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# ZEB2 reduction contributes to pre-eclampsia via Wnt/β-Catenin pathway

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## Abstract

**Background** Pre-eclampsia (PE) is a pregnancy specific disease characterized by hypertension and proteinuria. The aim of this study was to investigate the effects of Zinc finger E-box binding homologous box 2 (ZEB2) on PE mice and on placental trophoblast cells, as well as to elucidate its role in Wnt/β-Catenin pathway.

**Methods** The PE mice models were established through L-NAME administration. RT-qPCR and western blot assay were used to detect the expression of ZEB2 in human serum, placental tissues, HTR8/Sveno cells, and mice models. Edu assay, flow cytometry, and Transwell analysis were applied for determining HTR8/Sveno cells proliferation, apoptosis, migration, and invasion ability, respectively. The expression levels of related proteins in the Wnt/β-Catenin pathway were detected by western blot analysis. The systolic blood pressure (SBP) of mice was analyzed by the noninvasive tail cuff method. Proteinuria was detected using CBB kits and TUNEL method was used to measure apoptosis of placental tissue cells in PE mice.

**Results** The significant increase SBP and urinary protein in L-NAME treated mice indicated the successful construction of the PE mice model. We found that ZEB2 was down-regulated in the serum and placental tissues of PE patients. Further in vitro experiments showed that ZEB2-plasmid enhanced cell proliferation, migration, and invasion, as well as reduced cell apoptosis, compared with the control-plasmid group. In addition, up-regulation of ZEB2 promoted the protein level of Bcl-2 in HTR-8/SVneo cells and inhibited Bax expression. We also found that ZEB2-plasmid activated Wnt/β-Catenin signaling pathway, as confirmed by enhanced Wnt3a, β-Catenin, p-GSK3β, C-Myc, and Cyclin D1 expression. Importantly, the Wnt/β-Catenin signaling inhibitor (XAV939) partially reversed the effects of ZEB2-plasmid on HTR-8/SVneo cells. We also observed similar findings in in vivo mice models as in vitro cell experiments.

**Conclusion** ZEB2 was involved in the pathological and physiological processes of PE through Wnt/β-Catenin pathway, which may provide a useful perspective for exploring new therapies for PE.

**Keywords** ZEB2, Pre-eclampsia, Wnt/β-Catenin pathway

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## Introduction

PE is a clinically important complication of human pregnancy, characterized by the emergence of hypertension and proteinuria in the late pregnancy, which is also the main cause of maternal death, fetal growth restriction, and even death (1–2). The pathogenesis of PE may involve various factors such as maternal, placental, and fetal factors, including abnormal invasion of trophoblasts, abnormal immune regulatory function, endothelial cell damage, genetic and nutritional factors (3–4). Ou et al. have revealed that circular RNA circ_0111277 attenuates human trophoblast cell invasion and migration by regulating miR-494/HTRA1/Notch-1 signal pathway in PE [5]. Gao et al. suggested that up-regulation of miR-299 suppressed HTR-8/SVneo trophoblast cells invasion and migration via targeting HDAC2 in PE [6]. However, the exact pathogenesis of PE is still largely unknown. Therefore, exploring its regulatory role on placental trophoblasts may provide clues for the development of new biomarkers and therapeutic targets for PE diagnosis.

ZEB2 belongs to the ZEB family and is an important nuclear transcription factor. It is located on the second chromosome of humans and consists of 10 exons and 9 introns, encoding 1214 amino acids [7]. Previous reports have shown that ZEB2 is abnormally expressed in many cancers, including liver cancer [8], colorectal cancer [9], and non-small cell lung cancer [10]. Besides, DaSilva-Arnold et al. have identified ZEB2 as a master regulator of the epithelial-mesenchymal transition in mediating trophoblast differentiation [11]. ZEB2 begins to be expressed in the early stages of embryo formation and development, and its transcription is activated during the formation stage. The expression is more pronounced during the mature stage, indicating that ZEB2 plays an important role in embryo formation and development [12]. The expression and molecular mechanism of ZEB2 in patients with PE still need to be elucidated.

The Wnt signaling pathway is a growth control pathway that can regulate many biological processes from developmental evolution to adult homeostasis [13]. The classic Wnt/β-Catenin pathway is widely recognized for regulating cell growth, apoptosis, and metastasis in various cancers (14–15). Furthermore, report from Chen et al. suggested that LASP2 inhibits trophoblast cell migration and invasion in PE through inactivation of the Wnt/β-Catenin signaling pathway [16]. However, the roles of the relationship between ZEB2 and PE in the Wnt/β-Catenin signaling pathway remains to be studied.

Therefore, our report was designed to (i) explore whether ZEB2 was regulated in the PE development by influencing the HTR-8/SVneo cell proliferation, apoptosis, and invasion; (ii) explain the roles of ZEB2 in Wnt/β-Catenin signaling pathway and illustrate potential mechanisms in PE, as to find a novel therapeutic target for PE treatment.

## Results

### ZEB2 was sensibly down-regulated in the serum and placental tissue of PE patients

To achieve insights into the functions of ZEB2 in the serum of PE patients and healthy pregnant women, we collected the serum samples from 20 healthy individuals or PE patients. Results from RT-qPCR analysis suggested that ZEB2 levels in serum samples from PE patients was significantly lower than that in healthy individuals (Fig. 1A). Our data revealed that ZEB2 was remarkably down-regulated in the PE patients serum. Furthermore, we determined the expression of ZEB2 in the healthy and PE placentas using RT-qPCR and western blot assay. As presented in Fig. 1B-D, the ZEB2 expression was clearly decreased in PE placentas than that in normal placental tissues. These data demonstrated that ZEB2 was low-expression in PE blood and placentas. The down-regulation of ZEB2 may be related to the progression of PE.

### Up-regulation of ZEB2 substantially increased HTR8/Sveno cells proliferation and suppressed cells apoptosis

To further investigate the roles of ZEB2 in PE, control-plasmid or ZEB2-plasmid were transfected into HTR-8/SVneo cells. The proliferation capacity and apoptosis rate of cells after transfection were determined using EdU assay and flow cytometry analysis. We found that ZEB2 was up-regulated in ZEB2-plasmid transfected HTR-8/SVneo cells, compared to control-plasmid group (Fig. 2A-C). Moreover, as displayed in Fig. 2D-E, ZEB2-plasmid enhanced the growth rate of HTR-8/SVneo cells, compared to control-plasmid group. Further, flow cytometry analysis suggested that ZEB2-plasmid stimulated more apoptotic HTR-8/SVneo cells than that in control-plasmid group (Fig. 2F-G). Besides, it was found that ZEB2-plasmid up-regulated the the anti-apoptotic protein (Bcl-2) expression, and down-regulated the apoptotic protein (Bax) expression in HTR-8/SVneo cells (Fig. 2H-J). Hence, our data inferred that ZEB2 promotes HTR-8/SVneo cells proliferation and reduced cell apoptosis.

### Up-regulation of ZEB2 enhanced HTR-8/SVneo cells migration and invasion in PE

Previous researches have revealed that migration ability of trophoblast cell is linked to the PE pathogenesis [17], thus then we explained whether ZEB2 was associated with the HTR-8/SVneo cells migration and invasion using Transwell assay. Our results revealed that the migration of HTR-8/SVneo cells were remarkably strengthened upon the transfection of ZEB2-plasmid (Fig. 3A-B), compared to control group. Besides, the invasive capacity

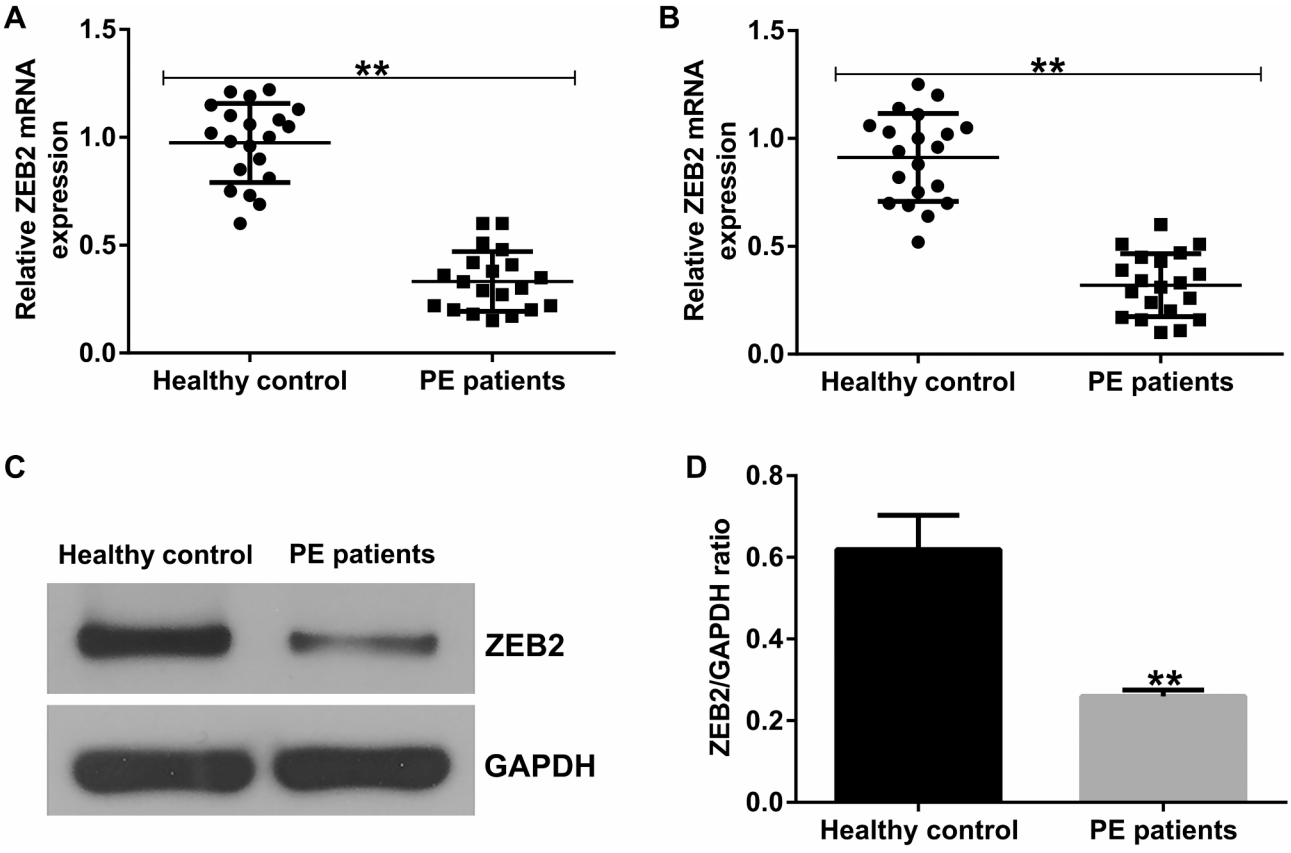


**Fig. 1** Expression of ZEB2 in the blood and placental tissues of PE patients. **A**: RT-qPCR analysis of ZEB2 levels in serum samples from PE patients and healthy individuals. Expression of ZEB2 in placental tissues of PE patients and healthy individuals were detected using (**B**) RT-qPCR and (**C** and **D**) Western blot assay. $^{**}P < 0.01$

of HTR-8/SVneo cells was enhanced significantly after ZEB2-plasmid treatment (Fig. 3C-D). Our findings above revealed that ZEB2-plasmid promoted trophoblast cells migration and invasion in PE.

### Up-regulation of ZEB2 activated Wnt/β-Catenin signal pathway in HTR-8/SVneo cells

Researches have shown that Wnt/β-Catenin signal pathway plays a vital role in the cell invasion and migration. Multiple studies have confirmed a close connection between ZEB2 and the Wnt/β-Catenin signal pathway [18]. Next, to illustrate the potential molecular mechanism by which ZEB2 regulated HTR-8/SVneo cells proliferation, apoptosis, migration, and invasion, the related protein expression in Wnt/β-Catenin signal pathway was determined, including Wnt3a, β-Catenin, GSK3β, p-GSK3β, C-Myc, and Cyclin D1. Our data suggested that ZEB2-plasmid group had significantly elevated Wnt3a, β-Catenin, p-GSK3β, C-Myc, and CyclinD1 expression, while there was no significant change in GSK3β expression in HTR-8/SVneo cells, compared with control-plasmid group (Fig. 4A-F). These findings indicated that ZEB2 regulated HTR-8/SVneo cells proliferation, apoptosis, migration, and invasion through Wnt/β-Catenin signal pathway in PE human placenta.

### XAV939 reversed the effects of ZEB2-plasmid on Wnt/β-Catenin signal pathway

To further investigate the effects of ZEB2 on Wnt/β-Catenin signal pathway in PE, rescue experiments were conducted. HTR-8/SVneo cells were transfected with control-plasmid or ZEB2-plasmid, followed by 10 μM XAV939. As displayed in Fig. 5A-F, XAV939 reversed the effects of ZEB2-plasmid on Wnt/β-Catenin signal pathway, as confirmed by suppressed Wnt3a, β-Catenin, p-GSK3β, C-Myc, and Cyclin D1 expression. Our data suggested that ZEB2 regulated the HTR-8/SVneo cells physiological functions through Wnt/β-Catenin signal pathway.

### XAV939 reversed the effects of ZEB2-plasmid on trophoblast cells proliferation, apoptosis, migration and invasion

Increasing researches have confirmed placental trophoblast cells proliferation and invasion were regulated through multiple signal pathways, including

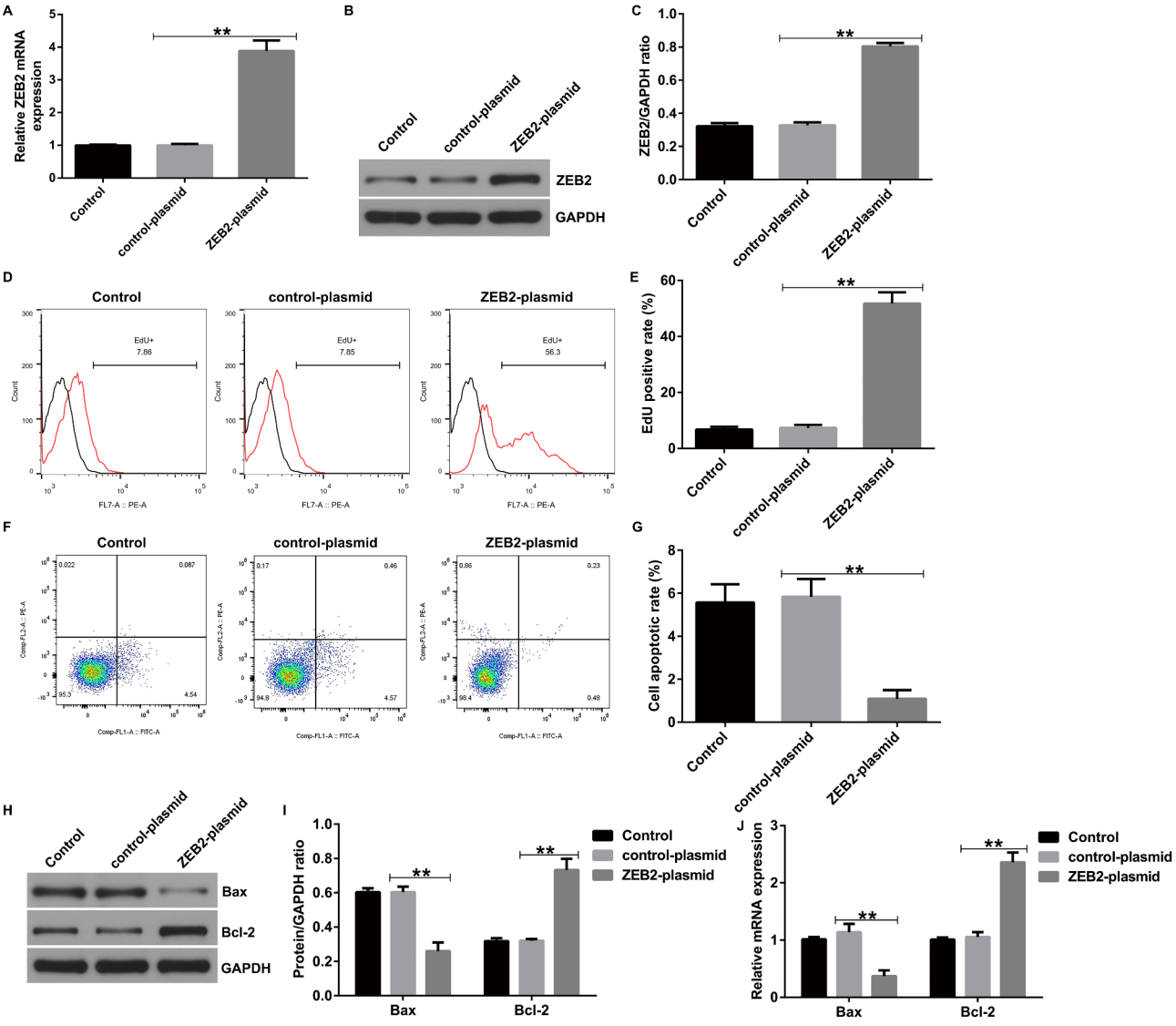


**Fig. 2** Effects of ZEB2-plasmid on HTR-8/SVneo cells proliferation and apoptosis. Trophoblast cells were transfected with control-plasmid or ZEB2-plasmid, and cells were divided into three groups: Control, control-plasmid and ZEB2-plasmid. (**A**) Relative ZEB2 levels were determined by RT-qPCR analysis. (**B**) Western blot analysis of ZEB2 expression. (**C**) Plot showing the quantification of ZEB2 expression. (**D**) The proliferation of trophoblast cells were determined using EdU assay. (**E**) Plot showing the quantification of $EdU^+$ cells. (**F**) Flow cytometry analysis of apoptotic cells. (**G**) Quantitation of apoptotic cells. (**H**) Western blot analysis of Bax and Bcl-2 expression. (**I**) the quantification of Bax and Bcl-2 protein expression. (**J**) Relative mRNA levels of Bax and Bcl-2 were assessed by RT-qPCR analysis. $^{**}P < 0.01$

Wnt/β-Catenin signal pathway [19, 20]. We further explored the roles of XAV939 in ZEB2 regulated HTR-8/SVneo cells biological behavior. We observed that XAV939 treatment led to inhibited cells growth (Fig. 6A-B), promoted cells apoptosis (Fig. 6C-D), as well as suppressed migration and invasion capabilities (Fig. 6H-K). Meanwhile, we also determined the expression of Bax and Bcl-2 using RT-qPCR and Western blot. Our data suggested that ZEB2-plasmid down-regulated Bax level and enhanced Bcl-2 expression in HTR-8/SVneo cells (Fig. 6E-G), as compared to control-plasmid group. However, we found an inverse result in XAV939 treated group. Based on these findings above, we observed that ZEB2 regulates the HTR-8/SVneo cells proliferation, apoptosis, migration, and invasion through Wnt/β-Catenin signal pathway.

### XAV939 reversed the effects of ZEB2-plasmid on preeclampsia-like symptoms in mice

It is well known that PE is characterized by markedly elevated systolic blood pressure and high proteinuria. PE mice models were constructed to determine the expression of ZEB2 in PE models and illustrated its potential mechanism. As presented in Fig. 7A-B, the

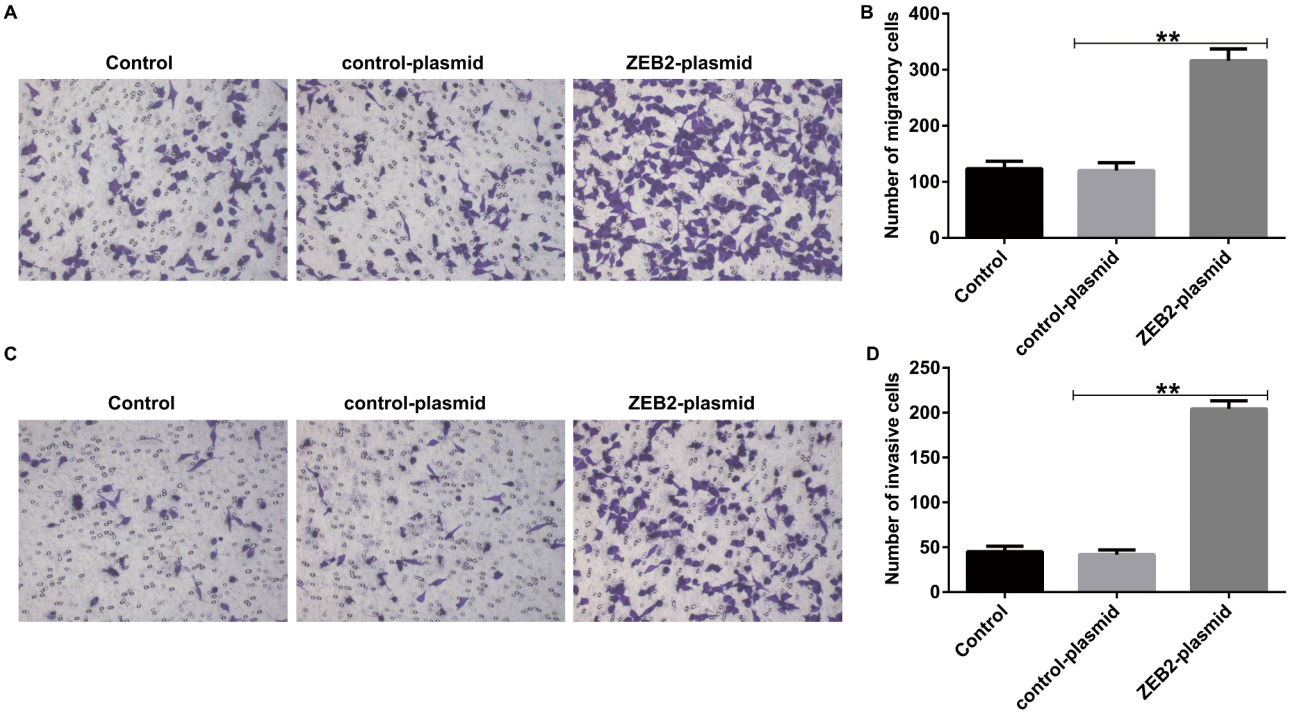


**Fig. 3** Effects of ZEB2-plasmid on HTR-8/SVneo cells migration and invasion Migration (**A**) and invasion (**C**) capacities of trophoblast cells with or without transfection of ZEB2-plasmid. Number of migratory (**B**) and invasive (**D**) HTR-8/SVneo cells were calculated. $^{**}P < 0.01$

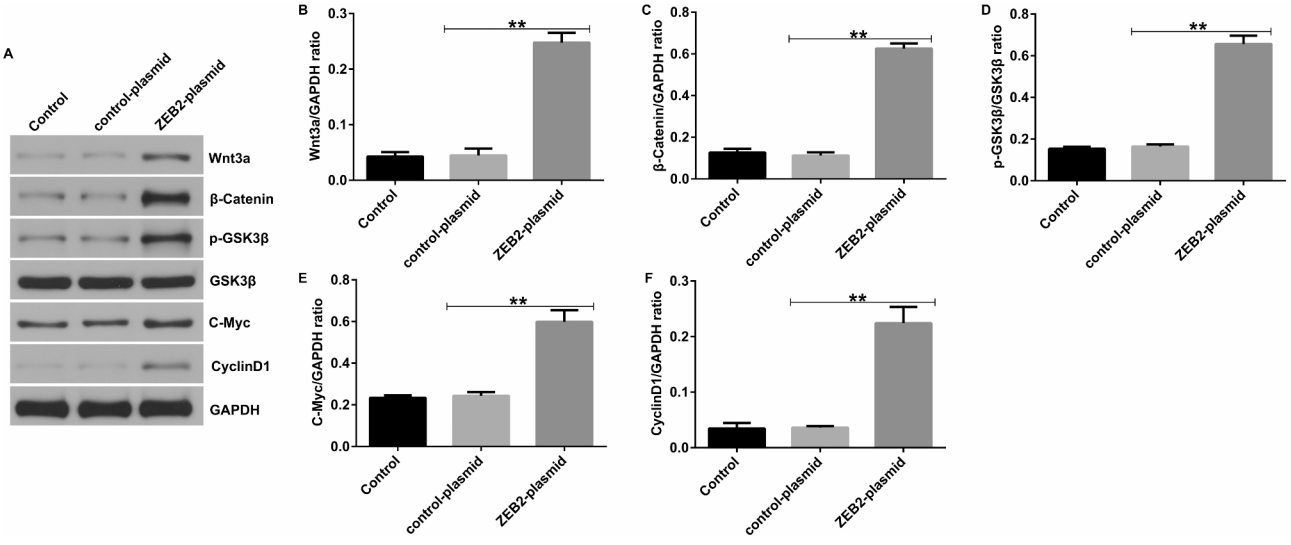


**Fig. 4** Effects of ZEB2-plasmid on Wnt/β-Catenin signal pathway in HTR-8/SVneo cells (**A**) Western blot analysis of Wnt3a, β-Catenin, GSK3β, p-GSK3β, C-Myc and Cycling D1 expression. (**B**-**F**) Quantification diagram of Wnt3a, β-Catenin, p-GSK3β/ GSK3β, C-Myc and CyclinD1 expression. $^{**}P < 0.01$

administration of ZEB2-plasmid successfully suppressed systolic pressure and urinary protein content. In addition, we found that ZEB2 was down-regulated in the serum and placental tissues of PE mice (Fig. 7C-F). Furthermore, TUNEL staining revealed that ZEB2-plasmid reduce the apoptotic cells in the placental tissues of PE mice (Fig. 7G). Nevertheless, all these findings were reversed by XAV939 treatment, demonstrating that ZEB2 may alleviate PE symptoms in vivo models.

### XAV939 reversed the effects of ZEB2-plasmid on Wnt/β-Catenin signal pathway in mice

To further illustrate the latent mechanism of ZEB2 and Wnt/β-Catenin signal pathway in PE mice, PE mice were treated with control-plasmid, ZEB2-plasmid and

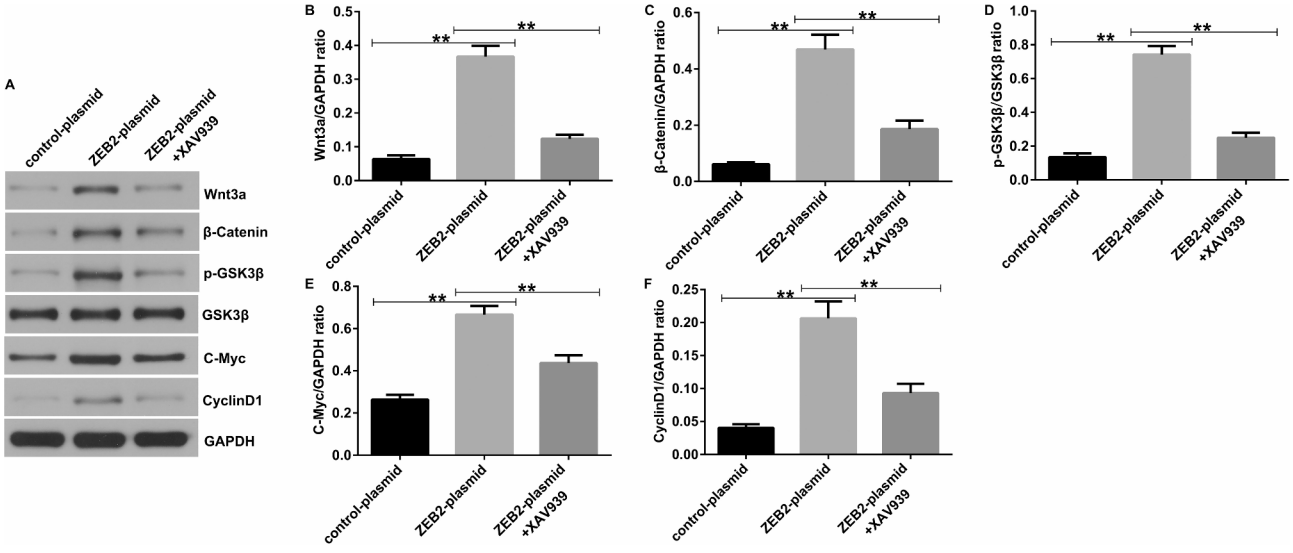


**Fig. 5** Influence of AV939 and ZEB2-plasmid on Wnt/β-Catenin signal pathway in HTR-8/SVneo cells Trophoblast cells were transfected with control-plasmid or ZEB2-plasmid, and treated with 10 μM XAV939. Cells were divided into three groups: Control-plasmid, ZEB2-plasmid, and ZEB2-plasmid + XAV939 group. (**A**) Detection of Wnt3a, β-Catenin, GSK3β, p-GSK3β, C-Myc and Cyclin D1 expression using Western blot assay. (**B**-**F**) Quantification diagram of Wnt3a, β-Catenin, p-GSK3β/GSK3β, C-Myc and Cyclin D1 expression. $^{**}P < 0.01$

ZEB2-plasmid + XAV939. Results from western blot assay suggested that Wnt3a, β-Catenin, p-GSK3β, C-Myc, and CyclinD1 was down-regulated in the placental tissues of PE mice, and these reductions were reversed by ZEB2-plasmid, as compared to the control-plasmid group. Further observations suggested that XAV939 reversed the effects of ZEB2-plasmid on Wnt/β-Catenin signal pathway in mice, as confirmed by inhibited Wnt3a, β-Catenin, p-GSK3β, C-Myc, and CyclinD1 expression. However, there was no significant difference in GSK3β expression among all groups (Fig. 8A-F). Our observations in this research revealed the protective role of ZEB2 in PE through regulating trophoblast cells proliferation, apoptosis, migration, and invasion via Wnt/β-Catenin signal pathway.

## Discussion

To our knowledge, this research is the first to illustrate the expression and roles of ZEB2 in placental tissues. We found that ZEB2 is down-regulated in PE, possibly stimulating trophoblast cells proliferation, migration, and invasion through the Wnt/β-Catenin signal pathway.

PE is a complex placental related disease that affects 5–8% of pregnant women worldwide. Most researchers believed that trophoblast cells dysfunction is the main cause of PE [21]. During pregnancy, the infiltration ability of placental trophoblasts decreases, leading to insufficient placental perfusion, ischemia, hypoxia, metabolic disorders, and ultimately developing into PE [22]. Previous reports have confirmed that PE is mainly characterized by proteinuria and hypertension [23]. Our in vivo experiments have displayed an increase in urine protein content and blood pressure in PE mice, indicating the successful construction of the PE mice model. ZEB2, a vital nuclear transcription factor, is abnormally expressed in various kidney diseases [24]. In addition, Guo et al. suggested that ZEB2 interacted with MDM2, contributes to the dysfuntion of brain microvascular endothelial cells and brain injury after intracerebral hemorrhage [25]. Manders et al. identified ZEB2 as RNA marker for non-invasive presymptomatic screening of pre-eclampsia [26]. Nevertheless, the expression and specific molecular mechanisms of ZEB2 in pre-eclampsia patients need to be further elucidated.

In our study, we first verified the expression of ZEB2 in the serum and placenta of PE pregnant women through RT-qPCR and Western blot analysis, and we observed that the expression of ZEB2 protein was significantly reduced in the serum and placenta of PE pregnant women, further in vivo experiment suggested the expression level of ZEB2 was negatively correlated with systolic blood pressure and urinary protein levels in PE patients. Our findings revealed the possible relationship between ZEB2 expression and PE progression. Previous reports have shown that PE typically involves multiple factors, including excessive apoptosis and abnormal invasion of trophoblasts. Qin et al. revealed the expression of lncRNA TINCR in the placenta of patients with PE and its effect on the biological behaviors of trophoblasts [27]. Jiang et al. suggested that elevated microRNA-520 g in PE inhibits migration and invasion of trophoblasts [28]. Therefore, elucidating the molecular mechanisms related to trophoblast behaviors and identifying new therapeutic targets for PE are crucial for the treatment of PE.

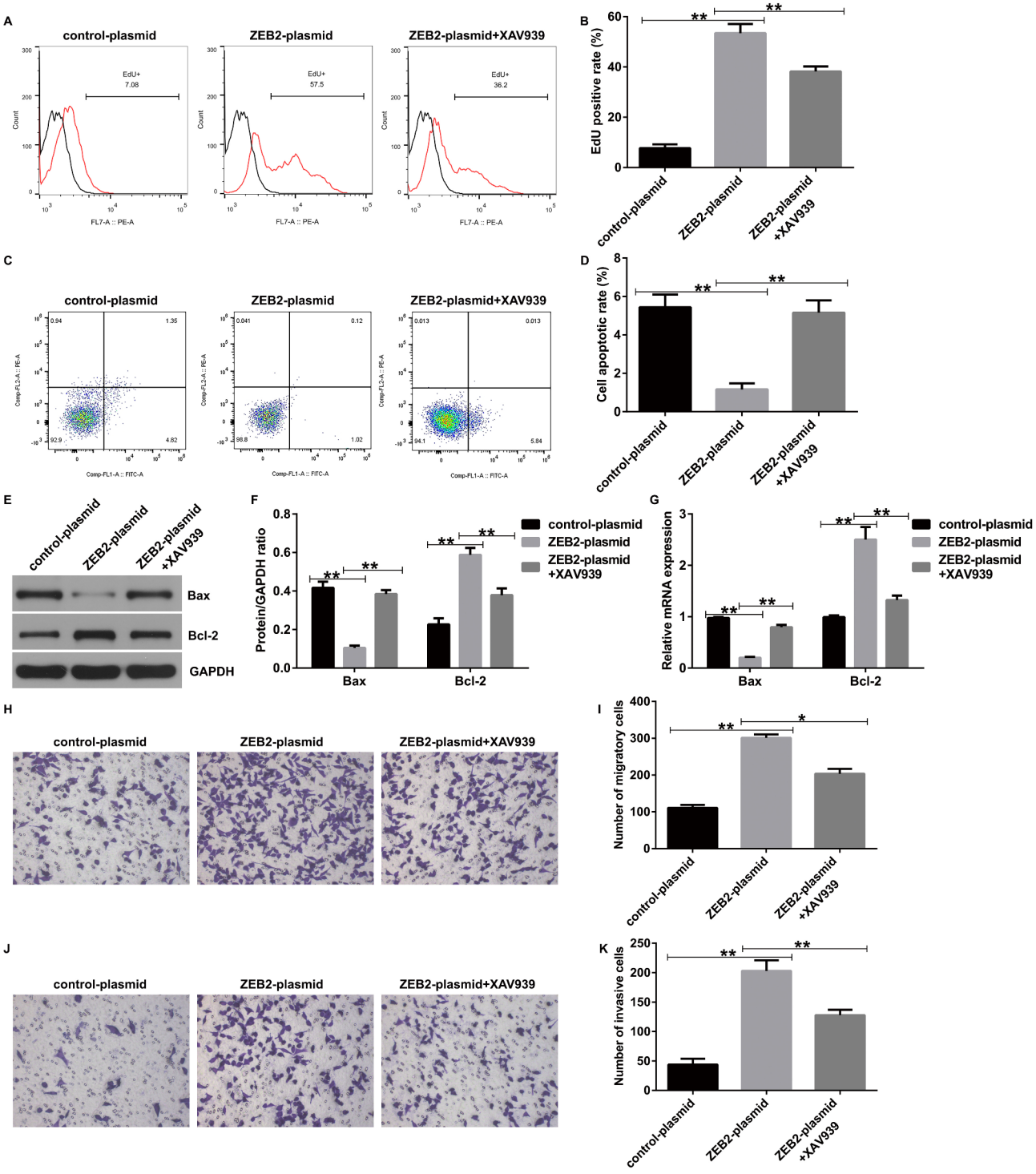


**Fig. 6** Influence of AV939 and ZEB2-plasmid on trophoblast cells proliferation, apoptosis, migration and invasion EdU assay was applied for determining trophoblast cells growth. (**E**) Plot showing the quantification of EdU$^+$ cells. (**C**) Flow cytometry analysis of apoptotic cells. (**D**) Quantitation of apoptotic cells. (**E**) Western blot analysis of Bax and Bcl-2 expression. (F) the quantification of Bax and Bcl-2 protein expression. (**G**) Relative mRNA levels of Bax and Bcl-2 were assessed by RT-qPCR analysis. **H** and **I**) Cells migration was detected using transwell assay. (**J** and **K**) Invasion capacity of HTR-8/SVneo cells was assessed by transwell assay. * $P < 0.05$, ** $P < 0.01$

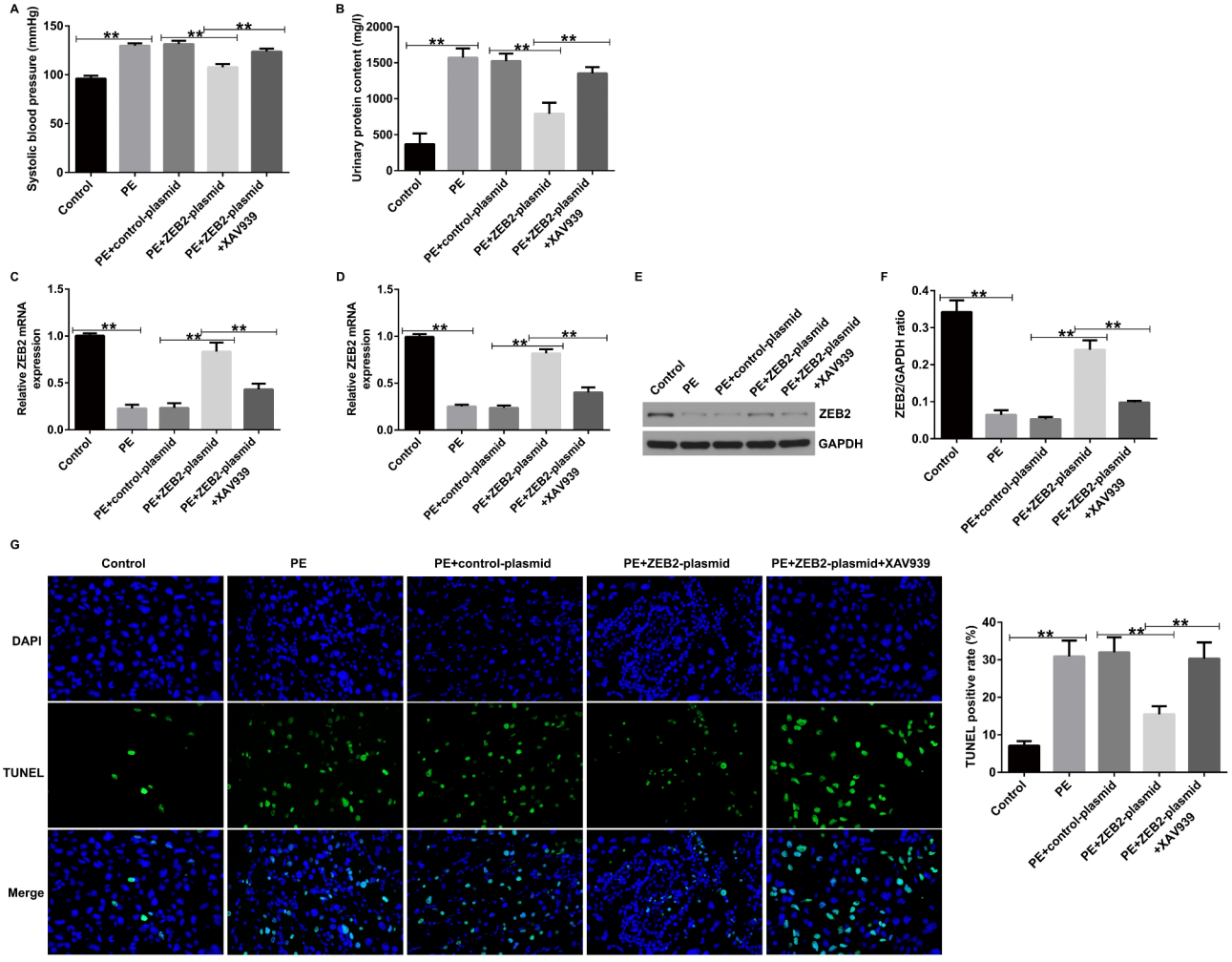


**Fig. 7** Influence of AV939 and ZEB2-plasmid on preeclampsia-like symptoms in mice. PE mice were exposed to control-plasmid, ZEB2-plasmid and 10 μM XAV939. Mice were divided into five groups: C57 mice, PE mice, PE + control-plasmid, PE + ZEB2-plasmid, and PE + ZEB2-plasmid + XAV939 group. (**A**) Plot showing the SBP in different groups were measured. (B) Plot showing the urinary protein concentrations. (**C**-**D**) Relative mRNA levels of ZEB2 in the serum and placental tissues of PE mice. (**E** and **F**) Detection of ZEB2 protein expression in the placental tissues of PE mice. (**G**) TUNEL staining was applied to detect the apoptotic cells in the placental tissues of PE mice. $^{**}P < 0.01$

In this research, the functions of ZEB2 in PE were investigated, and it was observed that ZEB2 was up-regulated in ZEB2-plasmid transfected HTR-8/SVneo cells, compared to Control and control-plasmid group. Moreover, ZEB2 over-expression increased cell proliferation and suppressed cell apoptosis in HTR-8/SVneo cells. Bcl-2, as a cytoplasmic protein, targets the nucleus and inhibits cell apoptosis through lipid anchored domains [29]. Bax, a member of the Bcl family, forms a heterodimer with the homologous dimer of Bcl-2 protein, thereby reducing the anti-apoptotic effect of Bcl-2 and leading to apoptotic death [30]. In addition, we also detected the expression levels of Bax and Bcl-2 proteins in HTR-8/SVneo cells. Compared with the control plasmid group, up-regulation of ZEB2 increased Bcl-2 expression and depressed Bax expression. Additionally, ZEB2 was also verified as a vital regulator of EMT in cancer cells. For instance, report from Ji suggested that miR-124 regulates EMT based on ZEB2 target to inhibit invasion and metastasis in triple-negative breast cancer [31]. Moreover, knockdown of VASH2 inhibited the stemness and EMT procession by regulating ZEB2 in colorectal cancer [32]. Our results are in accordance with these researches confirming the function of ZEB2 in enhancing cells invasion, and demonstrated that up-regulation of ZEB2 may be vital in the PE development.

The Wnt/β-Catenin signaling pathway is a well-known pathway involved in regulating cell survival, metastasis, and apoptosis in various diseases (13–14). In addition, increasing research have found that the Wnt/β-Catenin signaling pathway plays a crucial role in regulating the growth and invasion of trophoblasts. For example, Chen et al. suggested that LASP2 inhibited trophoblast cells migration and invasion in PE through inactivation of the

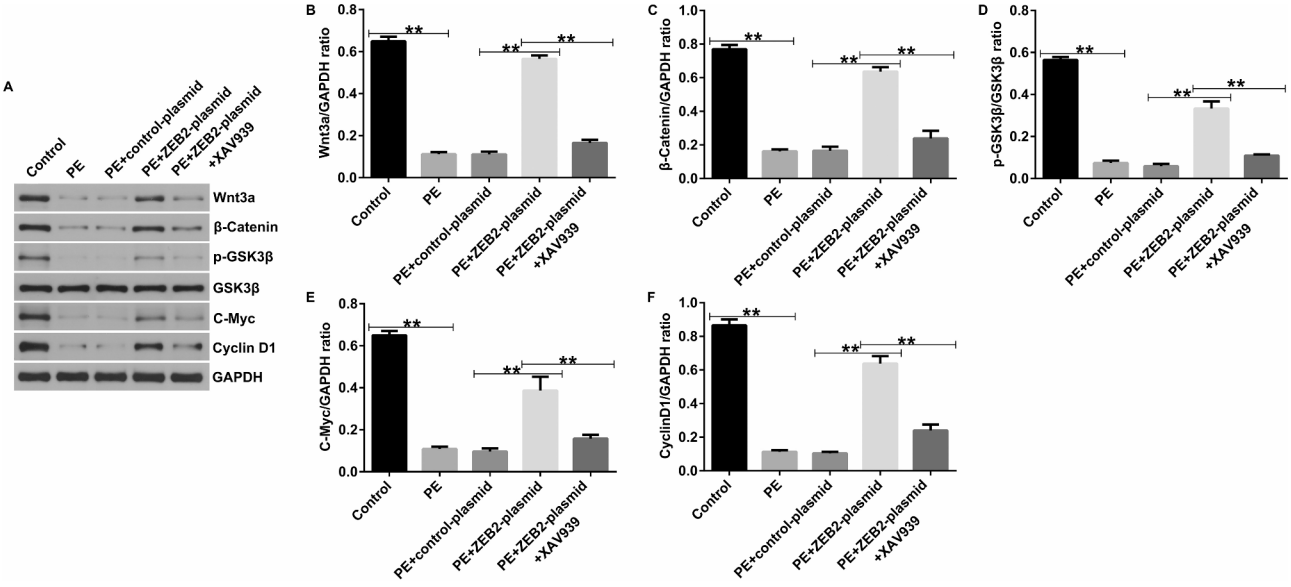


**Fig. 8** Influence of AV939 and ZEB2-plasmid on Wnt/β-Catenin signal pathway in mice. (**A**) Detection of Wnt3a, β-Catenin, GSK3β, p-GSK3β, C-Myc and Cycling D1 expression using Western blot assay. (**B-F**) Quantification diagram of Wnt3a, β-Catenin, p-GSK3β/GSK3β, C-Myc and Cyclin D1 expression. $^{**}P < 0.01$

Wnt/β-Catenin signaling pathway [16]. Furthermore, Li et al. confirmed that LRP6 regulates Rab7-mediated autophagy through the Wnt/β-Catenin pathway to modulate trophoblast cell migration and invasion [33]. Interestingly, previous investigations have shown the relationship between ZEB2 and Wnt/β-Catenin signaling pathway. For instance, Cui et al. also suggested that microRNA-545 targets ZEB2 to inhibit the development of non-small cell lung cancer by inactivating Wnt/β-Catenin pathway [34]. Therefore, we want to make sure whether ZEB2 act as a potential regulator of the Wnt/β-Catenin signaling pathway in trophoblast cells. Our findings revealed that up-regulation of ZEB2 remarkably increased Wnt3a, β-Catenin, p-GSK3β, C-Myc, and CyclinD1 expression in HTR-8/SVneo cells, compared with the control-plasmid group, indicating that ZEB2 regulates trophoblast cells proliferation and invasion through Wnt/β-Catenin pathway in PE. To further illustrate the functions of ZEB2 and Wnt/β-Catenin pathway in PE, rescue experiments were carried out by XAV939. XAV939, a Wnt/β-Catenin pathway modulator, was evidenced to have inhibitory effects on inflammatory response and promoting cells apoptosis in neuroblastoma cell lines [35, 36]. Further in vitro experiments revealed that inhibition of the Wnt/β-Catenin pathway by XAV939 reversed the ZEB2 over-expression-regulated trophoblast cells proliferation, apoptosis, migration, and invasion via the Wnt/β-Catenin pathway. Our further in vivo assay revealed that XAV939 treatment reversed the effects of ZEB2-plasmid on the apoptotic cells in the placental tissues of PE mice and Wnt/β-Catenin signal pathway in mice. These findings supported that activation of Wnt/β-Catenin signal pathway was participated in the ZEB2 over-expression-regulated alleviation of PE progression.

In summary, our findings demonstrated that ZEB2 is down-regulated in human placentas in PE. Up-regulation of ZEB2 may enhance trophoblast cell proliferation and invasion via the Wnt/β-Catenin signal pathway. In addition, ZEB2 over-expression alleviated PE progression in PE mice, related to activation of the Wnt/β-Catenin signal pathway. Our research provides a theoretical basis for PE pathogenesis, thereby providing a promising therapeutic target for PE.

## Materials and methods

### Patients and specimens

A cohort of 20 PE patients and 20 subjects with normal pregnancies admitted to Northern Jiangsu People's Hospital were separately participated in this research. Serum and placental tissues were collected in a timely manner from pregnancies undergoing elective cesarean delivery in the absence of labor and preserved in a freezer at −80 °C for later use. Healthy pregnant women matched PE patients in terms of age and gestational age. The study protocol was approved by the Ethics Committee of the Northern Jiangsu People's Hospital (Approval number: 2023ky244) with the oral and written consent of each participant.

### Cell culture

The HTR8/Sveno cells were cultured in RPMI-1640 culture medium with 10% FBS (Gibco, Rockville, MD, USA)

and 1% penicillin/streptomycin at 37℃ in an incubator containing 5% $CO_2$.

#### Cell transfection

The HTR8/Sveno cells were transfected with control-plasmid and ZEB2-plasmid using Lipofectamine® 3000 reagent (Thermo) for 24 h following the instructions. Then RT-qPCR and Western blot were conducted to evaluate cell transfection efficiency.

#### RT-qPCR assay

Total RNA was separated from cultured HTR8/Sveno and tissues which used TRIpure Total RNA Extraction Reagent (EP013, ELK Biotechnology) according to the specifications. Total RNA was eluted and stored at −80 °C. Then, cDNA was synthesized using the EntiLink™ 1st Strand cDNA Synthesis Super Mix (Eq. 031, ELK Biotechnology). Quantitative real-time PCR (qPCR) was executed using the EnTurbo™ SYBR Green PCR SuperMix (Eq. 001, ELK Biotechnology). The level of ZEB2, Bax and Bcl-2 were determined using QuantStudio 6 Flex System (Life technologies).

#### Western blot assay

Total protein from HTR8/Sveno cells and tissues was harvested and lysed in RIPA lysis buffer (AS1004, ASPEN) and protease inhibitor cocktail (04693159001, ROCHE) for 30 min at 4 °C. The lysate was centrifuged for 15 min at 12,000 rpm and 4 °C, the upper supernatant was collected, and the protein was separated by 10% SDS-PAGE and transferred onto PVDF membranes (IPVH00010, Millipore). The membranes were blocked with 5% nonfat milk for 60 min and then washed in TBS-Tween 20 buffer for 15 min three times and incubated with specific primary antibodies against ZEB2, Bax, Bcl-2, Wnt3a, β-Catenin, GSK3β, p-GSK3β, C-Myc, CyclinD1 or GAPDH (1:1000) at 4℃ overnight. Then, the membranes were washed three times with TBS-Tween-20 buffer and incubated with secondary antibodies for 1 h and then developed onto the chemiluminescence Western blotting detection system using an ECL chemiluminescent kit (AS1059, ASPEN) according to the synopsis.

#### EdU assay

After transfected for 24 h, HTR8/Sveno cells were cultivated in 96 well plates and pre-warmed EdU staining solution was added to each cell and incubated for 2 h at 37 °C. The culture medium was removed after centrifugation for 5 min and the cells were fixed with 2% paraformaldehyde for 20 min at room temperature. Then the cells were washed with PBS. The 0.5% Triton X-100 in PBS was added to each well and incubated for 10 min at room temperature. Then cells were stained using the Apollo reaction kit (ribobio, C10310-3) and photographed under a fluorescence microscope.

#### Flow cytometer assay

After transfected for 24 h, HTR8/Sveno cells were cultured into 96-well plates. Then cells were collected by centrifugation at 4 °C for 5 min. Then the cells were washed twice with PBS and assessed using the Annexin-V/propidium iodide (PI) Apoptosis Detection Kit (Beyotime). Then cells were gently mixed and were cultivated for 20 min at room temperature without light. Finally, apoptotic cells were checked using Flow cytometer (Beckman coulter) and analyzed using Kaluza following the direction.

#### Migration and invasion assays

The cell migration and invasion abilities of HTR8/SVneo cells were determined by Transwell assays. For the cell invasion assay, the HTR8/Sveno cells were incubated in RPMI-1640 medium for starvation and seeded into the upper chamber of transwell chambers with Matrigel (354248, Corning). For the cell migration assay, the HTR8/Sveno cells were incubated in serum-free RPMI 1640 medium for starvation and seeded into the upper chamber of transwell chambers without Matrigel. The lower chamber was filled with complete medium. After48 h of incubation, the cells in the lower chamber were fixed in 4% paraformaldehyde and stained with crystal violet for 10 min. The remaining HTR8/Sveno cells on the upper chamber were removed with a cotton swab. Images were obtained under an inverted microscope (IX51, OLYMPUS).

#### PE mice model

In this study, 8-week-old female C57BL/6 mice (weighing 25–30 g) were applied to conduct PE mice model. During the estrus period, female mice mated with male mice of the same weight in a ratio of 2:1. The time of discovery of the vaginal plug was defined as GD0.5. Subsequently, pregnant mice were divided into 5 groups: control group (normal pregnancy), PE group, PE+control-plasmid group, PE+ZEB2-plasmid group, and PE+ZEB2-plasmid+XAV939 group. Starting from the 12th day of pregnancy, the mice in PE model group received subcutaneous injection of L-NAME for 7 days, while the control group mice received subcutaneous injection of equivalent volume of physiological saline. Mice were euthanized at GD20 and placental or blood samples were collected. All experimental plans have been approved by the Ethics Committee of the Bestcelll Model Biological Center (Approval number: BSMS-2023-10-09 F).

### Tunel analysis

Apoptotic cells in the placentas were detected by TUNEL kit (G1504, Servicebio). In brief, the tissue was fixed with 4% paraformaldehyde for 20 min, and 2–3 μm paraffin sections were taken after dehydration. After that, the slices were dewaxed in xylene for 15 min, then washed with anhydrous ethanol for 5 min and soaked in 0.2% Triton X-100 for 15 min and soaked in DAPI solution(D8417-1MG, Sigma) for 20–30 min. Then the slices were photographed and counted under an optical microscope (Eclipse Ci-L, Nikon).

### Blood pressure measurement and urine analysis

The systolic blood pressure (SBP) of each rat was measured by the noninvasive tail cuff method. Each rat was exposed to 37 ℃ for 5 min before each measurement was taken. The 24-hour urine volume of each rat was acquired. CBB reagent kit (Nanjing Jiancheng Institute, China) was used to detect proteinuria.

### Statistical analysis

Statistical analyses were conducted using SPSS 20.0. All results are expressed by mean ± SD from three independent experiments. Differences among groups were estimated with unpaired Student's t-test or one-way ANOVA. $P<0.05$ indicated statistically significant difference.

## Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13008-024-00137-7.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

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Not applicable.

**Author contributions**
Yanxin Zhang and Fangle Gu: Conceptualization, Formal analysis, Project administration, Writing-original draft, and Writing-review & editing. Yan Liu, Yujie Sun, and Liying Zhang: Investigation, Methodology, and Software. Dan Lu: Investigation, Validation, and Writing-review & editing. All authors have read and approved the final manuscript.

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**Data availability**
No datasets were generated or analysed during the current study.

## Declarations

**Human Ethics and Consent to Participate declarations**
This study was approved by the Ethics Committee of the Northern Jiangsu People's Hospital (Approval number: 2023ky244) in accordance with the Declaration of Helsinki. Written informed consent was obtained from the patient before surgery.

**Competing interests**
The authors declare no competing interests.

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