pregnant women are usually with normal blood pressure before pregnancy. PE is one of the five conditions of hypertensive disorder complicating pregnancy, which can affect the system of body organs. Its incidence is about 3.9% of all pregnancies[2]. PE is one of the main causes of pregnancy and fetal death. Clinical evidence has suggested that long-term or pregnancy-induced hypertension may develop into PE. More seriously, PE may even progress into severe conditions, such as eclampsia, HELLP (hemolysis, elevated liver enzymes and low platelets) syndrome[3]. However, the etiology and mechanism of PE have not been fully elucidated, threatening the health and living of pregnant women and fetus. Therefore, researches on pathogenesis and molecular mechanism of PE have theoretical and clinical significance to prevent its occurrence.

Some scholars[4-7] believed that the pathogenesis of PE is related to the pathological changes in placenta, including oxidative stress, inflammatory immune over-activation, lack of vascular remodeling, trophoblast apoptosis, maternal fetal interface immune abnormalities, placental local coagulation and anticoagulation mechanism imbalance. Placenta is an important organ during pregnancy, which is related to many pregnancy-related diseases. Placenta mediates the nutrition absorption and gas exchange of the developing fetal. The placenta regulates the temperature, produces hormones, protects and prevents internal infection during pregnancy. The placenta is mainly composed of trophoblasts, decidual cells, endothelial cells and mesenchymal cells. The biological functions of these cells, such as trophoblast proliferation, differentiation and invasion, as well as mesenchymal cell differentiation, decidualization and angiogenesis, are critical for healthy pregnancies[8,9]. Pathological changes during pregnancy contribute to the pathogenesis of PE. In recent years, a lot of researchers have proven the interaction between microRNAs and the pathogenesis of PE[10]. Hence, we proposed that miRNAs alter the phenotypes

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of placenta cells and respond to the changes of physiological condition during pregnancy. The abnormal expression of miRNAs during pregnancy may lead to disordered cellular functions. MiRNAs, a kind of non-coding regulatory factors, have been identified in regulating a lot of biological processes such as differentiation, proliferation, apoptosis and metabolism[11-13]. With the advanced miRNA sequencing technology, a lot of placenta-specific miRNAs have been found to regulate pregnancy process. For example, miR-141 and miR-519d-3p could regulate trophoblast cell proliferation, migration, invasion and intercellular communication[14,15]. MiR-517a, miR-517b, miR-518b, and miR-519a were the four C19MC members observed in complete hydatidiform moles (CHM)[15]. Besides, miR-210 expression was upregulated in placental tissues from PE patients than those of normal pregnancies[16]. MiR-517-5p was previously demonstrated to be exclusively expressed in placenta[17]. In the study of circulating C19MC microRNAs in PE, the research found the upregulated miR-517-5p in placenta, suggesting its functional role in the generation of PE. In this report, we mainly focused on the potential role of miR-517-5p in PE development. In this report, we focused on the potential role of miR-517-5p in the pathogenesis of PE and its specific mechanism. The result showed that the expression level of miR-517-5p increased in the placenta of PE pregnancies compared with healthy pregnancies. In order to further explore the mechanism of miR-517-5p in the pathogenesis of PE, we explored the biological function of miR-517-5p in JAR cell line.

Materials and Methods

Chemicals and Materials

JAR cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). Fetal bovine serum (FBS) was obtained from Gibco (Rockville, MD, USA). Cell Counting Kit-8 (CCK-8) Assay Kit was obtained from MedChem Express (Monmouth Junction, NJ, USA). Antibodies anti-β-actin, ERK, p-ERK and MMP-2 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Lipofectamine® 3000 Transfection Reagent was obtained from Invitrogen (Carlsbad, CA, USA).

JAR Cell Culture

JAR cells were maintained in RPMI-1640 (Roswell Park Memorial Institute-1640) supplemented with 10% fetal bovine serum (FBS), antibiotics (penicillin and streptomycin), 2 mM L-glutamine, and 25 μg/mL gentamicin at 37°C in a humidified atmosphere of 5% CO_2. Cell passage was performed every 2-3 days.

RNA Extraction and Real-Time quantitative Polymerase Chain Reaction (RT-qPCR)

After JAR cells were transfected with miRNA-517-5p mimics or inhibitors, total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Briefly, 1 μg of total RNA was reversely transcribed using reverse transcription kit. Real-time PCR was conducted using the ABI PRISM 7500 sequence detection system. The reverse transcription reaction program was as follows: 25°C for 10 min, 37°C for 120 min and 85°C for 5 min. Real-time PCR amplification conditions were 95°C for 10 min, followed by 50 cycles of denaturation at 95°C for 15 s and annealing at 62°C for 1 min. All reactions were repeated for three times, and the relative mRNA expression levels for target genes were normalization by β-actin.

Protein Extraction and Western Blotting Analysis

After JAR cells were transfected with miR-517-5p mimics or inhibitors and treated with ERK inhibitor, JAR cells were harvested for protein isolation. Whole-cell lysates were prepared by cell lysis buffer containing protease inhibitors. The protein samples were separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to the polyvinylidene difluoride (PVDF). The membranes were incubated with 5% defatted milk in phosphate-buffered saline tween (PBST) for 1 hours, and were then incubated with primary antibodies overnight at 4°C. After washing with Tris-buffered saline and Tween (TBST) buffer on the next day, membranes were incubated with peroxidase-conjugated individual secondary antibodies for 1 hour. Finally, membranes were exposed using electrochemiluminescence (ECL) solution for detecting fluorescence intensity.

Cell Proliferation Viability

JAR cells were suspended in complete RPMI-1640 medium and adjusted to 5×10^6 cells/mL. Cells were seeded into 96-well plates with 100 μL of suspension per well. Cell proliferation was detected by CCK-8 assay kit according to the manu-

facturer's steps. Absorbance was detected at the wavelength of 450 nm using a microplate reader (Bio-Rad, Hercules, CA, USA).

Cell Invasion Viability

The invasive ability of JAR cells was detected using Matrigel transmembrane invasion assay. The transwell chambers were coated with Matrigel. JAR cells were harvested for calculating and plating into the upper chamber. The bottom wells were filled with complete medium. After incubation, non-adherent cells on the upper chamber were scraped with a cotton swab. The invaded cells were stained with crystal violet solution after fixed with methanol. Finally, five randomly selected fields were captured for counting the number of penetrated cells.

Statistical Analysis

Each experiment was repeated at least in triplicate. Results were expressed as the mean value ± standard deviation (SD). Statistical analysis was carried out using Student's *t*-test. *p*-value less than 0.05 was thought to be with significance.

Results

The Expression Level of miR-517-5p Increased in Placenta Tissues of Preeclampsia Patients

Placenta tissues were obtained from 20 women with preeclampsia and 20 women with normotensive pregnancies for RNA isolation. After reverse transcription, the expression level of miR-517-5p was detected by Real-time qPCR. The relative expression level of miR-517-5p was higher in preeclampsia placenta tissues compared with normotensive placenta tissues. The average expression level of miR-517-5p was nearly two-fold higher in preeclampsia patients (Figure 1).

MiR-517-5p Inhibited the Proliferative and Invasive Abilities of JAR Cells

In order to investigate the effect of the increased expression of miR-517-5p in preeclampsia patients, we selected placental cell line JAR for the following experiments. According to the previous report, dysregulation of placental cells may contribute to the pathogenesis of PE. We tried to verify whether miR-517-5p could regulate the proliferative and invasive abilities of placental cells. JAR cells were first transfected with miR-517-5p mimics or inhibitors, respectively. Proliferative and invasive abilities of JAR cells were evaluated by CCK-8 assay and transwell assay. The results showed that the proliferative and invasive abilities of JAR cells were inhibited by miR-517-5p overexpression (Figure 2).

MMP-2 was the Target Gene of miR-517-5p

To investigate the mechanism by which miR-517-5p regulates the proliferation of JAR cells, we searched for the target gene of miR-517-5p. According to prediction result by TargetScan, MMP-2 was a candidate target for miR-517-5p. MMP-2 is a member of matrix metalloproteinase (MMP) family, which is involved in the breakdown of extracellular matrix (ECM) in biological processes[18]. It is reported that MMP-2 is also involved in cell proliferation ability[19]. In the present study, JAR cells were transfected with miR-517-5p mimics or inhibitors for 24 h. The Real-time PCR result showed that the mRNA level of MMP-2 significantly decreased after JAR cells were transfected with miR-517-5p mimics. Expression level of MMP-2 significantly increased, on the contrary by transfection of miR-517-5p inhibitor (Figure 3A). In addition, the protein level of MMP-2 showed the same change (Figure 3B). In order to find the direct evidence for the interaction between miR-517-5p and MMP-2, we constructed the luciferase plasmid containing 3'UTR of MMP-2 gene named PGL3/MMP2-3'UTR. Transfection of miR-517-5p mimics significantly suppressed the luciferase activity of PGL3/MMP2-3'UTR, but has no effect on the luciferase activity of PGL3/MMP2-3'UTR mutant plasmid (Figure 3C). These results clearly displayed that MMP2 was the direct target gene of miR-517-5p.

MMP-2 Expression Level Decreased in Placenta Tissues of Preeclampsia Patients

In order to verify the result that MMP-2 could be regulated by miR-517-5p in placenta tissues, we detected the mRNA level of MMP-2 by Real-time PCR. According to the Real-time PCR result, the relative expression level of MMP-2 was higher in preeclampsia placenta tissues compared with normotensive placenta tissues. Furthermore, we analyzed the correlation between MMP2 and miR-517-5p in each sample. The correlation analysis result showed that the expression level of MMP2 was negatively correlated to the expression level of miR-517-5p (Figure 1B and Figure 1C).

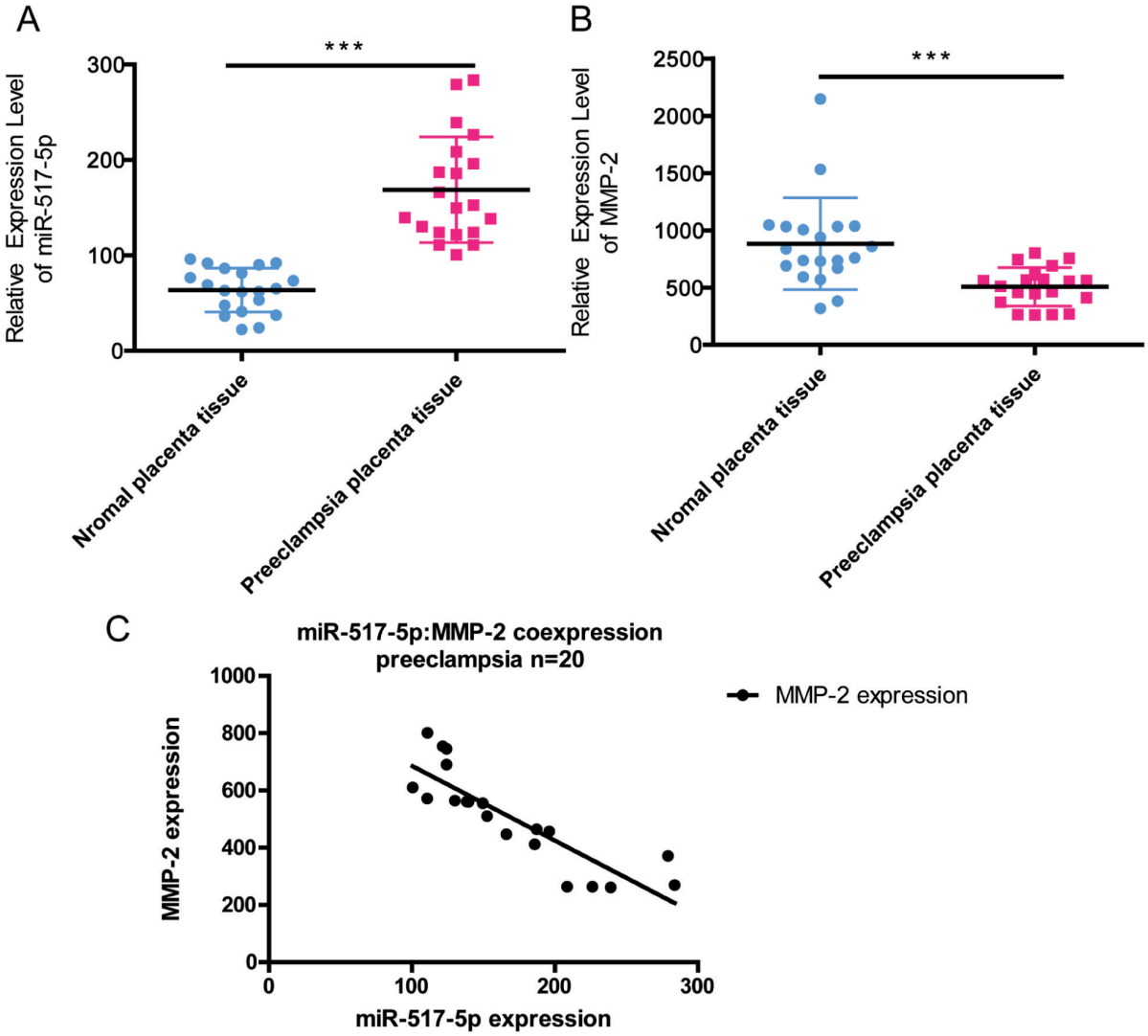

Figure 1. The expression levels of miR-517-5p and MMP-2 in placental tissues of preeclampsia. The placental tissues of 20 women with preeclampsia and 20 women with normotensive pregnancies were collected for RNA isolation. ***A,*** The expression level of miR-517-5p was analyzed in preeclampsia placental tissues compared with normotensive placental tissues. ***B,*** The expression level of MMP-2 was analyzed in preeclampsia placental tissues compared with normotensive placental tissues. ***C,*** The correlation analysis of the expression level of MMP2 and the expression level of miR-517-5p (** $p<0.01$).

ERK Pathway was Involved in MiR-517-5p Induced MMP-2 Down Regulation in JAR Cells

It is reported that MMP-2 expression is regulated by MEK-ERK signaling pathway in a lot of diseases[20]. In this study, MMP-2 was downregulated in placental tissues of preeclampsia patients. Besides, MMP-2 expression was regulated by miR-517-5p. In order to verify the contribution of ERK pathway to MMP-2 transcription and placental cell proliferative ability, we investigated the effect of miR-517-5p on regulating ERK pathway in placental cells. We transfected JAR cells with miR-517-5p mimics or inhibitors, respectively, followed by protein expression detection of ERK pathway-related genes. Western blot results showed inhibition of ERK pathway in miR-517-5p mimics transfected cells and activation of ERK pathway in miR-517-5p inhibitor transfected cells (Figure 4). Taken together, these results suggested that miR-517-5p inhibits placental cells proliferation by inhibiting ERK/MMP-2 pathway.

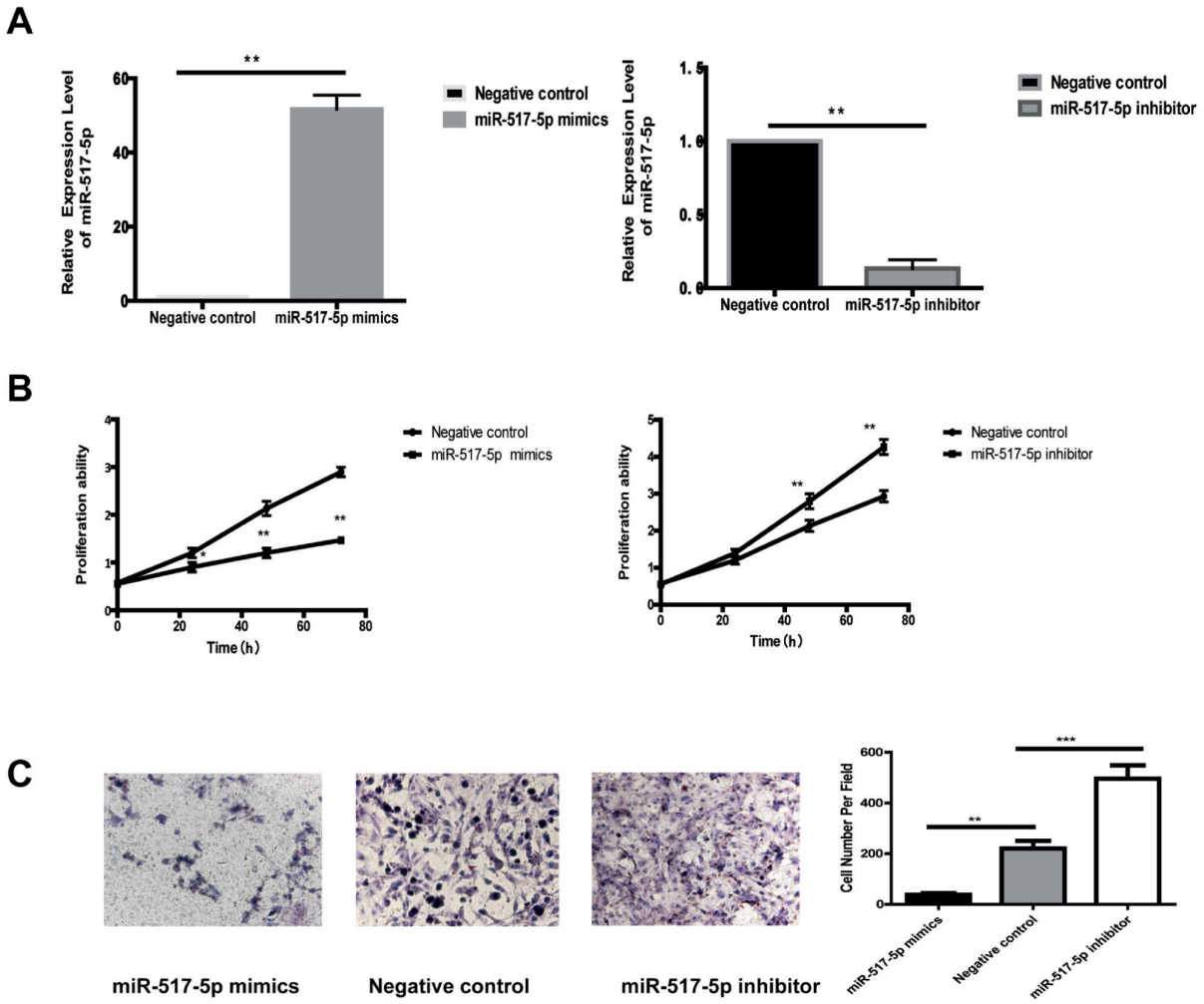

Figure 2. MiR-517-5p inhibited the proliferation and invasion of JAR cells. JAR cells were transfected with miR-517-5p mimics or inhibitors, and the expression level of miR-517-5p was analyzed by Real-time PCR. ***A,*** Transfection effects of miR-517-5p mimics and miR-517-5p inhibitor were evaluated by Real-time qPCR. ***B,*** The CCK-8 assay result showed that the proliferation of JAR cells decreased in miR-517-5p mimics group compared with control, but increased in miR-517-5p inhibitor group compared with control**.** ***C,*** Transwell assay result showed that the invasion of JAR cells decreased in miR-517-5p mimics group compared with control, but increased in miR-517-5p inhibitor group compared with control ($^{**}p<0.01$, $^{***}p<0.001$).

Inhibition of the ERK/MMP-2 Pathway Reduced JAR Cells Proliferation Ability

To further verify whether ERK/MMP-2 pathway could regulate proliferative ability of placental cells, JAR cells were treated with PD98059 (ERKi), a specific inhibitor of ERK1/2. PD98059 treatment inhibited ERK1/2 phosphorylation and MMP-2 expression in JAR cells. This result also indicated that the decreased expression level of MMP-2 was caused by ERK1/2 phosphorylation inhibition (Figure 5). The CCK-8 result showed that the proliferative ability of JAR cells is inhibited by ERK inhibitor. This result also suggested that miR-517-5p regulates proliferative ability of placental cells by regulating ERK/MMP-2 pathway.

Upregulation of MMP-2 Partly Reversed Proliferative and Invasive abilities in JAR Cells

MMP-2 plays an essential role in regulating cell proliferation and invasion. The expression level of MMP-2 significantly decreased in miR-517-5p mimics transfected JAR cells. Besides, inhibition of

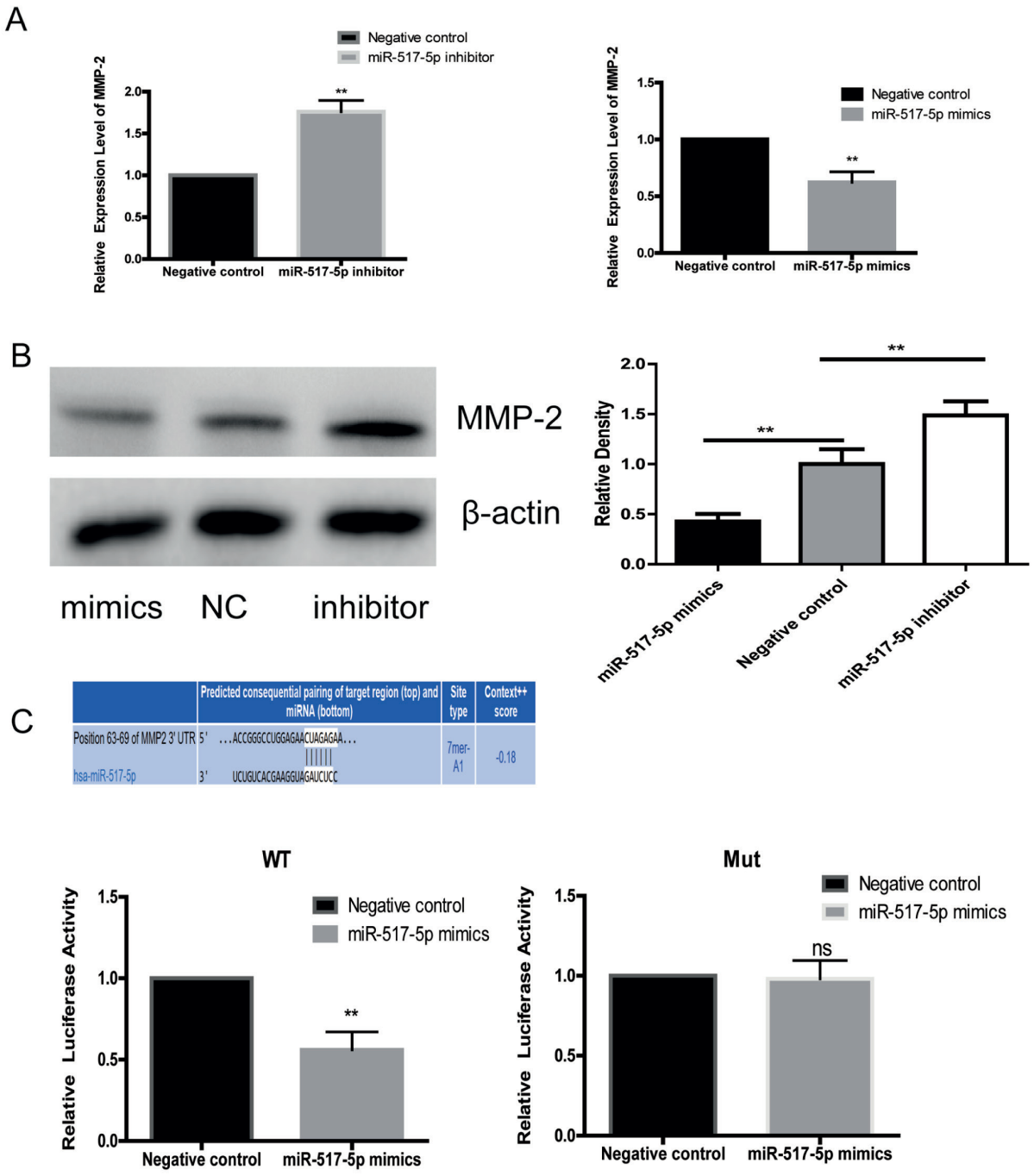

Figure 3. MMP-2 was the target gene of miR-517-5p. JAR cells were transfected with miR-517-5p mimics or inhibitor, and the expression level of MMP-2 was analyzed by Real-time PCR and Western-blot. *A,* The expression level of MMP-2 decreased in miR-517-5p mimics transfected cells compared with control, but increased in miR-517-5p inhibitor transfected cells compared with control. *B,* Western-blot showed miR-517-5p mimics decreased protein expression of MMP-2, but miR-517-5p inhibitor increased protein expression of MMP-2. *C,* Luciferase plasmid of wild-type PGL3/MMP2-3'UTR 3'UTR was transfected with miR-517-5p. The luciferase activity of wild-type PGL3/MMP2-3'UTR 3'UTR significantly decreased. The luciferase activity of mutant PGL3/MMP2-3'UTR 3'UTR remained unchanged (** $p<0.01$, ns, none significant).

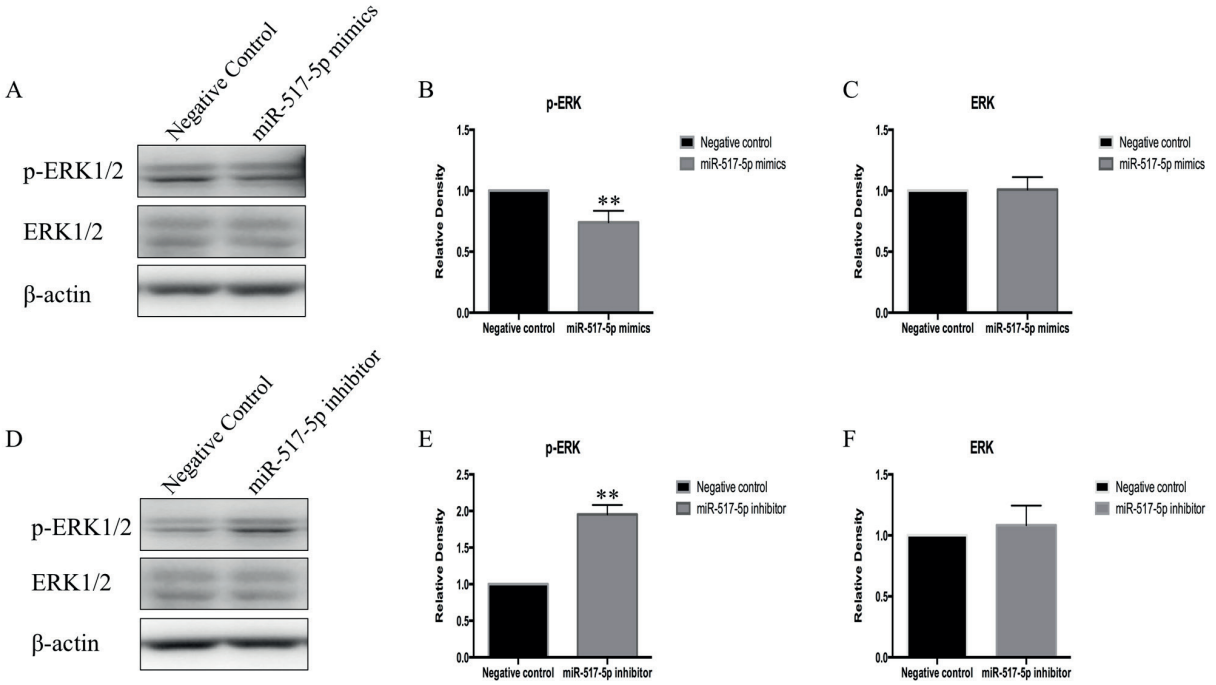

Figure 4. ERK pathway was regulated by miR-517-5p. JAR cells were transfected with miR-517-5p mimics or inhibitor, and the activity of ERK pathway was analyzed by Western Blot. ***A,*** The protein level of p-ERK in miR-517-5p mimics transfected JAR cells significantly decreased and the protein level of ERK remained unchanged. ***B,*** Density analysis of p-ERK in miR-517-5p mimics transfected JAR cells. ***C,*** Density analysis of ERK in miR-517-5p mimics transfected JAR cells. ***D,*** The protein level of p-ERK in miR-517-5p inhibitor transfected JAR cells significantly increased and the protein level of ERK remained unchanged. ***E,*** Density analysis of p-ERK in miR-517-5p inhibitor transfected JAR cells. ***F,*** Density analysis of ERK in miR-517-5p inhibitor transfected JAR cells (** $p<0.01$).

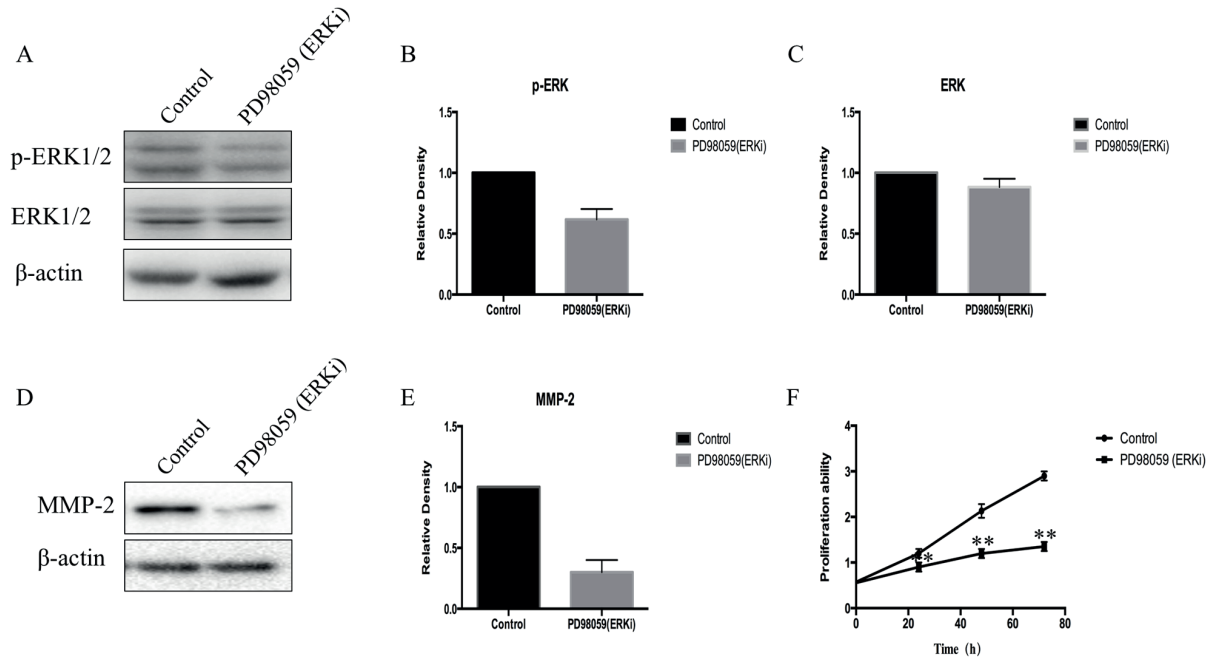

Figure 5. Inhibition of the ERK/MMP-2 pathway reduced proliferation of JAR cells. JAR cells were treated with 2 μM PD98059 (ERKi) for 72 hour. The cells were collected for protein isolation and CCK-8 analysis. *A,* The protein level of p-ERK and ERK in PD98059 treated JAR cells were evaluated by Western blot. *B,* Density analysis of p-ERK in PD98059 treated JAR cells. *C,* Density analysis of ERK in PD98059 treated JAR cells. *D,* The protein level of MMP-2 in PD98059 treated JAR cells was evaluated by Western Blot. *E,* Density analysis of MMP-2in PD98059 treated JAR cells (** $p<0.01$).

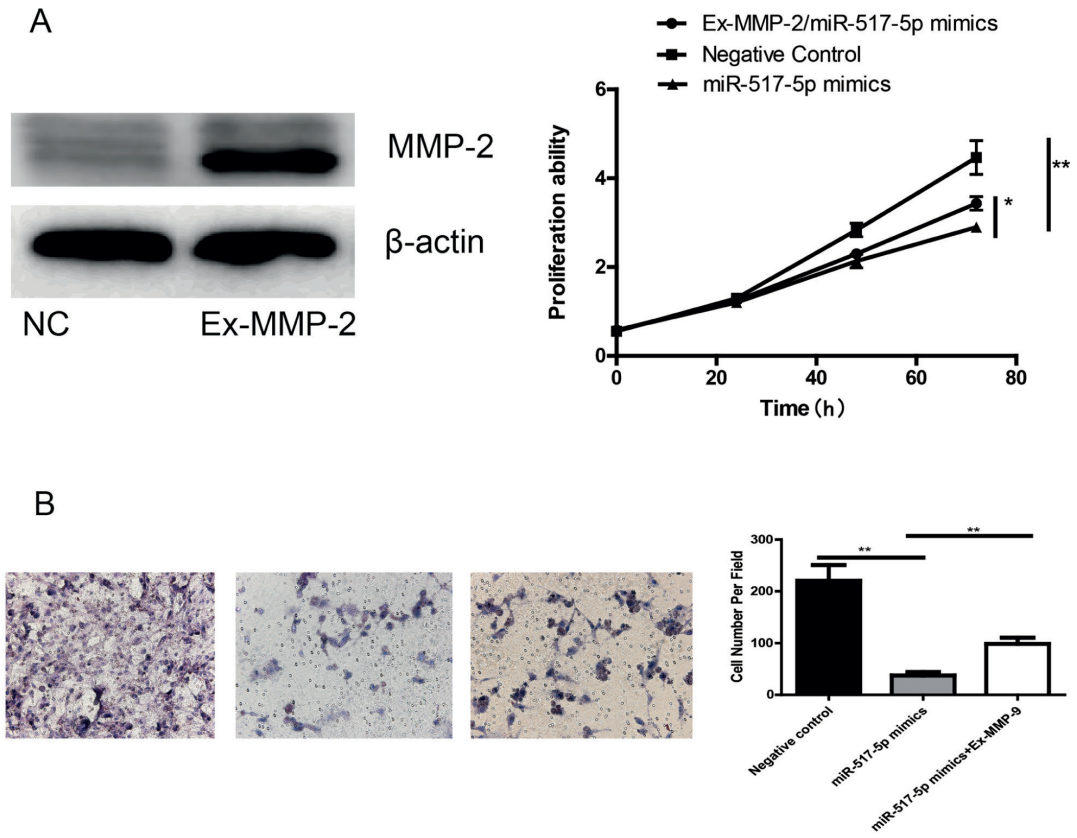

Figure 6. Upregulation of MMP-2 partly reversed inhibited proliferation and invasion induced by miR-517-5p in JAR cells. JAR cells were transfected with MMP-2 overexpression plasmid and miR-517-5p mimics. *A,* The overexpression effect of MMP-2 was confirmed by Western blot. The cell proliferation of JAR cells was evaluated by CCK-8 assay. *B,* The cell invasion of JAR cells was evaluated by transwell assay (** $p<0.01$).

ERK pathway also inhibited the expression level of MMP-2. Therefore, we suggested that MMP-2 is a key factor in the phenotype change of placental cells. JAR cells were co-transfected with miR-517-5p mimics and MMP-2 overexpression plasmid. Overexpression of MMP-2 partly restored the inhibited proliferation by miR-517-5p in JAR cells (Figure 6A). In addition, overexpression of MMP-2 partly restored the inhibited invasion by miR-517-5p in JAR cells (Figure 6B). These results showed that miR-517-5p inhibits proliferation and invasion of placental cells by inhibiting ERK/MMP-2 pathway.

Discussion

Preeclampsia is one of the most serious diseases in late pregnancy with high morbidity and mortality. However, the cause of PE is unknown. To date, the potential function of miR-517-5p in PE has been rarely reported In this study, the miR-517-5p expression level was higher in placenta tissues of PE pregnancies compared with those without hypertension, which was consistent with the previous reports[17,21]. Many reports[22-24] have found that miR-517 plays a crucial role in various diseases, such as PE, lung cancer and bladder cancer. Previous studies[22] have shown that miR-517a/b contributes to the development of PE by altering extra villous trophoblast function at the first trimester. MiR-517a accelerates lung cancer cell invasion and proliferation through inhibiting FOXJ3 expression[23]. MiR-517a could also regulate cell apoptosis in bladder cell lines[24]. In this report, we found of the highly expressed miR-517-5p could inhibit proliferation and invasion of placental cells. However, the other functions of miR-517-5p should also be investigated in placental cells. According to the previous studies, the researchers also observed its higher expression in the plasma of preeclampsia[25]. We considered that

miR-517-5p may serve as a biomarker for PE. In placental cell lines, our results demonstrated that miR-517-5p could decrease cell proliferation and invasion ability by inhibiting ERK/MMP-2 pathway, which has not been reported before. This result suggested that miR-517-5p expression is associated with proliferative and invasive abilities of placental cells. Furthermore, we predicted the target gene of miR-517-5p. According to the target gene prediction, MMP-2 was screened out. The MMP-2 gene is an important member of the matrix metalloproteinase family and closely related to cell proliferation and apoptosis. From the reported articles, viability of vascular smooth muscle cells (VSMCs) were significantly inhibited by the treatment of high-concentration MMP-2[26]. MMP-2 was also reported to regulate cardiomyocyte dedifferentiation and proliferation, which contribute to cardiomyocyte regeneration[27]. MMP-2 could regulate cell migration and invasion of various cells, such as endometriotic cells, cervical cancer cells and trophoblast cells[28-30].

Conclusions

We found that miR-517-5p was highly expressed in preeclampsia placenta tissues. MMP-2, as the target gene of miR-517-5p, was dysregulated in the placental tissues of preeclampsia. MiR-517-5p inhibits proliferation and invasion of placental cells by inhibiting ERK/MMP-2 pathway. Our study suggest that miR-517-5p could be used as a predictor of preeclampsia.

Conflict of Interest

The Authors declare that they have no conflict of interest.

References

1) Lambert G, Brichant JF, Hartstein G, Bonhomme V, Dewandre PY. Preeclampsia: an update. Acta Anaesthesiol Belg 2014; 65: 137-149.
2) Hansen AT, Kesmodel US, Juul S, Hvas AM. Increased venous thrombosis incidence in pregnancies after in vitro fertilization. Hum Reprod 2014; 29: 611-617.
3) Nirmala CK, Nor AM, Harry SR, Lim PS, Shafiee MN, Nur AA, Omar MH, Hatta MD. Outcome of molar pregnancies in Malaysia: a tertiary centre experience. J Obstet Gynaecol 2013; 33: 191-193.
4) Guerby P, Vidal F, Garoby-Salom S, Vayssiere C, Salvayre R, Parant O, Negre-Salvayre A. [Oxidative stress and preeclampsia: a review]. Gynecol Obstet Fertil 2015; 43: 751-756.
5) Staff AC, Johnsen GM, Dechend R, Redman CW. Preeclampsia and uteroplacental acute atherosis: immune and inflammatory factors. J Reprod Immunol 2014; 101-102: 120-126.
6) Saito S, Nakashima A. A review of the mechanism for poor placentation in early-onset preeclampsia: the role of autophagy in trophoblast invasion and vascular remodeling. J Reprod Immunol 2014; 101-102: 80-88.
7) He G, Xu W, Chen Y, Liu X, Xi M. Abnormal apoptosis of trophoblastic cells is related to the up-regulation of CYP11A gene in placenta of preeclampsia patients. PLoS One 2013; 8: e59609.
8) Ji L, Brkic J, Liu M, Fu G, Peng C, Wang YL. Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. Mol Aspects Med 2013; 34: 981-1023.
9) Liu H, Li Y, Zhang J, Rao M, Liang H, Liu G. The defect of both angiogenesis and lymphangiogenesis is involved in preeclampsia. Placenta 2015; 36: 279-286.
10) Jiang F, Li J, Wu G, Miao Z, Lu L, Ren G, Wang X. Upregulation of microRNA335 and microRNA584 contributes to the pathogenesis of severe preeclampsia through downregulation of endothelial nitric oxide synthase. Mol Med Rep 2015; 12: 5383-5390.
11) Hou WZ, Chen XL, Wu W, Hang CH. MicroRNA-370-3p inhibits human vascular smooth muscle cell proliferation via targeting KDR/AKT signaling pathway in cerebral aneurysm. Eur Rev Med Pharmacol Sci 2017; 21: 1080-1087.
12) Maalouf SW, Smith CL, Pate JL. Changes in MicroRNA expression during maturation of the bovine corpus luteum: regulation of luteal cell proliferation and function by MicroRNA-34a. Biol Reprod 2016; 94: 71.
13) Maul J, Baumjohann D. Emerging roles for MicroRNAs in t follicular helper cell differentiation. Trends Immunol 2016; 37: 297-309.
14) Xie L, Sadovsky Y. The function of miR-519d in cell migration, invasion, and proliferation suggests a role in early placentation. Placenta 2016; 48: 34-37.
15) Cai M, Kolluru GK, Ahmed A. Small molecule, big prospects: MicroRNA in pregnancy and its complications. J Pregnancy 2017; 2017: 6972732.
16) Muralimanoharan S, Maloyan A, Mele J, Guo C, Myatt LG, Myatt L. MIR-210 modulates mitochondrial respiration in placenta with preeclampsia. Placenta 2012; 33: 816-823.
17) Hromadnikova I, Kotlabova K, Ivankova K, Krofta L. First trimester screening of circulating C19MC microRNAs and the evaluation of their potential to predict the onset of preeclampsia and IUGR. PLoS One 2017; 12: e171756.
18) Naruse K, Lash GE, Innes BA, Otun HA, Searle RF, Robson SC, Bulmer JN. Localization of matrix metalloproteinase (MMP)-2, MMP-9 and tissue inhibitors for MMPs (TIMPs) in uterine natural killer

cells in early human pregnancy. Hum Reprod 2009; 24: 553-561.

19) McEnery MW, Pedersen PL. Diethylstilbestrol. A novel F0-directed probe of the mitochondrial proton ATPase. J Biol Chem 1986; 261: 1745-1752.

20) Xiao LJ, Lin P, Lin F, Liu X, Qin W, Zou HF, Guo L, Liu W, Wang SJ, Yu XG. ADAM17 targets MMP-2 and MMP-9 via EGFR-MEK-ERK pathway activation to promote prostate cancer cell invasion. Int J Oncol 2012; 40: 1714-1724.

21) Hromadnikova I, Kotlabova K, Ivankova K, Krofta L. Expression profile of C19MC microRNAs in placental tissue of patients with preterm prelabor rupture of membranes and spontaneous preterm birth. Mol Med Rep 2017; 16: 3849-3862.

22) Anton L, Olarerin-George AO, Hogenesch JB, Elovitz MA. Placental expression of miR-517a/b and miR-517c contributes to trophoblast dysfunction and preeclampsia. PLoS One 2015; 10: e122707.

23) Jin J, Zhou S, Li C, Xu R, Zu L, You J, Zhang B. MiR-517a-3p accelerates lung cancer cell proliferation and invasion through inhibiting FOXJ3 expression. Life Sci 2014; 108: 48-53.

24) Yoshitomi T, Kawakami K, Enokida H, Chiyomaru T, Kagara I, Tatarano S, Yoshino H, Arimura H, Nishiyama K, Seki N, Nakagawa M. Restoration of miR-517a expression induces cell apoptosis in bladder cancer cell lines. Oncol Rep 2011; 25: 1661-1668.

25) Hromadnikova I, Kotlabova K, Krofta L, Hron F. Follow-up of gestational trophoblastic disease/neoplasia via quantification of circulating nucleic acids of placental origin using C19MC microRNAs, hypermethylated RASSF1A, and SRY sequences. Tumour Biol 2017; 39: 1393392116.

26) Liu T, Lin J, Ju T, Chu L, Zhang L. Vascular smooth muscle cell differentiation to an osteogenic phenotype involves matrix metalloproteinase-2 modulation by homocysteine. Mol Cell Biochem 2015; 406: 139-149.

27) Fan X, Hughes BG, Ali MA, Chan BY, Launier K, Schulz R. Matrix metalloproteinase-2 in oncostatin M-induced sarcomere degeneration in cardiomyocytes. Am J Physiol Heart Circ Physiol 2016; 311: H183-H189.

28) Ding J, Huang F, Wu G, Han T, Xu F, Weng D, Wu C, Zhang X, Yao Y, Zhu X. MiR-519d-3p suppresses invasion and migration of trophoblast cells via targeting MMP-2. PLoS One 2015; 10: e120321.

29) Ahn JH, Choi YS, Choi JH. Leptin promotes human endometriotic cell migration and invasion by up-regulating MMP-2 through the JAK2/STAT3 signaling pathway. Mol Hum Reprod 2015; 21: 792-802.

30) Zhu D, Ye M, Zhang W. E6/E7 oncoproteins of high risk HPV-16 upregulate MT1-MMP, MMP-2 and MMP-9 and promote the migration of cervical cancer cells. Int J Clin Exp Pathol 2015; 8: 4981-4989.