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Original Research Article

USP22 promotes aspirin-induced trophoblast cell proliferation as well as migration via Wnt/β-catenin pathway in preeclampsia

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Abstract

Purpose: *To investigate the involvement of ubiquitin-specific peptidase 22 (USP22) in the pathogenesis of preeclampsia (PE), and the potential mechanism of action.*
Methods: *To investigate the role of USP22 in preeclampsia, immunoblot assay as well as quantitative real-time polymerase chain reaction (qRT-PCR) were conducted to determine USP22 expression levels in placental tissues from both the control (non-preeclampsia normal) and the PE cases. Furthermore, cell counting kit-8 as well as EdU assays were performed to assess the impact of USP22 on the viability of HTR-8/Svneo and TEV-1 cells. Furthermore, wound closure and Transwell assays were used to examine the effect of USP22 on cell motility, while tube formation assay was used to determine the effect of USP22 on the angiogenesis of trophoblast cells. Finally, the effect of USP22 on Wnt/β-catenin pathway in HTR-8/Svneo and TEV-1 human trophoblast cells was evaluated by immunoblot analysis.*
Results: *USP22 expression was significantly reduced in PE placental tissue compared to control tissues. In addition, USP22 promoted trophoblast cell proliferation, increased cell motility as well as enhanced angiogenesis capacity. USP22 regulated the proliferation as well as migration of trophoblast cells via Wnt/β-catenin pathway. Notably, USP22 enhanced cell proliferation and migration in aspirin-induced PE.*
Conclusion: *USP22 plays a crucial role in inhibiting trophoblast cell growth by regulating Wnt/β-catenin pathway. Therefore, targeting USP22 may hold promise for the treatment of PE.*

Keywords: *Preeclampsia, USP22, Proliferation, Angiogenesis, Trophoblast cells, Wnt/β-catenin pathway, Aspirin*

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INTRODUCTION

Preeclampsia (PE) is a significant clinical complication of human pregnancy [1]. It affects approximately 2 - 8 % of pregnant women, triggering fetal morbidity and maternal mortality [2]. The clinical characteristics of PE include the onset of hypertension and proteinuria in women with normal blood pressure after 20 weeks of gestation. The pathogenesis of PE involves several factors such as abnormal reconstruction of the spiral artery, placental dysfunction, maternal vascular injury, and oxygen regulation disorders [3]. In addition, the abnormal migration

and invasion of extravillous trophoblast cells are considered crucial factors in the development of PE [4].

An elevated expression of ubiquitin-specific peptidase 22 (USP22) is often associated with the poor prognosis of tumors [5], including colorectal, breast, pancreatic, and prostate cancers, due to its ability to promote tumor migration and invasion [6]. Furthermore, USP22 has been shown to mediate chemoresistance in colorectal cancer cells through the Wnt/β-catenin pathway [7]. Trophoblast cells exhibit histological and behavioral characteristics similar to those of cancer cells [8]. In addition, USP22 is involved in various biological processes, such as transcription [9]. Interestingly, USP22 is reduced in placentas with fetal growth restriction [10], and its knockout in mice leads to placental vascular dysplasia and fetal death [11]. Despite these findings, the precise role of USP22 in preeclampsia remain unclear.

The Wnt/β-catenin signaling is fundamental to controlling human growth and development, including organogenesis, carcinogenesis, and tissue homeostasis [12]. During pregnancy, this pathway contributes to blastocyst adhesion, making it a critical regulator of placental formation [13]. Any defects in this key signaling pathway can lead to abnormal trophoblast invasion and ultimately result in PE [14]. To investigate the potential USP22 role in the progression of PE, an *in vitro* cell model was utilized.

METHODS

Tissues and ethical approval

This study was conducted with approval from the Ethics Committee of Qinghai Provincial People's Hospital (approval no. 2020001) in accordance with the ethical guidelines of 1964 Helsinki Declaration and its later amendments [15]. Informed consent was obtained from participants. Thirty (30) normal pregnancy and 30 PE patients were recruited. Placental tissue was rapidly collected from pregnancies undergoing elective cesarean sections and kept in at -80 °C in a refrigerator for later use. The diagnostic criteria for PE included systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg. Proteinuria ≥300 mg/day (or protein-creatinine ratio ≥0.3 mg/dL or proteinuria ≥1) was defined in women who previously had normal blood pressure, or severe clinical features in the absence of proteinuria after 20 weeks of gestation.

Exclusion/inclusion criteria

This study excluded patients with comorbidities such as diabetes, lupus, urinary tract infection, or chronic kidney disease, which are known to affect pregnancy outcomes. The control group consisted of subjects with normal pregnancies, and they were matched with PE patients for both age and gestational age to ensure an accurate comparison between the groups.

Antibodies and siRNAs

The following antibodies were purchased from Abcam: anti-USP22 (1:500 dilution; ab195289), anti-β-catenin (1:500; ab32572), anti-Axin (1:500; ab109307), anti-C-myc (1:500; ab32072), anti-MMP7 (1:500; ab207299) and anti-GAPDH (1:2000; ab8245).

USP22 small interfering RNAs (siRNAs) were purchased from Riobio plc (Guangzhou, China). The USP22 overexpression plasmids were obtained from Addgene (Cambridge, MA, USA). USP22 siRNA sequence was 5'-CACGGACAGUCUCAACAAUTT-3', while USP22-HA overexpression vector was constructed by inserting cDNA fragments encoding USP22 into the vector.

Cell culture

The HTR-8/Svneo and TEV-1 human trophoblast cells were purchased from American Type Culture Collection (ATCC, USA). Cell lines were maintained in DMEM, supplemented with 10 % fetal bovine serum, and incubated at 37 °C in a 5 % CO_2 incubator. The plasmids and siRNA were transfected into cells using Lipofectamine 3000.

Quantitative polymerase chain reaction (qPCR)

Total RNA was extracted from cells using TRIzol reagent (15596-018; Invitrogen, USA). Quantitative PCR was subsequently conducted using an SYBR mixture (RR420A; Takara). The USP22 mRNA levels were normalized to GAPDH. USP22 PCR primer sequences were: forward, 5’- GGACAACTGGAAGCAGAACC-3’; reverse, 5’--TGAAACAGCCGAAGAAGACA-3’.

Immunoblot assay

Cell or tissue samples were lysed using RIPA lysis buffer (Beyotime, China), the protein samples were separated using 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and transferred onto

polyvinylidene fluoride membranes (Millipore, USA). The PVDF membranes were blocked with 5 % dry milk in Tris-buffered saline using Tween 20 buffer, and then incubated with primary antibodies for 2 h. The membranes were incubated with HRP-labeled secondary antibodies for 45 min. Each blot was visualized using an enhanced chemiluminescence kit (GE, SA).

Cell viability assay

To conduct Cell Counting Kit-8 (CCK-8) assays, 1000 cells per well were seeded into 96-well plates, and maintained in 10 % FBS medium at 37 °C. Next, the cells were treated with CCK-8 reagent at 37 °C for 4 h. The relative cell viability was assessed over 3 days using a microplate spectrophotometer at the wavelength of 490 nm (Bio-Rad, USA).

For 5-ethynyl-2′-deoxyuridine (EdU) staining, after the indicated treatments, cells were fixed with formaldehyde, rinsed with TBS buffer, and then stained using EdU kit (Abcam, Cambridge, UK) and as well by both Edu and DAPI.

Transwell migration and invasion assays

Cell culture inserts (BD Falcon, USA) were used as upper chambers, while 24-well plates were used as lower chambers. Cell culture inserts were coated with or without 100 µL of Matrigel and incubated for 24 h. A total of 10^5 cells were placed in the upper chambers while invaded cells on the underside were fixed using 4 % paraformaldehyde, stained with 0.2 % crystal violet, and the image taken.

Statistical analysis

GraphPad 6.0 was used for statistical analysis and the data presented as means ± SD. Statistical difference was determined using Student's t-test and $p < 0.05$ was considered statistically significant.

RESULTS

USP22 expression was downregulated in PE placental tissues

An analysis of the clinical features indicated elevated levels of systolic and diastolic blood pressure, proteinuria, and birth weight in both PE patients and normal controls (Table 1). USP22 expression was significantly lower in 30 PE placental tissue samples when compared to control samples (Figure 1 A), which was further confirmed by immunoblot assays of 4 PE tissue samples (Figure 1 B). Therefore, our findings suggest that the expression of USP22 was downregulated in the PE placental tissues.

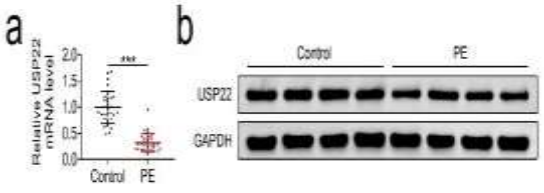

Figure 1: USP22 expression was downregulated in the PE placental tissues. (a) The mRNA levels of USP22 in the samples; (b) Expression of USP22 in theol samples. ***$P < 0.001$

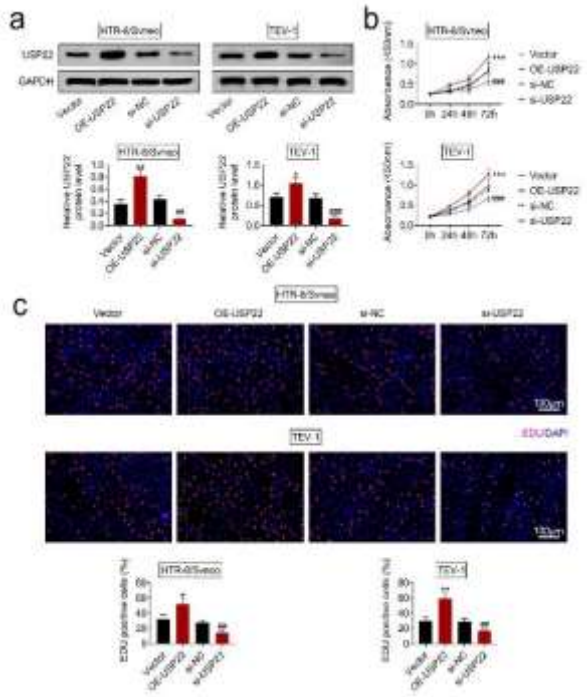

Figure 2: USP22 promoted the viability of trophoblast cells. (a) Expression of USP22 in both HTR-8/Svneo and TEV-1 cells following transfection. (b) Viability of both HTR-8/Svneo and TEV-1 cells transfection. (c) Viability of both HTR-8/Svneo and TEV-1 cells following transfection and calculation of EdU-positive cells. *$P < 0.05$, **$p < 0.01$, ***$p < 0.001$, USP22 *vs.* control; ##$p < 0.01$, ###$p < 0.001$, siUSP22 *vs.* si-control

USP22 enhanced the viability of trophoblast cells

To investigate the impact of USP22 on trophoblast cell growth, both siRNA and plasmids were utilized to manipulate USP22 expression in HTR-8/Svneo, as well as TEV-1 cells. The transfection of USP22 siRNA effectively reduced UDP22 expression in both cell lines, whereas the overexpression plasmids increased USP22 expression (Figure 2 A). Furthermore, CCK-8

assays revealed that the depletion of USP22 suppressed the growth of trophoblast cells, whereas its overexpression promoted cell viability (Figure 2 B). Similarly, EdU assay results showed that USP22 knockdown led to a reduction in the number of EdU-positive cells (Figure 2 C) while USP22 overexpression promoted cell viability. Collectively, findings in this study suggest that USP22 promoted the viability of trophoblast cells.

USP22 stimulates the motility of trophoblast cells

The depletion of USP22 was found to impede the migration of HTR-8/Svneo as well as TEV-1 cells, whereas its overexpression facilitated cell migration (Figure 3 A). Additionally, USP22 knockdown was observed to suppress HTR-8/Svneo and TEV-1 cell invasion, while its overexpression stimulated cell invasion (Figure 3 B). Therefore, results in this study suggest that USP22 stimulates the motility of trophoblast cells.

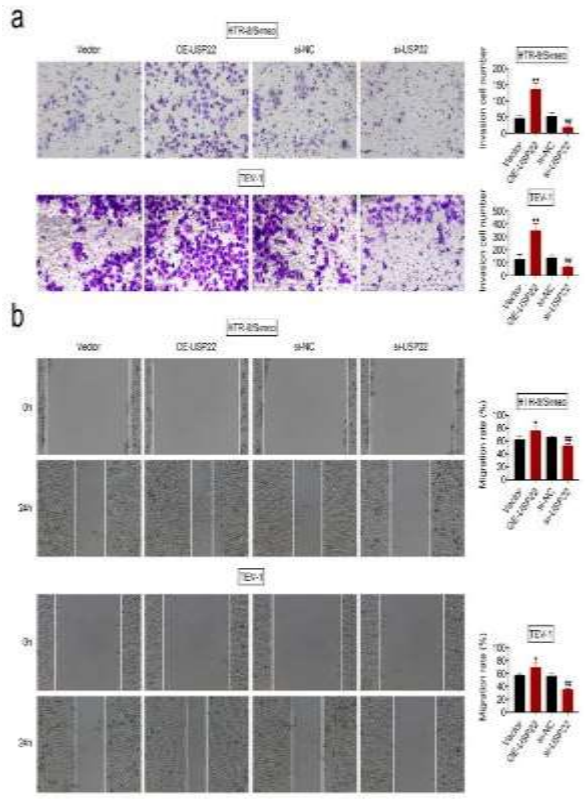

Figure 3: USP22 stimulated the motility of trophoblast cells. Transwell-migration assays (a) Wound healing and invasive of HTR-8/Svneo and TEV-1 cells following the indicated transfection. ***Note***: representative images and invasive cell numbers are shown. *$P < 0.05$, **$p < 0.01$, USP22 *vs.* control; ##$p < 0.01$, siUSP22 *vs.* si-control

USP22 enhanced cell proliferation and migration in aspirin-induced PE

Aspirin treatment significantly increased cell proliferation, which was further induced by USP22 overexpression (Figure 4 A). Aspirin facilitated the migration of HTR-8/Svneo as well as TEV-1 cells, while USP22 overexpression further promoted cell migration (Figure 4 B). In addition, Transwell-invasion assays showed that aspirin promoted HTR-8/Svneo as well as TEV-1 cell invasion, which was further induced by USP22 overexpression (Figure 4 C). Therefore, USP22 enhanced the proliferation and migration in aspirin-induced PE.

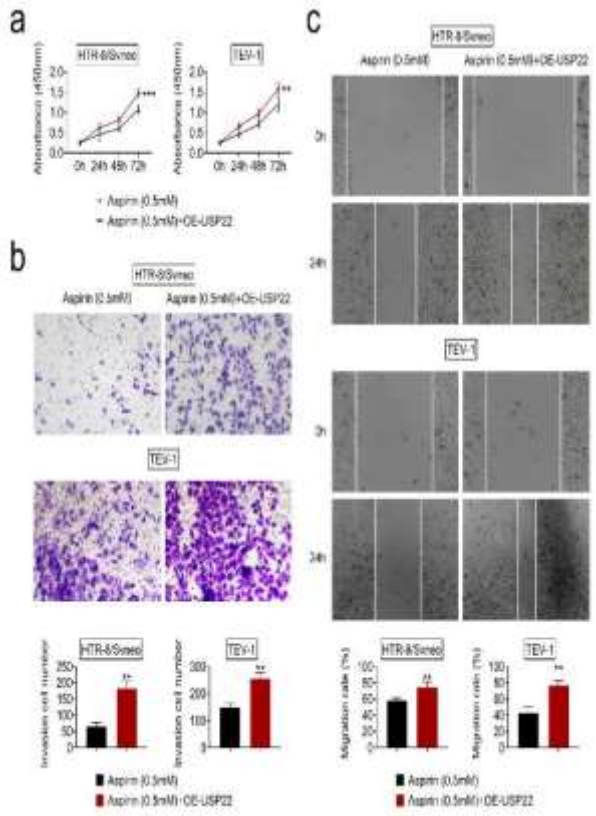

Figure 4: USP22 enhanced proliferation and migration in aspirin-induced PE. (a) Viability and degree of wound healing and invasion of both HTR-8/Svneo and TEV-1 cells following aspirin treatment and USP22 transfection. **$P < 0.01$, ***$p < 0.001$, USP22+aspirin *vs.* aspirin only

DISCUSSION

PE is defined as elevated blood pressure and proteinuria after 20 weeks of gestation. Eclampsia progresses from preeclampsia to a more serious condition that causes convulsions or coma [16]. The etiology remains unknown and it often leads to severe maternal and infant complications. No effective treatment exists other than pregnancy termination. Developing more

effective drugs are crucial to improving therapy. Low USP22 expression was found in the PE placental tissues. The USP22 inhibits trophoblast cell proliferation and migration, and therefore, it may affect PE progression.

The results of *in vitro* assay have demonstrated that USP22 promotes trophoblast cell proliferation, and plays a significant role in the motility and angiogenesis of trophoblast cells, highlighting its critical involvement in the progression of PE. In addition to these findings, USP22 has been found to possess a wide range of biological functions. For example, USP22 contributes to lipidome accumulation by stabilizing peroxisome proliferator-activated receptor γ in hepatocellular carcinoma (HCC) tissues. USP22-mediated de-ubiquitination of phosphatase and tensin homolog has been shown to suppress the progression of pancreatic cancer by stimulating p21 expression [13]. Furthermore, USP22 is a deubiquitinase that can modulate the function of regulatory T-cells [17]. A previous study reported that USP22 positively mediated FOXP3 activity in mouse regulatory T cells.

Several studies have demonstrated the role of USP22 in the formation and function of the placental vasculature during fetal growth restriction development, as well as its expression in mouse uterus tissue during early pregnancy. Interestingly, recent research has revealed its involvement in mediating the progression of PE. Furthermore, studies on *Drosophila* have shown that USP22 polarizes the cytoskeleton during collective border cell motility, which is similar to its role in promoting cell motility in trophoblast cells. Additionally, USP22 has been found to ameliorate chronic alcohol-associated liver diseases by mediating BRD4 expression [9] , and it also contributes to the progression of melanoma and BRAF inhibitor resistance by promoting YAP stabilization. These studies, together with other findings, confirm the critical role of USP22 in the development of several diseases.

Angiogenesis is a physiological process that occurs during embryogenesis, wound repair, tumor growth, and metastasis. It involves the formation of new blood vessels from existing ones, and is regulated by angiogenic precursor cells [10]. During fetal development, the placenta undergoes high levels of angiogenesis, and defects in this process have been associated with the development of preeclampsia. In the present study, USP22 was found to mediate the angiogenesis of trophoblast cells, making it a potential therapeutic target for PE.

Moreover, the Wnt pathway, which is involved in blastocyst adhesion, implantation, and early trophoblast determination during pregnancy, was also found to be regulated by USP22, thus influencing the progression of PE [11]. Therefore, this pathway may act as a target for PE therapy. Additionally, the beneficial effects of aspirin in preventing PE by promoting the proliferation and invasion of human trophoblast cells during early pregnancy were identified. In this study, USP22 was found to enhance the proliferation and migration of trophoblast cells in aspirin-induced PE.

CONCLUSION

Low expression of USP22 has been observed in the placental tissue of women with PE. This deubiquitinase protein plays a crucial role in regulating trophoblast cell proliferation and migration via Wnt/β-catenin pathway in PE. USP22 also enhances the proliferation and migration of trophoblast cells in aspirin-induced PE. These findings suggest that USP22 may serve as a promising target for managing PE in clinical practice.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Changcun Zhang and Qiping Han designed and performed the experiments; Luoji E analyzed and

interpreted the data, and prepared the manuscript with contributions from all the co-authors.

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