

Knockdown of JARID2 inhibits the viability and migration of placenta trophoblast cells in preeclampsia

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Abstract. Protein Jumonji (JARID2) is a member of the Jumonji family of proteins and has been demonstrated to regulate cell proliferation and invasion. However, little is known about the role of JARID2 in the metastasis of placenta trophoblast cells. In the present study, the effect and the underlying molecular mechanism of JARID2 on trophoblast cell viability and invasion was investigated. The expression of JARID2 in placental tissues was analyzed by reverse transcription-quantitative polymerase chain reaction and western blotting. HTR8/SVneo cells were transfected with si-JARID2 or scramble for 24 h. Cell viability, migration and invasion in HTR8/SVneo cells were then evaluated. The expression levels of matrix metalloproteinase 2 (MMP2), MMP9, phosphorylated phosphatidylinositol 3-kinase (p-PI3K), PI3K, phosphorylated AKT serine/threonine kinase 1 (p-Akt) and Akt in HTR8/SVneo cells were also detected using western blotting. The results of the present study demonstrated that JARID2 is underexpressed in human preeclamptic placentas. The knockdown of JARID2 significantly inhibited the viability, migration and invasion of HTR8/SVneo cells. In addition, the knockdown of JARID2 significantly decreased the levels of phosphorylated PI3K and Akt in HTR8/SVneo cells. The results of the present study demonstrated that JARID2 may serve a role in the progression of preeclampsia. The knockdown of JARID2 inhibited the viability and invasion of trophoblast cells in preeclampsia by suppressing the PI3K/Akt signaling pathway. Therefore, JARID2 may serve as a novel potential target for treating preeclampsia.

Introduction

Preeclampsia is a serious pregnancy-related syndrome that remains a leading cause of maternal and perinatal morbidity and

mortality. It is characterized by hypertension and proteinuria, with symptoms usually arising after 20 weeks of gestation (1). Despite research into preeclampsia, the molecular mechanisms underlying the onset and progression of the syndrome remain to be completely elucidated (2-4). Trophoblast cells are able to proliferate, migrate and invade the pregnant uterus during normal placental development. However, trophoblasts fail to invade the uterus in preeclamptic placentas (5). Therefore, an improved understanding of the molecular mechanisms underlying trophoblast invasion is required.

Protein Jumonji (JARID2) is a member of the Jumonji family of proteins (6). Previous studies have demonstrated that JARID2 acts as an accessory component of Polycomb repressive complex 2 (PRC2), which regulates important gene expression patterns during fetal development (7-9). JARID2 is associated with the maintenance of pluripotency and differentiation of embryonic stem cells (10). It has previously been observed that JARID2 is involved in the development and progression of a number of types of tumor such as lung and colon cancer (11,12). Recently, Lei *et al* (13) reported that down-regulation of JARID2 inhibits hepatocellular carcinoma cell migration, invasion and proliferation *in vitro*, and metastasis *in vivo*. However, little is known about the role of JARID2 in the metastasis of placenta trophoblast cells. In the present study, the effect and underlying molecular mechanism of JARID2 in trophoblast cell viability and invasion was examined. The expression of JARID2 in placental tissues was analyzed by performing reverse transcription-quantitative polymerase chain reaction and western blotting. HTR8/SVneo cells were transfected with si-JARID2 or scramble for 24 h, then cell viability, migration and invasion were evaluated. The expression levels of matrix metalloproteinase 2 (MMP2), MMP9, phosphorylated phosphatidylinositol 3-kinase (p-PI3K), PI3K, phosphorylated AKT serine/threonine kinase (p-Akt) 1 and Akt were also detected in HTR8/SVneo cells using western blotting. It was demonstrated that the knockdown of JARID2 inhibited trophoblast cell viability and migration through inactivation of the Rac- α Akt signaling pathway.

Materials and methods

Tissue specimens. Fresh placental tissues at 26-28 weeks of gestation were obtained from 3 healthy pregnant women (age, 24.2 \pm 2.4 years) and from 3 female patients with preeclampsia (age, 25.7 \pm 1.8 years) who were recruited from the Department

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of Obstetrics and Gynecology, Fujian Provincial Hospital (Fujian, China), between July 2015 and December 2015. The specimens were immediately snap-frozen, and stored in liquid nitrogen until use. The present study was conducted with the approval of the Ethics Committee of Fujian Provincial Hospital (Fuzhou, China), and informed consent was obtained from all patients.

Cell culture. The human trophoblast cell line HTR8/SVneo was obtained from the American Type Culture Collection (Manassas, VA, USA). The cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany), 100 U/ml penicillin and 100 mg/ml streptomycin (Sigma-Aldrich; Merck KGaA) at 37°C in a humidified 5% CO₂/95% air atmosphere.

RNA interference and transfection. The small interfering RNA against JARID2 (siRNA-JARID2; 5'-AGGAAGAGGAGGAGGACAA-3') and control siRNA (scramble; 5'-GAGUGGGUCUGGGUCUUCCCGUAGA-3') were chemically synthesized by Shanghai GenePharma Co., Ltd. (Shanghai, China). HTR8/SVneo cells were transfected for 24 h with siRNA-JARID2 or scramble using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from frozen tissues or cells using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). cDNA was synthesized from the extracted RNA (5 µg) using the EasyScript First-Strand cDNA Synthesis SuperMix kit (Invitrogen; Thermo Fisher Scientific, Inc.). qPCR analysis was performed using a 7500 Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) with Fast Start Universal SYBR Green Master (Roche Diagnostics, Basel, Switzerland). PCR amplification was performed using the following primers: JARID2, 5'-GACACCAAACCCAATCACCAC-3' (sense) and 5'-GTTCAACCTGCCACTGACCTT-3' (antisense); and β-actin, 5'-TTAGTTGCGTTACACCCTTTC-3' (sense) and 5'-ACCTTCACCGTTCCAGTTT-3' (antisense). The thermocycling conditions were as follows: 95°C for 4 min, followed by 40 cycles of 95°C for 25 sec, 55°C for 30 sec and 72°C for 20 sec with 2 sec for plate reading, then melting curve analysis from 65 to 95°C. Data were analyzed using the formula: $R = 2^{-[\Delta Cq_{\text{sample}} - \Delta Cq_{\text{control}}]}$ (14).

Western blotting. Total protein was extracted from frozen tissues or cells using radioimmunoprecipitation assay lysis buffer (Beyotime Institute of Biotechnology, Haimen, China). Protein content was determined using a bicinchoninic acid protein assay (Pierce; Thermo Fisher Scientific, Inc.). Proteins (30 µg/lane) were separated using SDS-PAGE on a 12% gel and transferred onto a nitrocellulose membrane (EMD Millipore, Billerica, MA, USA). Following blocking with 5% fat-free milk in PBS containing 0.1% (v/v) Tween-20 at room temperature for 1 h, membranes were incubated with one of the following mouse primary antibodies: anti-JARID2

(1:3,000; cat. no. SAB2105079; Sigma; Merck KGaA), anti-MMP2 (1:2,000; cat. no. sc-13594), anti-MMP9 (1:3,000; cat. no. sc-21733), anti-PI3K (1:2,000; cat. no. sc-365290), anti-p-PI3K (1:2,500; cat. no. sc-293115), anti-Akt (1:3,000; cat. no. sc-5298), anti-p-Akt (1:2,500; cat. no. sc-52940) or anti-GAPDH (1:2,000; cat. no. sc-47724; all Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at 4°C overnight. Membranes were subsequently incubated with goat anti-mouse horseradish peroxidase (HRP)-conjugated immunoglobulin (Ig)G (1:2,500; cat. no. sc-2005; Santa Cruz Biotechnology, Inc.) or goat anti-rabbit HRP-conjugated IgG (1:3,000; cat. no. sc-2004; Santa Cruz Biotechnology, Inc.) for 1 h at room temperature. The target protein was visualized using enhanced chemiluminescence (Pierce; Thermo Fisher Scientific, Inc.) and densitometry was performed using Gel-Pro Analyzer software version 4.0 (Media Cybernetics, Inc., Rockville, MD, USA).

Cell viability assay. Cell viability was measured using the Cell Counting Kit-8 assay (CCK-8; Dojindo Molecular Technologies, Inc. Kumamoto, Japan). HTR8/SVneo cells were plated at a density of 1x10⁴ cells/well in 96-well plates and transfected with siRNA-JARID2 or scramble. Following incubation for 24 h, the CCK-8 reagents were added and incubated with the cells for 1 h. Subsequently, the absorbance at 450 nm was measured using an ELISA plate reader.

Cell invasion and migration assays. Cell invasion assays were performed using Transwell™ chambers (Costar; Corning Incorporated, Corning, NY, USA). Transfected HTR8/SVneo cells (1x10⁴ cells/well) were plated in the top chamber of the insert, which had been precoated with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA; 8 µm pore size), and 500 µl DMEM containing 10% FBS was added to the lower chamber. Following 24 h of incubation, cells transferred to the lower surface of the base membrane were stained with hematoxylin and eosin (Sigma-Aldrich; Merck KGaA), and counted per 4 high power fields under a light microscope (magnification, x100). The migration assay was performed by the same procedure, except that the inserts were not pre-coated with Matrigel.

Statistical analysis. Data are expressed as the mean ± standard deviation. All experiments were repeated at least three times. Statistical significance was analyzed using one-way factorial analysis of variance followed by a Dunnett's post hoc test or Student's two-tailed t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

JARID2 is underexpressed in preeclamptic placentas. In order to investigate the role of JARID2 in preeclampsia, the expression levels of JARID2 in placental tissues were examined by RT-qPCR analysis and western blotting. As presented in Fig. 1A, JARID2 mRNA levels were significantly decreased in placentas obtained from preeclamptic patients compared with placentas from healthy subjects. Similarly, the results of the western blot analysis indicated that the protein expression of JARID2 was significantly decreased

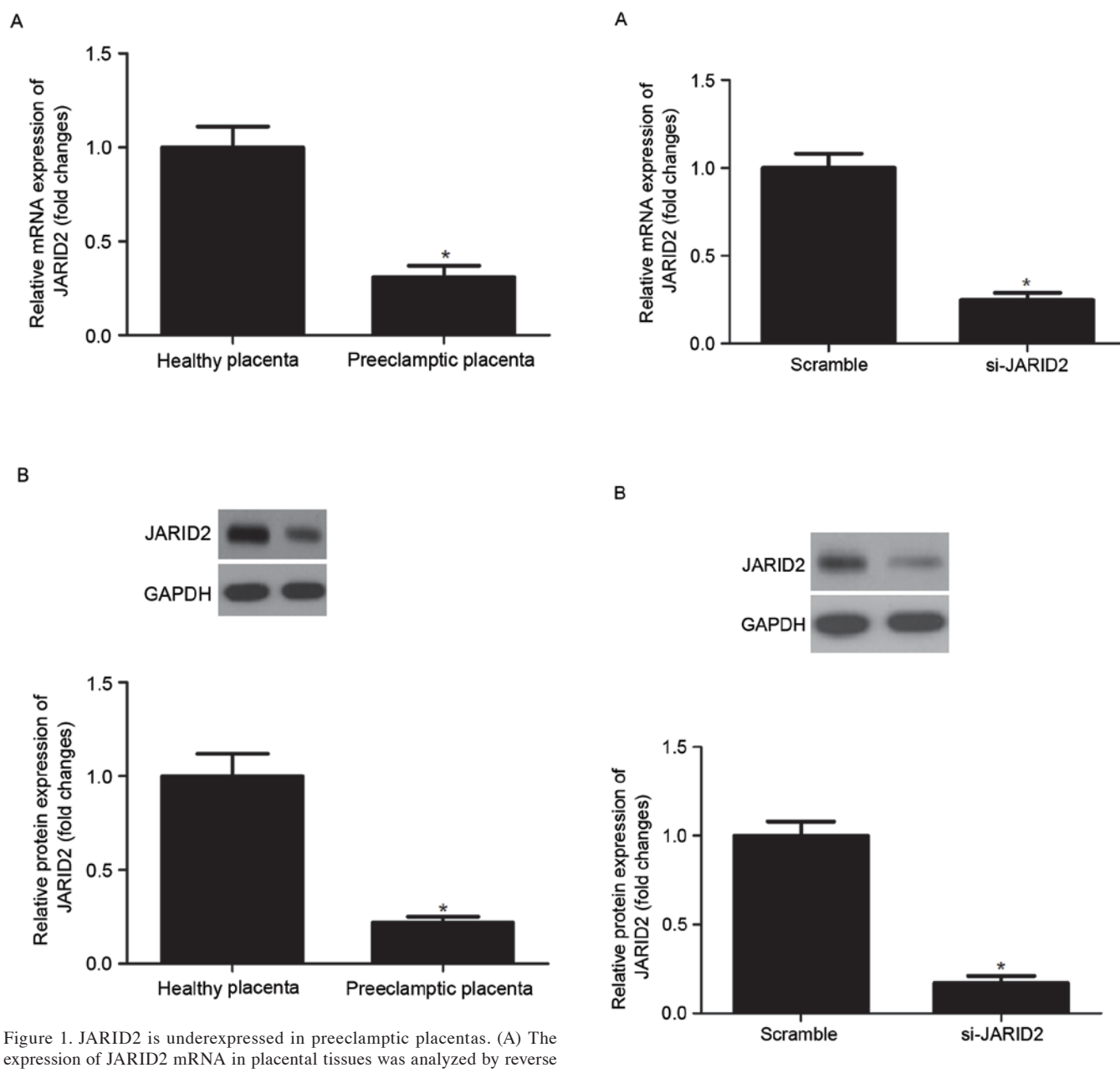


Figure 1. JARID2 is underexpressed in preeclamptic placentas. (A) The expression of JARID2 mRNA in placental tissues was analyzed by reverse transcription-quantitative polymerase chain reaction, with quantification relative to β -actin. (B) The expression of JARID2 protein in placental tissues was analyzed by western blotting, with quantification relative to GAPDH. Data are presented as the mean \pm standard deviation of three independent experiments. * $P < 0.05$ vs. healthy placenta. JARID2, protein Jumonji.

in preeclamptic placentas compared with the control group (Fig. 1B).

Knockdown of JARID2 inhibits HTR8/SVneo cell viability. In order to gain further insight into the effect of JARID2 on cell viability, JARID2 was downregulated using siRNA against JARID2 in HTR8/SVneo cells. As demonstrated in Fig. 2A and B, respectively, siRNA knockdown of JARID2 significantly decreased the expression of JARID2 at the mRNA and protein levels in HTR8/SVneo cells. In addition, the effects of si-JARID2 on HTR8/SVneo cell viability were examined. The results of the CCK-8 assay demonstrated that, compared with the scramble group, knockdown of JARID2 significantly inhibited the viability of HTR8/SVneo cells (Fig. 2C).

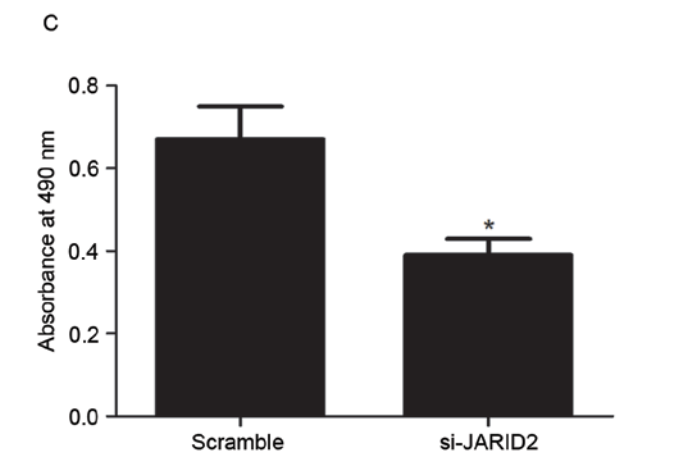


Figure 2. Knockdown of JARID2 reduces HTR8/SVneo cell viability. (A) mRNA and (B) protein expression of JARID2 was significantly decreased in HTR8/SVneo cells transfected with si-JARID2 compared with cells transfected with scramble. (C) Cell viability was assessed by Cell Counting Kit-8 assay. Data are presented as the mean \pm standard deviation of three independent experiments. * $P < 0.05$ vs. scramble. si, small interfering; JARID2, protein Jumonji.

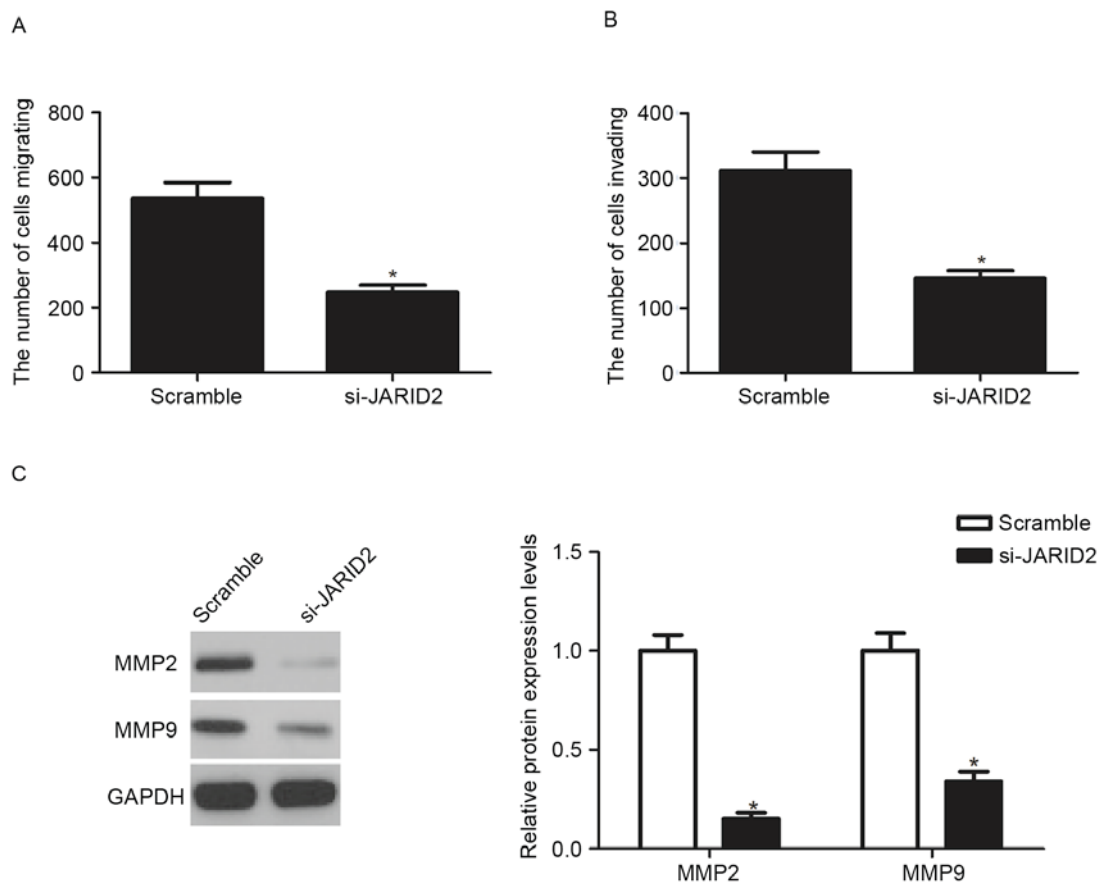


Figure 3. Knockdown of JARID2 inhibits the migration and invasion of HTR8/SVneo cells. HTR8/SVneo cells were transfected with si-JARID2 or scramble for 24 h. (A) Cell migration was evaluated using the Transwell migration assay. (B) Cell invasion was determined using the Matrigel invasion assay. (C) Western blotting was used to detect the levels of MMP2 and MMP9 protein expression in transfected HTR8/SVneo cells. Data are presented as the mean \pm standard deviation of three independent experiments. * $P < 0.05$ vs. respective scramble. JARID2, protein Jumonji; si, small interfering; MMP, matrix metalloproteinase.

Knockdown of JARID2 inhibits the migration and invasion of HTR8/SVneo cells. The present study investigated the effects of JARID2 on HTR8/SVneo cell migration and invasion. The Transwell migration assay demonstrated that the knockdown of JARID2 significantly inhibited the migratory capacity of HTR8/SVneo cells, compared with the scramble group (Fig. 3A). The Matrigel assay demonstrated that the number of cells that passed through the Matrigel-coated membrane into the lower chamber was significantly decreased in si-JARID2-transfected cells compared with scramble-transfected cells (Fig. 3B). In addition, the present study analyzed the effect of JARID2 on MMP expression in HTR8/SVneo cells. The results of the western blot analysis demonstrated that knockdown of JARID2 significantly decreased the expression of MMP2 and MMP9 protein, compared with the scramble group (Fig. 3C).

Knockdown of JARID2 inhibits the activation of PI3K/Akt pathway in HTR8/SVneo cells. In order to gain insights into the downstream signaling pathways modulated by si-JARID2 in preeclampsia inhibition, the present study investigated the effect of si-JARID2 on the activation of the PI3K/Akt signaling pathway in HTR8/SVneo cells. As presented in Fig. 4, the knockdown of JARID2 significantly decreased the levels of phosphorylated PI3K and Akt in HTR8/SVneo cells, compared with the scramble group.

Discussion

Preeclampsia is a pregnancy-specific complication and is associated with insufficient extravillous trophoblast invasion. To the best of our knowledge, the present study is the first to investigate the expression and role of JARID2 in preeclampsia. The results of the present study demonstrated that JARID2 is underexpressed in human preeclamptic placentas. The knockdown of JARID2 significantly inhibited the viability and migration/invasion of HTR8/SVneo cells. Additionally, the knockdown of JARID2 reduced the activation of PI3K and Akt in HTR8/SVneo cells.

JARID2 has been observed to regulate the proliferation of a number of types of cells. Mejetta *et al* (10) confirmed that silencing JARID2 decreases the proliferation of rhabdomyosarcoma cells by inducing myogenic differentiation. By contrast, it has been reported that the knockdown of JARID2 promotes leukemia cell proliferation via acceleration of the G1/S transition (15). These previous findings suggest that JARID2 may serve promotive or inhibitory functions depending on the cell type or context. In the present study, it was observed that JARID2 is underexpressed in human preeclamptic placentas, and that the knockdown of JARID2 inhibited the viability of HTR8/SVneo cells. The present results suggested that JARID2 may act as a suppressor in the development and progression of preeclampsia.

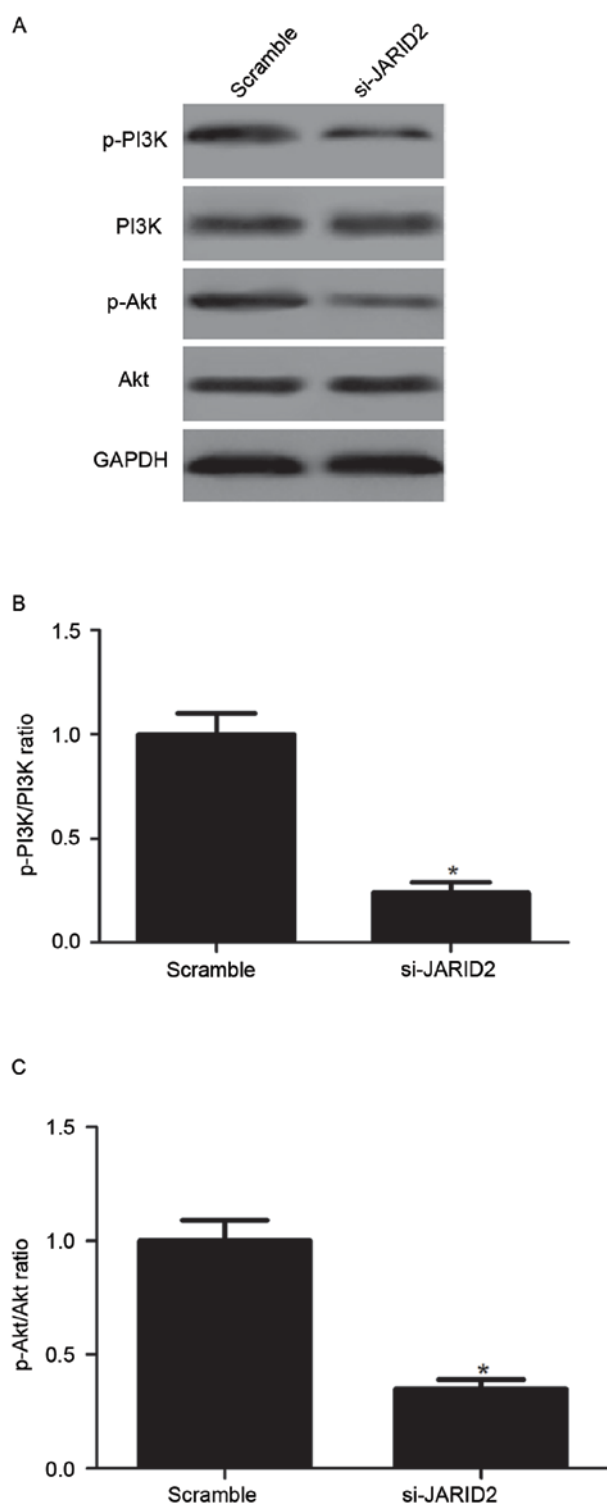


Figure 4. Knockdown of JARID2 inhibits the activation of the PI3K/Akt signaling pathway in HTR8/SVneo cells. HTR8/SVneo cells were transfected with si-JARID2 or scramble for 24 h. (A) Western blotting was used to detect the protein expression levels of p-PI3K, PI3K, p-Akt and Akt in HTR8/SVneo cells, with quantification relative to GAPDH. PI3K and Akt activation was assessed by examination of relative levels of (B) p-PI3K/PI3K and (C) p-Akt/Akt. Data are presented as the mean \pm standard deviation of three independent experiments. * $P < 0.05$ vs. scramble. JARID2, protein Jumonji; si, small interfering; p, phosphorylated; PI3K, phosphatidylinositol 3-kinase; Akt, AKT serine/threonine kinase 1.

Previous studies have demonstrated that deficient migration and shallow invasion of trophoblasts may lead to

preeclampsia (16,17). JARID2 has been suggested to regulate tumor cell migration and invasion; a previous study revealed that knockdown of JARID2 markedly inhibits hepatocellular carcinoma cell migration and invasion (13). Consistent with the results of this previous study, in the present study, it was observed that the knockdown of JARID2 significantly inhibited the viability and migration/invasion of HTR8/SVneo cells. Additionally, MMPs serve important roles in cell migration and invasion by remodeling the extracellular matrix (18,19). During early pregnancy, for embryo implantation and placentation, the invasion of human trophoblast cells depends on the secretion of MMPs, primarily MMP2 and MMP9 (20,21). In the present study, it was observed that the knockdown of JARID2 significantly inhibited the expression of MMP2 and MMP9 in HTR8/SVneo cells. The present study provides evidence to suggest that the knockdown of JARID2 may lead to decreased MMP2 and MMP9 expression, which may lead to insufficient trophoblast cell migration and invasion, thereby contributing to preeclampsia.

Previous studies have reported that the PI3K/Akt signaling pathway may serve important roles in regulating the proliferation, migration and invasion of trophoblast cells (22-24). Akt is a serine/threonine protein kinase, and the phosphorylation of Akt has been observed to be decreased in the preeclamptic placenta (25). In addition, activated Akt is able to promote cell motility and invasion (26,27). Therefore, the suppression of PI3K/Akt signaling may be a promising approach for treating preeclampsia. A recent study reported that JARID2 promotes the activation of Akt in hepatocellular carcinoma (13). In the present study, it was observed that the knockdown of JARID2 suppressed the levels of phosphorylated PI3K and Akt in HTR8/SVneo cells. The results of the present study suggested that the knockdown of JARID2 inhibited the viability and invasion of trophoblast cells in preeclampsia by suppressing the PI3K/Akt signaling pathway.

In conclusion, the results of the present study demonstrated that JARID2 may serve an important role in the progression of preeclampsia. The knockdown of JARID2 inhibited the viability and invasion of trophoblast cells in preeclampsia by suppressing the PI3K/Akt signaling pathway. Therefore, JARID2 may serve as a novel potential target for treating preeclampsia.

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