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lncRNA FOXD2-AS1 affects trophoblast cell proliferation, invasion and migration through targeting miRNA

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Research Article

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Summary

The abnormal expression of lncRNAs and miRNAs has been found in the placentas of patients with preeclampsia (PE). Therefore, we determined the role of lncRNA FOXD2-AS1/miR-3127 in trophoblast cells. The expression of lncRNA FOXD2-AS1 was detected by qRT-PCR. The proliferation, migration and invasion ability of trophoblast cells were evaluated using CCK-8, wound healing and transwell assays. The target gene of lncRNA FOXD2-AS1 was determined by StarBase and luciferase reporter assays. Western blotting was used to analyze the expression of matrix metalloproteinase 2 (MMP2) and matrix metalloproteinase 9 (MMP9). The results showed that FOXD2-AS1 affected trophoblast cell viability *in vitro*, while the expression of miR-3127 was decreased. FOXD2-AS1 silencing decreased the promotion effects on trophoblast cell induced by miR-3127 inhibition. In addition, FOXD2-AS1 and miR-3127 presented the same effect on MMP2 and MMP9 levels. lncRNA FOXD2-AS1 modulated trophoblast cell proliferation, invasion and migration through downregulating miR-3127 expression. Therefore, lncRNA FOXD2-AS1 could act as a latent therapeutic marker in preeclampsia.

Introduction

Preeclampsia (PE) is characterized by blood pressure higher than 140/90 mmHg after 20 weeks gestation that seriously affects the perinatal survival rates of mothers and children (Tangeras *et al.*, 2015). Inadequate trophoblastic invasion was believed to play a role in the pathogenesis of PE (Wu *et al.*, 2019).

Extravillous trophoblasts (EVTs) are involved in the establishment of maternal and fetal circulation (Ashton *et al.*, 2005; Harris *et al.*, 2006). EVTs migrating and invading the decidua, and then entering the maternal spiral arteries of the myometrium, could initiate vascular remodelling process in early pregnancy. This process is crucial for decreased blood flow resistance and increased uteroplacental perfusion (Brosens and Renaer, 1972). However, defects in this process would lead to serious pregnancy syndromes, such as PE (Meekins *et al.*, 1994). Changes in the microenvironment *in vivo* could affect the vascular remodelling process. Matrix metalloproteinases (MMPs) could degrade extracellular matrix and help tissue remodelling during pregnancy (Soundararajan and Rao, 2004), among which MMP2 and MMP9 have been regarded as the key enzymes during implantation (Rahat *et al.*, 2016). A review demonstrated that reduction in MMP-2 and MMP-9 levels in early gestation reduced the remodelling of spiral arteries (Apicella *et al.*, 2019).

The mechanisms related to the progression of cell invasion and migration in PE remain unclear. Some studies have shown that genetic events could play vital roles in the development and improvement of the PE. lncRNAs are RNAs of more than 200 nucleotides in length, involving multiple physiological activities such as cell growth, differentiation and apoptosis (Zhao *et al.*, 2018). Some researchers have demonstrated that lncRNA levels were reduced in PE placentas (Xu *et al.*, 2018a; Wu *et al.*, 2019) and implied that lncRNA plays a vital role in the progression of PE. For example, lncRNA HOXA11-AS1 could affect trophoblast cell proliferation by modulating HOXA7 expression via sponged miR-15b-5p (Xu *et al.*, 2018b). Search of the Ensembl database showed that lncRNA FOXD2-AS1, FOXD2 adjacent opposite strand RNA 1, had one non-coding exon. It was reported that FOXD2-AS1 was involved in tumour and OA progression. FOXD2-AS1 could reverse resistance to sorafenib in hepatocellular carcinoma mediated by miR-150-5p (Sui *et al.*, 2019). Furthermore, the effects of FOXD2-AS1 were reversed via miR-206 inhibitors on chondrocytes viability in osteoarthritis (Cao *et al.*, 2018). The review showed that some miRNAs are thought to be involved in placental development by suppressing genes associated with regulation of trophoblast fate, invasion and proliferation (Apicella *et al.*, 2019). However, the role of lncRNA FOXD2-AS1 in trophoblast cells remains unclear. In this study we determined the role of lncRNA FOXD2-AS1 in trophoblast cell proliferation, invasion and migration and elucidated the underlying mechanism.

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Materials and methods

Tissue samples and patients

Patients diagnosed with PE in Huai'an First People's Hospital from 2018 to 2019 were chosen for this study. The plasma from patients with PE or unaffected pregnancies were collected from the relevant research studies and stored at −80°C. In total, 52 PE patients and 52 healthy pregnancies were included in the study. Each patient in the study submitted informed written consents. The study was authorized by the Ethic Committee of Huai'an First People's Hospital of Nanjing Medical University, China.

Cell culture

HTR-8/SVneo cells were cultured in RPMI 1640 medium (Gibco, USA) containing 10% fetal bovine serum (Thermo Scientific, USA), 100 IU/ml penicillin and 100 µg/ml streptomycin (Invitrogen, USA). Cells were cultured in a 5% CO_2 in air atmosphere at 37°C.

Plasmids, microRNAs and cell transfection

shRNA plasmids vector against FOXD2-AS1-1 were obtained from Shanghai GEMAR Pharmaceutical Technology (Shanghai, China), which were respectively shown as shRNA-FOXD2-AS1-1 and shRNA-FOXD2-AS1-2. The plasmid overexpressing lnc-FOXD2-AS1 was provided by the same company. miRs including miR-3127 mimics and corresponding negative control (miR-NC), as well as miR-3127 inhibitors and corresponding negative control were purchased from Guangzhou Rubio Biotechnology (Guangzhou, China). The plasmid and oligonucleotide were transfected into cells using Lipofectamine 2000 reagent (Invitrogen, USA). Cells were then cultured continuously for 36 h.

RNA isolation and qRT-PCR

Total RNA extraction kit was used to extract total RNA (Beyotime, Shanghai, China) following the manufacturer's instructions. RNA concentration was measured using an ultraviolet light spectrophotometer; FOXD2-AS1 and miRNA-3127 were reverse transcribed into cDNA using the cDNA Reverse Transcription Kit and the miRNA and Reverse Transcription Kit (Beyotime, Shanghai, China). The qPCR reactive system was prepared using the SYBR Select Master Mix (Thermo Scientific, USA). Finally, relative expression levels of FOXD2-AS1 and miR-3127 were detected using a RT-PCR system.

Cell proliferation assay

Cell proliferation was determined using the CCK-8 assay (KeyGen, Nanjing, China). HTR-8/SVneo cells post-transfection were seeded into 96-well plates. Here, 10 µl of CCK-8 solution was added to each well, which was incubated for 24, 48 or 72 h. Later, the cells were incubated at 37°C for 4 h. Absorbance at 450 nm was measured using a microplate reader (Bio-Rad, Hercules, CA, USA).

Cell migration and invasion assays

Cell migration was detected using the wound healing assay. HTR-8/SVneo cells were cultured in 6-well plates for 24 h, then a sterile plastic tip was used to create a wound by the scratching cells. The remaining cells were continuously cultured for 24 h. ImageJ software was used to analyze the photographs taken at 0 and 24 h under an inverted microscope. BD Matrial matrix was put on the upper surface of a transwell chamber. HTR-8/SVneo cells suspended in serum-free medium after 36 h of transfection, were added into the upper chamber. The lower chamber contained 600 µl of medium with 10% FBS. The cells on the upper chamber were cleaned out a cotton swab. The cells that invaded the low chamber through the BD Matrial matrix were fixed with paraformaldehyde and stained with crystal violet. A inverted microscope was used to take photographs for the cells and for cell counting.

Dual-luciferase reporter gene assay

The wild-type FOXD2-AS1 luciferase plasmid (FOXD2-AS1–3′UTR-WT) in which was included the DNA sequence of FOXD2-AS1-1 3′ untranslated region (UTR) containing the miR-3127 binding site was purchased from Promega (Madison, WI, USA). Mutant-type FOXD2-AS1 luciferase plasmid (FOXD2-AS1–3′UTR-MUT) containing the mutant region of miR-3127 binding site was also purchased. The constructed luciferase reporter vectors, FOXD2-AS1–3′UTR-WT/MUT and the miR-3127 mimic or miR-NC were cotransfected into HTR-8/SVneo cells using Lipofectamine 2000. After 48 h, the fluorinase activity of each group of cells was detected using a Dual-Luciferase Reporter Assay (Promega).

Western blot assays

Total proteins from each group were extracted using RIPA protein cell lysate buffer. The BCA method was used to detect protein concentration. Target proteins were separated using 10% SDS-PAGE and transferred into PVDF membranes by electrophoretic transfer. Then, the corresponding primary antibodies (rabbit anti-MMP2, 1:1000; rabbit anti-MMP9, 1:1000; mouse anti-GAPDH, 1:1500; abcam, Cambridge, USA) were added and membranes were incubated overnight at 4°C after sealing. After washing, the blots were incubated at 37°C for 1 h with the corresponding secondary antibody (anti-rabbit, 1:2500; anti-mouse, 1:10,000) coupled to horseradish peroxidase (HPR). Electrochemiluminescence (ECL) was used to develop the colour, and the blot was scanned and analyzed using a gel imager. The intensities of the grey levels of the target band were analyzed using ImageJ software.

Statistical analysis

Data analysis was carried out using GraphPad Prism software. Data from the groups were analyzed using one-way analysis of variance (ANOVA) followed by post hoc tests. A P-value <0.05 was considered to be statistically significant.

Results

FOXD2-AS1 expression was decreased in PE patients

In the present study, we explored FOXD2-AS1 expression in plasma using qRT-PCR. The results showed that FOXD2-AS1 expression was significantly decreased in PE patients and

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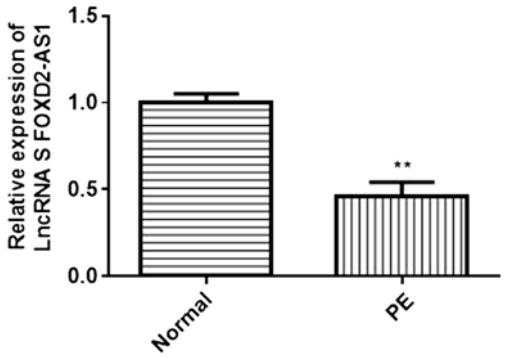


Figure 1. FOXD2-AS1 expression was upregulated in pre-eclampsia patients compared with patients with unaffected pregnancies. $^{**}P < 0.01$ compared with the control group.

implied that FOXD2-AS1 might play a pivotal role in PE progression (Fig. 1).

FOXD2-AS1 silencing inhibits cell proliferation, invasion and migration in the trophoblast cell

Cell proliferation, migration and invasion are important features of PE progression. FOXD2-AS1 was silenced in HTR-8/SVneo cells by transfection with shRNA-FOXD2-AS1-1 and shRNA-FOXD2-AS1-2. We found that HTR-8/SVneo cells transfected with shRNA-FOXD2-AS1-1 and shRNA-FOXD2-AS1-2 significantly decreased FOXD2-AS1 expression compared with cells transfected with shRNA-NC. shRNA-FOXD2-AS1-1 exhibited greater effects on expression of FOXD2-AS1-1 in HTR-8/SVneo cells as confirmed at transcriptional level (Fig. 2*A*). Therefore, shRNA-FOXD2-AS1-1 was used as a candidate shRNA for all subsequently studies. CCK-8 assay showed that FOXD2-AS1 silencing in HTR-8/SVneo cells significantly reduced cell proliferation (Fig. 2*B*). The wound healing assay showed that FOXD2-AS1 silencing in HTR-8/SVneo cells attenuated cell migration abilities (Fig. 2*C*, *E*). Furthermore, the transwell invasion assay demonstrated that downregulation of FOXD2-AS1 in HTR-8/SVneo cells caused a significant decrease in the number of invaded cells (Fig. 2*D*, *F*).

FOXD2-AS1 overexpression promotes cell proliferation, invasion and migration in the trophoblast cell

FOXD2-AS1 was upregulated in HTR-8/SVneo cells (Fig. 3*A*). CCK-8, wound healing and transwell assays showed that FOXD2-AS1 overexpression significantly promoted the proliferation, migration and invasion of HTR-8/SVneo cells (Fig. 3*B*–*F*).

FOXD2-AS1 significantly affects the MMP2 and MMP9 levels

Elevations of MMP-2 and MMP-9 levels could help to maintain normal pregnancy (Eleuterio *et al.*, 2015). Western blot analysis indicated that FOXD2-AS1 overexpression or knockdown significantly increased or decreased levels of MMP2 and MMP9 (Fig. 4*A*, *B*).

miR-3127 is a target of FOXD2-AS1 in trophoblast cells

To determine whether FOXD2-AS1 bound miRNAs, the potential binding force of miRNA-3127 and FOXD2-AS1 was predicted using StarBase v.2.0 software (Li *et al.*, 2014) (Fig. 5*A*). Dual-luciferase assay showed that cotransfection of miR-3127 mimicked the FOXD2-AS1 WT, rather than the FOXD2-AS1 mutant, and significantly suppressed luciferase activity (Fig. 5*B*). Moreover, qRT-PCR data revealed that FOXD2-AS1 overexpression significantly decreased miR-3127 expression in HTR-8/SVneo cells (Fig. 5*C*). Taken together, we believe that miR-3127 is a target of FOXD2-AS1 in HTR-8/SVneo cells.

FOXD2-AS1 silencing inhibits cell progression in trophoblast cells by upregulating miR-3127

Effects of miR-3127 on proliferation, migration and invasion of HTR-8/SVneo cells were investigated. CCK-8, wound healing and transwell assays showed that the miR-3127 inhibitor significantly increased trophoblast cell proliferation, migration and invasion. Furthermore, downregulation of FOXD2-AS1 notably reversed these effects (Fig. 6*A*–*E*). Western blot results indicated that FOXD2-AS1-1 silencing could reduce the levels of MMP2 and MMP9 increased by miR-3127 inhibitors (Fig. 6*F*).

Discussion

Preeclampsia is caused by the interaction of both genetic and environmental factors (Janani and Changaee, 2017). Impaired migration and invasion of trophoblastic cells have been proposed as possible causes of pathogenesis of PE pathogenesis. Abnormal expression of lncRNAs and miRNAs has been found in the placentas of patients with PE. Therefore, exploration and identification of PE-associated lncRNAs and their functions may provide a new route for understanding PE pathogenesis and defining novel therapeutic targets, as well as provide diagnostic and prognostic markers for PE (Wu *et al.*, 2019).

In this study, we explored the potential molecular mechanism of lncRNA FOXD2-AS1 using trophoblastic cell lines. FOXD2-AS1 has been mainly researched for its functions in osteoarthritis and cancer. Our study found that expression of FOXD2-AS1 was decreased in the plasma of women with PE compared with women with unaffected pregnancies. *In vitro*, FOXD2-AS1 overexpression could effectively promote the progression of trophoblast cells, whereas silencing of FOXD2-AS1 expression caused the opposite results. These findings implied that FOXD2-AS1 could be used as a vital regulatory molecule involved in influencing pathologic and physiologic processes of PE and suggested that FOXD2-AS1 may be a promising biomarker for the diagnosis of PE.

Long non-coding RNAs were reported to exert their biological roles by functioning as competing endogenous RNAs (ceRNAs) to negatively regulate miRNA expression (Wang and Chang, 2011). To investigate the underlying mechanisms by which FOXD2-AS1 exerts its biological effects on trophoblast cell proliferation, migration and invasion, it was essential to identify its binding miRNAs.

In the present study, by luciferase reporter assay, miR-3127 was identified as a downstream target of FOXD2-AS1. miR-3127

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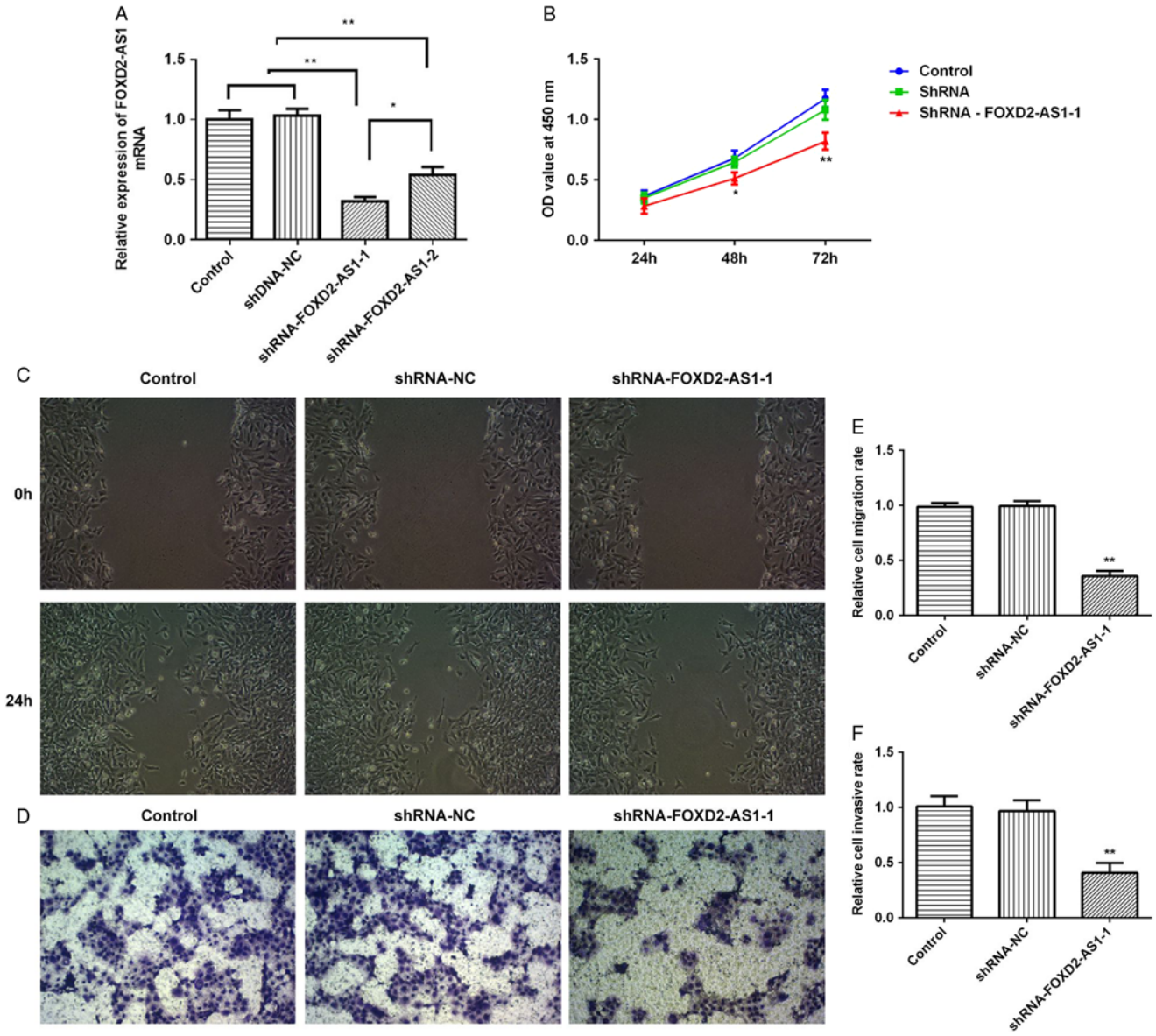


Figure 2. Downregulation of FOXD2-AS1 suppressed trophoblast cell proliferation, migration and invasion. (*A*) qRT-PCR analyzed the level of FOXD2-AS1 in HTR-8/SVneo cells transfected with ShRNA-FOXD2-AS1-1, ShRNA-FOXD2-AS1-2 or shRNA-NC. (*B*) CCK8 assay was used to evaluate the effect of FOXD2-AS1 silencing on HTR-8/SVneo cell proliferation. (*C–E*) Wound healing assay was used to investigate the effect of FOXD2-AS1 silencing on HTR-8/SVneo cell migratory capacity. (*D–F*) Transwell invasion assay was used to evaluate the effect of FOXD2-AS1 silencing on HTR-8/SVneo cell invasive capacity. $^{**}P < 0.01$ compared with the shRNA-NC group.

inhibitor significantly promoted the proliferation, invasion and migration in HTR-8/SVneo cells. This study found that miR-3127 could modulate cell proliferation in hepatocellular carcinoma (Jiang *et al.*, 2015). FOXD2-AS1 knockdown markedly altered the effect by which miR-3127 inhibitor increased trophoblast cell viability. Furthermore, miR-3127 inhibitor increased MMP2 and MMP9 levels in trophoblast cells, and upregulated MMP2 and MMP9 levels reduced by FOXD2-AS1 knockdown. A previous study showed that MMP2 and MMP9 levels were reduced in PE (Rahat *et al.*, 2016). These results suggested that miR-3127 plays a vital role in affecting the progression of PE and that miR-3127 plays a vital role in affecting the progression of PE. Previous studies have shown that miR-3127 could affect cell proliferation and invasion via signalling pathways such as PI3K/AKT, c-Abl/Ras/ERK or

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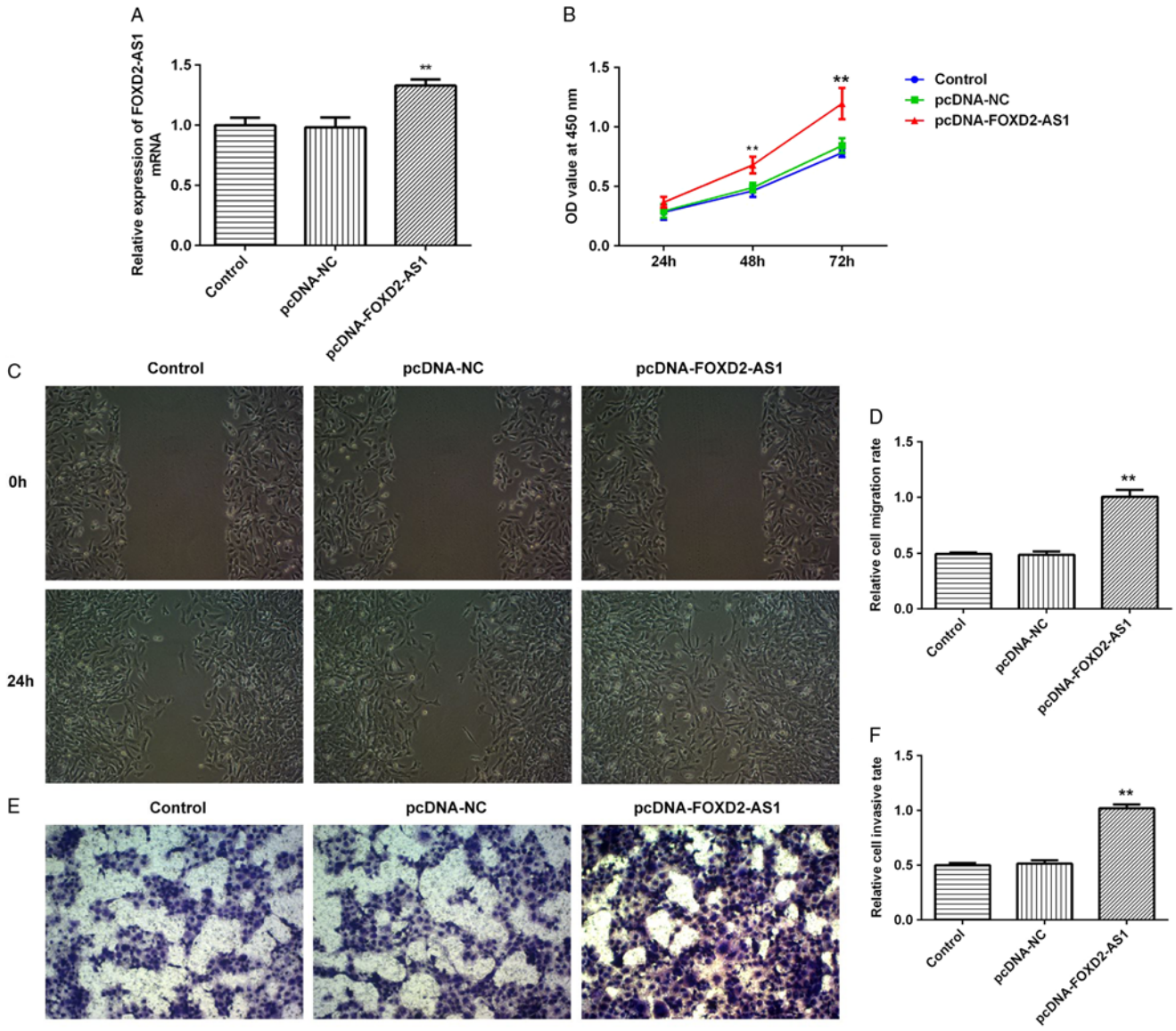


Figure 3. FOXD2-AS1 promoted cell proliferation, invasion and migration in the trophoblast cell. (*A*) FOXD2-AS1 expression in HTR-8/SVneo cells transfected with FOXD2-AS1 overexpression plasmids (PcDNA-FOXD2-AS1). (*B–F*) FOXD2-AS1 overexpression promoted HTR-8/SVneo cells proliferation, invasion and migration *in vitro*. $^{**}P < 0.01$ compared with the pcDNA-NC group.

Wnt/β-catenin signalling (Sun *et al.*, 2014; Jiang *et al.*, 2015; Yang *et al.*, 2018). However, further study is needed on how miR-3127 produces the effects in preeclampsia.

MMPs could regulate cellular migration, invasion, and angiogenesis during embryonic implantation under normal physiological conditions (Corthorn *et al.*, 2007). A previous study has indicated that overexpression of MMP2 and MMP9 could recover the invasion of HTR-8/SVneo cells decreased by BMAL1 siRNA treatment (Li *et al.*, 2017). In the present study, FOXD2-AS1 overexpression or knockdown significantly increased or decreased MMP2 and MMP9 levels in trophoblast cells. These results might imply that FOXD2-AS1 could modulate trophoblast cellular migration and invasion, partly mediated by influencing MMP2 and MMP9 levels.

In conclusion, our study showed that FOXD2-AS1 expression was lessened in placenta in the PE patients. Furthermore, FOXD2-AS1 significantly affected proliferation, invasion and migration of trophoblast cells *in vitro* by regulating miR-3127. FOXD2-AS1 regulated MMP2 and MMP9 levels by affecting miRNA-3127 levels. Further studies are required to investigate underlying mechanism for developing FOXD2-AS1 as a potential therapeutic target for PE.

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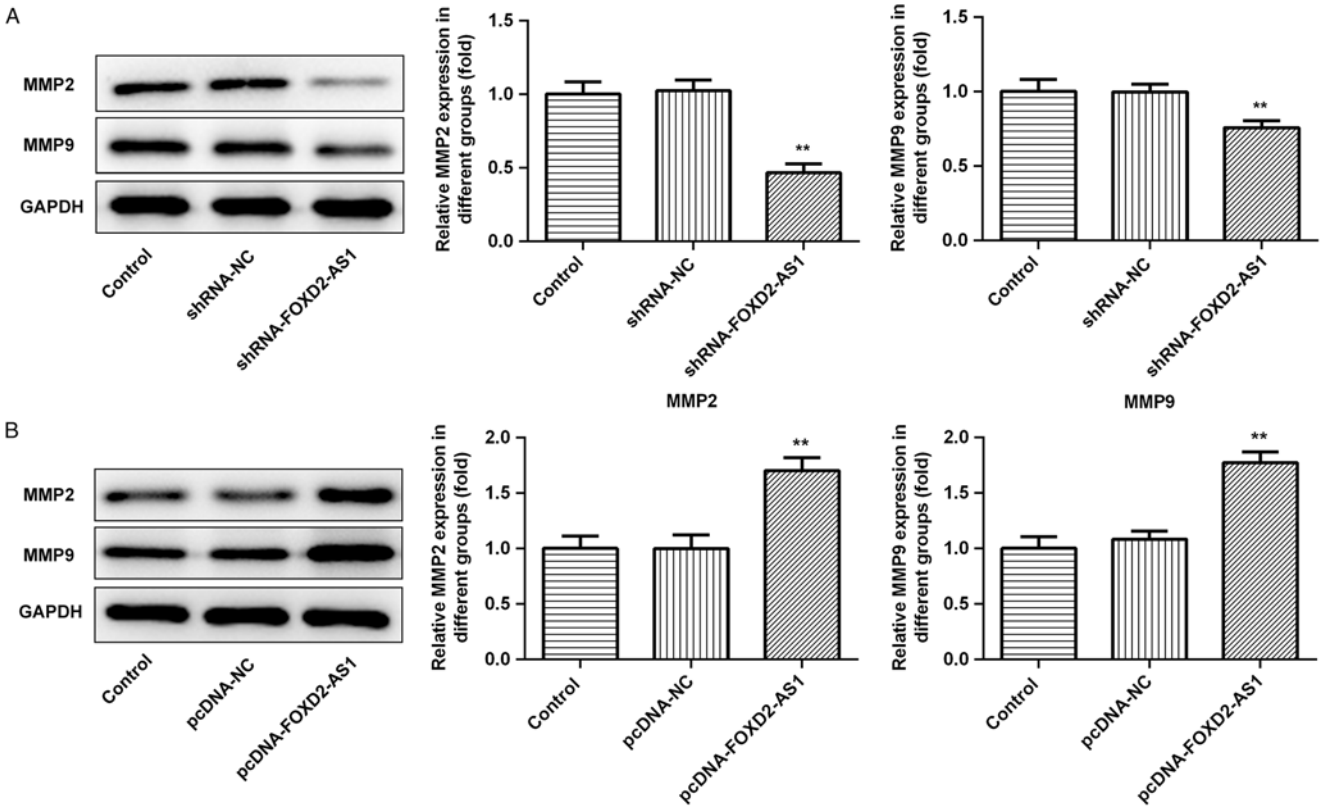


Figure 4. FOXD2-AS1 affected the MMP2 and MMP9 levels in trophoblast cells. (*A*) FOXD2-AS1 overexpression increased MMP2 and MMP9 levels. (*B*) FOXD2-AS1 knockdown decreased MMP2 and MMP9 levels. $^{**}P < 0.01$ compared with pcDNA-NC.

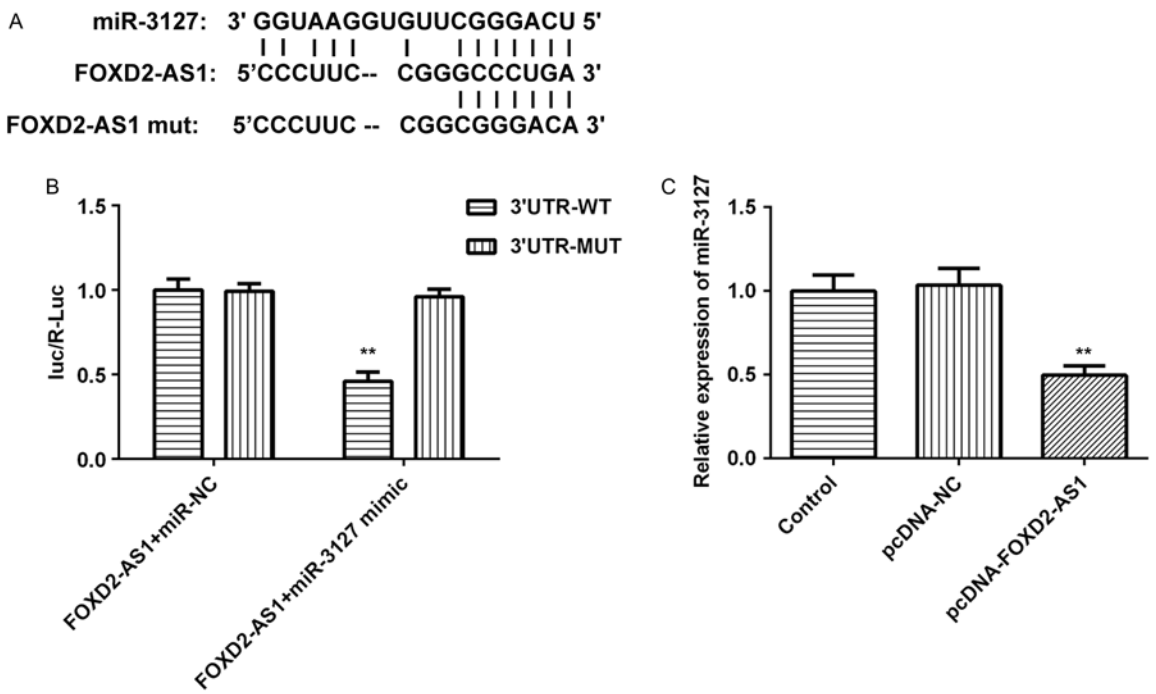


Figure 5. miR-3127 was a target of FOXD2-AS1 in trophoblast cells. (*A*) Binding and mutant sites between miR-3127 and FOXD2-AS1. (*B*) Luciferase activity in HTR-8/SVneo cells after cotransfection with miR-3127 mimic/miR-NC and FOXD2-AS1 WT or FOXD2-AS1 Mut reported plasmid. (*C*) Expression of miR-3127 in HTR-8/SVneo cells transfected with pcDNA-FOXD2-AS1 or pcDNA-NC. $^{**}P < 0.01$ compared with FOXD2-AS1 3′UTR-MUT + miR-3127 mimic in (*B*). $^{**}P < 0.01$ compared with in pcDNA-NC group in (*C*).

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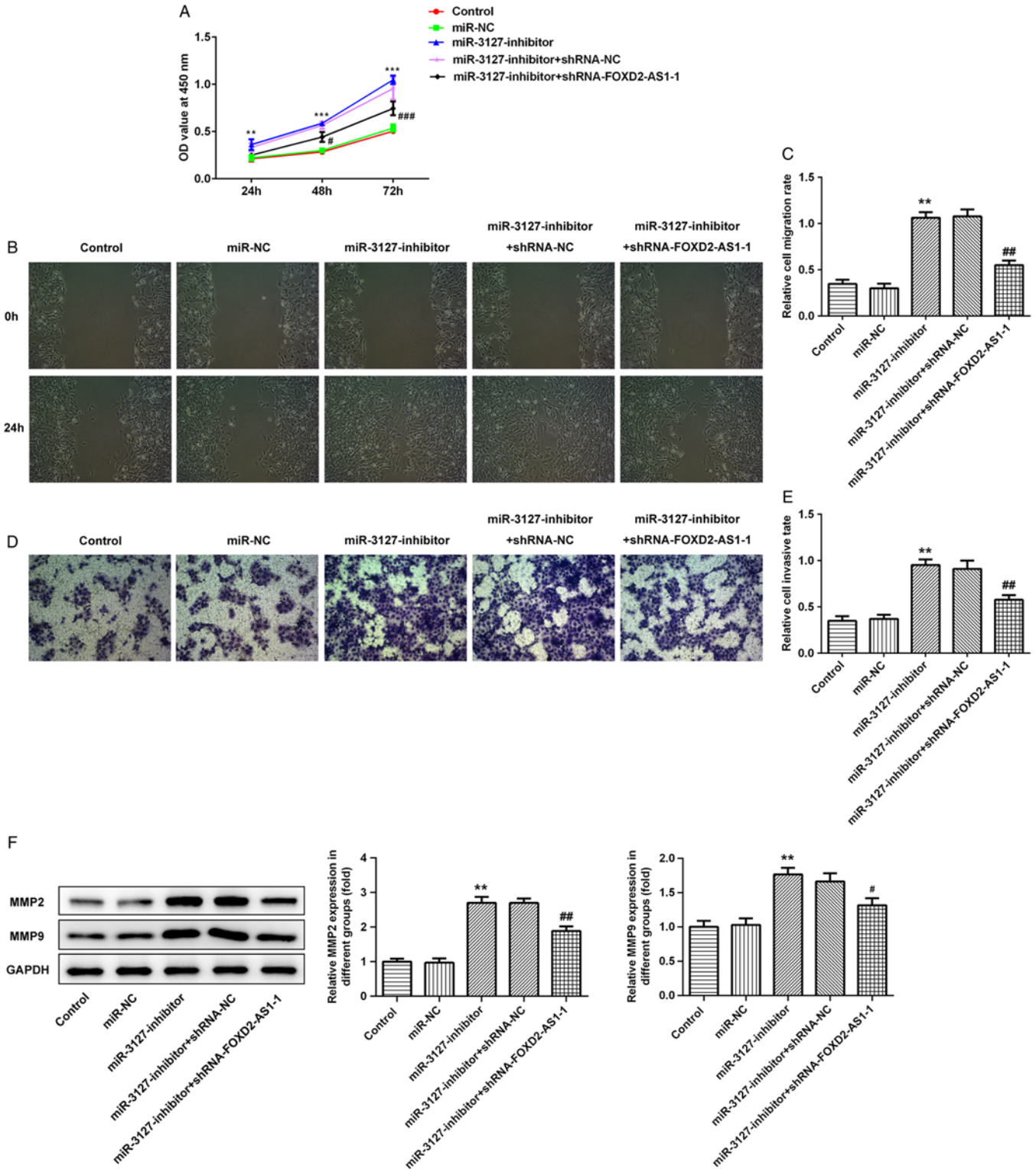


Figure 6. miR-3127 inhibitor rescued the inhibitory effect on proliferation, migration and invasion of trophoblast cells induced by FOXD2-AS1 silencing. (*A–E*) Cell proliferation, migration and invasion were detected in FOXD2-AS1 cells transfected with miR-NC, miR-3127 inhibitor, MiR-3127-inhibitor + shRNA-NC, MiR-3127-inhibitor + shRNA-FOXD2-AS1-1. (*F*) miR inhibitor increased the MMP2 and MMP9 levels. $^{**}P < 0.01$ compared with miR-NC. $^{\#\#}P < 0.01$ compared with miR-3127-inhibitor + shRNA-NC.

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Conflicts of interest. The authors declare no conflicts of interest.

References

Apicella C, Ruano CSM, Mehats C, Miralles F and Vaiman D (2019) The role of epigenetics in placental development and the etiology of preeclampsia. *Int J Mol Sci* **20**, 2837.

Ashton SV, Whitley GS, Dash PR, Wareling M, Crocker IP, Baker PN and Cartwright JE (2005) Uterine spiral artery remodeling involves endothelial apoptosis induced by extravillous trophoblasts through Fas/FasL interactions. *Arterioscler Thromb Vasc Biol* **25**, 102–8.

Brosens I and Renaer M (1972) On the pathogenesis of placental infarcts in pre-eclampsia. *J Obstet Gynaecol Br Commonw* **79**, 794–9.

Cao L, Wang Y, Wang Q and Huang J (2018) LncRNA FOXD2-AS1 regulates chondrocyte proliferation in osteoarthritis by acting as a sponge of miR-206 to modulate CCND1 expression. *Biomed Pharmacother* **106**, 1220–6.

Corthorn J, Rey S, Chacon C and Valdés G (2007) Spatio-temporal expression of MMP-2, MMP-9 and tissue kallikrein in uteroplacental units of the pregnant guinea-pig (*Cavia porcellus*). *Reprod Biol Endocrinol* **5**, 27.

Eleuterio NM, Palei AC, Rangel Machado JS, Tanus-Santos JE, Cavalli RC and Sandrim VC (2015) Positive correlations between circulating adiponectin and MMP2 in preeclampsia pregnant. *Pregnancy Hypertens* **5**, 205–8.

Harris LK, Keogh RJ, Wareing M, Baker PN, Cartwright JE, Aplin JD and Whitley GS (2006) Invasive trophoblasts stimulate vascular smooth muscle cell apoptosis by a Fas ligand-dependent mechanism. *Am J Pathol* **169**, 1863–74.

Janani F and Changaee F (2017) Seasonal variation in the prevalence of preeclampsia. *J Family Med Prim Care* **6**, 766–9.

Jiang J, Zhang Y, Guo Y, Yu C, Chen M, Li Z, Tian S and Sun C (2015) MicroRNA-3127 promotes cell proliferation and tumorigenicity in hepatocellular carcinoma by disrupting of PI3K/AKT negative regulation. *Oncotarget* **6**, 6359–72.

Li JH, Liu S, Zhou H, Qu LH and Yang JH (2014) starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. *Nucleic Acids Res* **42**, D92–7.

Li S, Zhai J, Liu J, Hong Y, Zhao W, Zhao A, Sun K, Du Y and Chen ZJ (2017) BMAL1 facilitates trophoblast migration and invasion via SP1-DNMT1/DAB2IP pathway in recurrent spontaneous abortion. *Oncotarget* **8**, 89451–64.

Meekins JW, Pijnenborg R, Hanssens M, McFadyen IR and van Asshe A (1994) A study of placental bed spiral arteries and trophoblast invasion in normal and severe pre-eclamptic pregnancies. *Br J Obstet Gynaecol* **101**, 669–74.

Rahat B, Sharma R, Bagga R, Hamid A and Kaur J (2016) Imbalance between matrix metalloproteinases and their tissue inhibitors in preeclampsia and gestational trophoblastic diseases. *Reproduction* **152**, 11–22.

Soundararajan R and Rao AJ (2004) Trophoblast 'pseudo-tumorigenesis': significance and contributory factors. *Reprod Biol Endocrinol* **2**, 15.

Sui C, Dong Z, Yang C, Zhang M, Dai B, Geng L, Lu J, Yang J and Xu M (2019) LncRNA FOXD2-AS1 as a competitive endogenous RNA against miR-150-5p reverses resistance to sorafenib in hepatocellular carcinoma. *J Cell Mol Med* **23**, 6024–33.

Sun Y, Chen C, Zhang P, Xie H, Hou L, Hui Z, Xu Y, Du Q, Zhou X, Su B and Gao W (2014) Reduced miR-3127-5p expression promotes NSCLC proliferation/invasion and contributes to dasatinib sensitivity via the c-Abl/Ras/ERK pathway. *Sci Rep* **4**, 6527.

Tangeras LH, Austdal M, Skrastad RB, Salvesen KA, Austqulen R, Bathen TF and Iversen AC (2015) Distinct first trimester cytokine profiles for gestational hypertension and preeclampsia. *Arterioscler Thromb Vasc Biol* **35**, 2478–85.

Wang KC and Chang HY (2011) Molecular mechanisms of long noncoding RNAs. *Mol Cell* **43**, 904–14.

Wu D, Yang N, Xu Y, Wang S, Zhang Y, Sagnelli M, Hu B, Huang Z and Sun L (2019) lncRNA HIF1A antisense RNA 2 modulates trophoblast cell invasion and proliferation through upregulating PHLDA1 expression. *Mol Ther Nucleic Acids* **16**, 605–15.

Xu Y, Lian Y, Zhang Y, Huang S, Zuo Q, Yang N, Chen Y, Wu D and Sun L (2018a) The long non-coding RNA PVT1 represses ANGPTL4 transcription through binding with EZH2 in trophoblast cell. *J Cell Mol Med* **22**, 1272–82.

Xu Y, Wu D, Liu J, Huang S, Zuo Q, Xia X, Jiang Y, Wang S, Chen Y, Wang T and Sun L (2018b) Downregulated lncRNA HOXA11-AS affects trophoblast cell proliferation and migration by regulating RND3 and HOXA7 expression in PE. *Mol Ther Nucleic Acids* **12**, 195–206.

Yang Y, Sun Y, Wu Y, Tang D, Ding X, Xu W, Su B and Gao W (2018) Downregulation of miR-3127-5p promotes epithelial-mesenchymal transition via FZD4 regulation of Wnt/beta-catenin signaling in non-small-cell lung cancer. *Mol Carcinog* **57**, 842–53.

Zhao L, Zhao Y, He Y, Li Q and Mau Y (2018) The functional pathway analysis and clinical significance of miR-20a and its related lncRNAs in breast cancer. *Cell Signal* **51**, 152–65.

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