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# CircHIPK3 is decreased in preeclampsia and affects migration, invasion, proliferation, and tube formation of human trophoblast cells

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ABSTRACT

*Introduction:* The migration and invasion of trophoblast cells into the endometrium and vasculature is a key step in human placentation. Preeclampsia, a devastating multi-system syndrome, is featured by shallow trophoblast invasion and unconverted narrow spiral arteries. Although the alteration in circHIPK3 expression is proved to regulate the invasion and metastasis of various malignant cells, its role in preeclampsia is unknown. Therefore, the aim of this study is to investigate the expression pattern of circHIPK3 in preeclampsia and its impact on trophoblast behavior.

*Methods:* The expression of circHIPK3 in placental tissues obtained from 70 women with preeclampsia and 43 healthy pregnancy controls using quantitative real-time PCR. Clinical information of participants was collected. The effects of circHIPK3 on trophoblast migration, invasion, proliferation, tube formation, and apoptosis were examined in trophoblast cell lines (HTR-8/SVneo) by transfected with knockdown siRNA and overexpression plasmid. Sanger sequencing was performed to verify circHIPK3 in placenta and HTR8/SVneo cells.

*Results:* Significantly decreased circHIPK3 levels were detected in preeclampsia compared with healthy pregnancy controls. The migration, invasion, proliferation, and tube formation capacities of HTR8/SVneo cells were inhibited via circHIPK3 silencing, but circHIPK3 overexpression effectively promotes these capacities except proliferation. No significant difference was observed in apoptosis between cells transfected with siRNA and overexpression plasmid.

*Discussion:* Our findings suggest for the first time that abnormal expression of circHIPK3 may contribute to the development of preeclampsia by leading to the aberrant biological behavior of trophoblast cells. The underlying mechanisms need further study.

## 1. Introduction

Preeclampsia is a devastating multi-system syndrome and remains a major cause of pregnancy-associated morbidity and mortality, affecting 5–8% of pregnancies globally [1]. The pathogenesis of preeclampsia is still not fully elucidated, but poor placentation is believed to play a key role in the development of this disorder [2]. Normal placentation is characterized by moderate trophoblast invasion and vascular transformation [3]. During the early placentation, extravillous trophoblast cells (EVTs) sprout from the cell columns at the anchoring villi, migrating and invading into the decidua and even the superficial myometrium (interstitial trophoblasts) or towards maternal blood vessels (endovascular trophoblasts), eventually transforming spiral arteries into larger diameter vessels with low resistance and high blood flow [1,2,4]. If this process is inhibited, consequent placental dysfunction will cause serious obstetrical disorders, especially preeclampsia [4].

Circular RNAs (circRNAs), a novel class of noncoding RNAs, are highly conserved and stable covalently closed RNA transcripts without 5′ to 3′ polarities and poly (A) tails [5]. These molecules are generated by back-splicing and ubiquitously expressed in eukaryotic cells during post-transcriptional processes [6]. CircRNAs have been shown to have a series of important biological functions and exhibit significant effects on human diseases, especially cancers [7]. Some studies have indicated that circRNAs may serve as novel cancer diagnostic and prognostic biomarkers [7,8]. Recently, Zeng et al. demonstrated that circHIPK3 (hsa_circ_0000284), derived from exon 2 of the HIPK3 gene, promotes

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the proliferation, migration, invasion, and reduced apoptosis of colorectal cancer cells [9]. Furthermore, this molecule was verified to be involved in bladder cancer [10], liver cancer [11], gastric cancer [12], and epithelial ovarian cancer [13].

Notably, trophoblasts are considered "pseudo-tumor cells" [14]. Because the regulation of trophoblast behaviors is believed to share certain similar mechanisms with malignant cells in tumorigenesis and metastasis [15], we speculate that circHIPK3 may also affect the proliferation, migration, invasion and other biological behaviors of trophoblast cells. However, the role of circHIPK3 in preeclampsia is unclear. Therefore, it is necessary to explore the alteration of circHIPK3 expression in preeclampsia placenta and the effect of circHIPK3 on trophoblast behaviors.

## 2. Materials and methods

### 2.1. Study subjects and specimen collection

The study was approved by the Ethics Committee of West China Second University Hospital, Sichuan University. Placental tissue samples were obtained with informed consent. A total of 113 participants, including 43 healthy pregnancy controls (term delivery) and 31 patients with early-onset and 39 patients with late-onset severe preeclampsia were recruited from the West China Second University Hospital during December 2015 and May 2018. Patients with severe preeclampsia were subdivided into term (23 cases) and preterm delivery (47 cases). Severe preeclampsia was defined as systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥110 mmHg on at least two occasions following 20 weeks of gestation, along with significant proteinuria (> 2 g/24 h and/or 2 + on dipstick testing) or evidence of multiorgan problems, including pulmonary edema, seizures, oliguria, thrombocytopenia, liver dysfunction or central nervous system perturbations [16]. Early-onset preeclampsia was diagnosed as manifestation prior to 34 gestational weeks and late onset preeclampsia thereafter [17].

All the participants delivered through elective cesarean section. The exclusion criteria consisted of multiple pregnancies, diabetes, chronic nephritis, chronic hypertension, heart diseases, autoimmune diseases, thrombophilic conditions, fetal malformation and HELLP syndrome (hemolysis, elevated liver enzymes and low platelet count). Placental tissue pieces (~1.0 $cm^3$) were excised from random regions immediately after extraction of the placenta from the uterus, avoiding areas of vessels, calcification or infarction. Samples were washed with sterilized saline water three times and stored at −80 °C for later use.

### 2.2. RNA extraction and qRT-PCR

Total RNA was isolated from placental tissues or cells by TRIzol Reagent (Invitrogen, USA). Complementary DNA (cDNA) was synthesized using a Reverse Transcription Kit (TOYOBO, Japan). QRT-PCR was performed using SYBR® Green Real-time PCR Master Mix (TOYOBO, Japan). The PCR primers were as follows: circHIPK3 forward 5′- GGG TCG GCC AGT CAT GTA TC-3′ and reverse 5′- ACT GCT TGG CTC TAC TTT GAG T -3′ and GADPH forward 5′- TTG GTA TCG TGG AAG GAC TCA -3′ and reverse 5′- TGT CAT CAT ATT GGC AGG TTT -3'. The PCR products of circHIPK3, amplified in placenta and HTR8/SVneo cells were tested by Sanger sequencing. The expression of circHIPK3 was calculated using the relative CT method ($2^{-\Delta\Delta Ct}$ method) and normalized to GAPDH.

### 2.3. Cell culture, transfection, small interfering RNAs and plasmids

The human first-trimester extravillous trophoblast cell line HTR8/SVneo cells (kindly provided by Dr Yali Hu, Affiliated Drum Tower Hospital, Medical College of Nanjing University, Nanjing, China) were cultured in RPMI 1640 growth medium (HyClone, USA) supplemented with 10% fetal bovine serum (Gibco, USA) at 37 °C with 5% $CO_2$. HTR-8/SVneo cells were transiently transfected with knockdown siRNA targeting circHIPK3 (siRNA-circHIPK3, Geneseed, China) or overexpression plasmid targeting circHIPK3 (pCDNA3.1-circHIPK3, Geneseed, China) using Lipofectamine 3000 transfection reagent (Invitrogen, USA). Negative control siRNA (siRNA-NC) and empty vector (pCDNA3.1) were provided as the control of siRNA-circHIPK3 and pCDNA3.1-circHIPK3, respectively. In addition, HTR-8/SVneo cells, treated with transfection reagent, served as the blank control of the study.

### 2.4. Cell proliferation assay

Transfected cells were seeded in 96-well flat bottom plates ($2.5 \times 10^3$ cells/well). Cell proliferation assays were conducted using Cell Counting Kit-8 (DOJINDO, Japan) according to the manufacturer's protocol. The absorbance of each well was measured at 450 nm using a spectrophotometer (TECAN, Mannedorf, Switzerland) at 0, 24, 48, 72, and 96 h after seeding.

### 2.5. Cell migration and invasion assays

For transwell migration and invasion assays, 24-well transwell chambers (Costar, USA) with or without precoated Matrigel matrix were conducted to observe cell migratory or invasive abilities. Transfected cells suspended in 200 μl serum-free medium ($5 \times 10^4$ cells/well) were pipetted into the upper chambers, and 600 μl medium supplemented with 10% FBS was added into the lower chambers. After incubating for 24 h at 37 °C, the cells that passed through the microporous membrane and adhered to the lower membrane surface were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. The migrated and invaded cells were counted in five randomly selected fields.

### 2.6. Tube formation assay

Tube formation assays were performed using 96-well flat bottom plates and each well was coated with 50 μl BD Matrigel matrix (BD Biosciences, USA). After solidification of the Matrigel matrix, a total of $5 \times 10^4$ transfected cells suspended in 10% FBS RPMI-1640 medium were planted into each well. After 4 h of incubation, the network formation was observed and photographed by a microscope (Olympus, USA). The branching point forming at least three tubules was counted in five random microscopic fields ( × 50).

### 2.7. Cell apoptosis assay

Transfected cells were harvested with 0.25% trypsin without EDTA, washed with PBS, double-stained with annexin V-APC/7-AAD (KeyGEN BioTECH, China) and then detected by flow cytometry (Cytomics FC 500, Beckman Coulter, USA). Finally, CXP Cytometer analysis software (Beckman Coulter) was used to sort viable, necrotic, early apoptotic, and late apoptotic cells.

## 3. Statistical analysis

Statistical analyses were carried out using GraphPad Prism 5.02 (GraphPad Software Inc., San Diego, CA, USA). Quantitative results of circHIPK3 expression in the placenta were not identical to the normal distribution, and values are represented as the median and interquartile range. Mann-Whitney tests were employed to evaluate the significant differences. Data from at least three independent experiments are expressed as the mean ± S.D. Statistically significant differences among three or more groups were evaluated using one-way ANOVA, and Student's t-tests were performed to analyze differences between two groups. A p value $< 0.05$ was identified as statistically significant.

**Table 1**
Demographic characteristics of study participants.

| | Control group | Early-onset group | Late-onset group |
|---|---|---|---|
| | ($n$ = 43) | ($n$ = 39) | ($n$ = 31) |
| Maternal age(years) | 32.35 ± 3.75 | 32.69 ± 5.33 | 31.16 ± 4.16 |
| Early pregnancy body mass index(Kg/m$^2$) | 22.80 ± 3.22 | 24.05 ± 4.09 | 24.11 ± 4.91 |
| Systolic blood pressure (mmHg) | 112.23 ± 9.56 | 161.41 ± 16.52☆ | 157.29 ± 12.41★ |
| Diastolic blood pressure (mmHg) | 71.63 ± 8.17 | 101.85 ± 12.16☆ | 99.74 ± 7.96★ |
| Gestation of delivery (weeks) | 39.61 ± 0.76 | 32.44 ± 3.23☆ | 37.31 ± 1.64★▲ |
| Smoking (%) | 0(0) | 0(0) | 0(0) |
| Birth weight (g) | 3445.35 ± 426.23 | 1486.03 ± 604.31☆ | 2669.68 ± 622.55★▲ |
| Birth length (cm) | 49.88 ± 1.66 | 38.13 ± 5.73☆ | 46.71 ± 4.41★▲ |

Data are presented as mean ± S.D or percentage.
☆: early-onset group compared with control group, $P < 0.05$; ★:late-onset group compared with control group, $P < 0.05$; ▲:late-onset group compared with early-onset group, $P < 0.05$.

## 4. Results

### 4.1. Decreased expression of circHIPK3 in severe preeclampsia

The study population characteristics are shown in Table 1. No significant difference was noted in maternal age, early pregnancy body mass index, and proportion of smokers among these three groups (P > 0.05). The control group showed lower systolic and diastolic blood pressure, later gestation of delivery, heavier birth weight, and longer birth length than the early- and late-onset groups, respectively (P < 0.05). In addition, the gestation of delivery, birth weight, and birth length of the late-onset group markedly differed from those of the early-onset group (P < 0.05).

The expression levels of circHIPK3 in placental tissues were examined using QRT-PCR. As shown in Fig. 1A, circHIPK3 was significantly decreased in patients with preeclampsia (2.61, 5.85) compared with healthy pregnancy controls (7.04, 14.04) (P < 0.01) (Fig. 1A). The circHIPK3 levels were markedly lower in early-onset preeclampsia (1.80, 4.78) and late-onset preeclampsia (5.15, 7.30) than in the healthy pregnancy controls (7.04, 14.04) (P < 0.05) (Fig. 1B). Additionally, no significant difference was observed in circHIPK3 levels between the term (4.71, 7.00) and preterm (2.23, 5.17) pregnant women in the severe preeclampsia group (P > 0.05). Furthermore, the circHIPK3 level of term delivery in the severe preeclampsia group (4.71, 7.00) was remarkably decreased compared with the healthy pregnancy controls (7.04, 14.04) (P < 0.05) (Fig. 1C). Women with preeclampsia were further divided into early-onset-preterm delivery (35 cases) and late-onset-term delivery (19 cases). CircHIPK3 levels were significantly lower in early-onset-preterm (1.80, 4.31) and late-onset-term delivery (4.71, 7.05) than in healthy pregnancy controls (7.04, 14.04) (P < 0.05). There was no significant difference in circHIPK3 levels between early-onset-preterm (1.80, 4.31) and late-onset-term delivery (4.71, 7.05) (P > 0.05) (Fig. S1 in supplementary file).

### 4.2. The effect of circHIPK3 on trophoblast cell migration

The number of cells that migrated through the Matrigel in the siRNA-circHIPK3 group (211.20 ± 18.63 cells) was lower than that in the siRNA-NC group (439.20 ± 11.80 cells) and the control group (520.80 ± 13.39 cells) (P < 0.01). Conversely, more migrating cells were observed in the pCDNA3.1-circHIPK3 group (785.60 ± 16.15 cells) than the pCDNA3.1 group (504.80 ± 8.67 cells) and the control group (520.80 ± 13.39 cells) (P < 0.01) (Fig. 2).

### 4.3. The effect of circHIPK3 on trophoblast cell invasion

Cell invasion capacities in the siRNA-circHIPK3 group (139.20 ± 12.46 cells) were inhibited compared with the siRNA-NC group (347.20 ± 16.35 cells) and the control group (410.40 ± 21.47 cells) (P < 0.01). Significantly increased invading cells were shown in the pCDNA3.1-circHIPK3 group

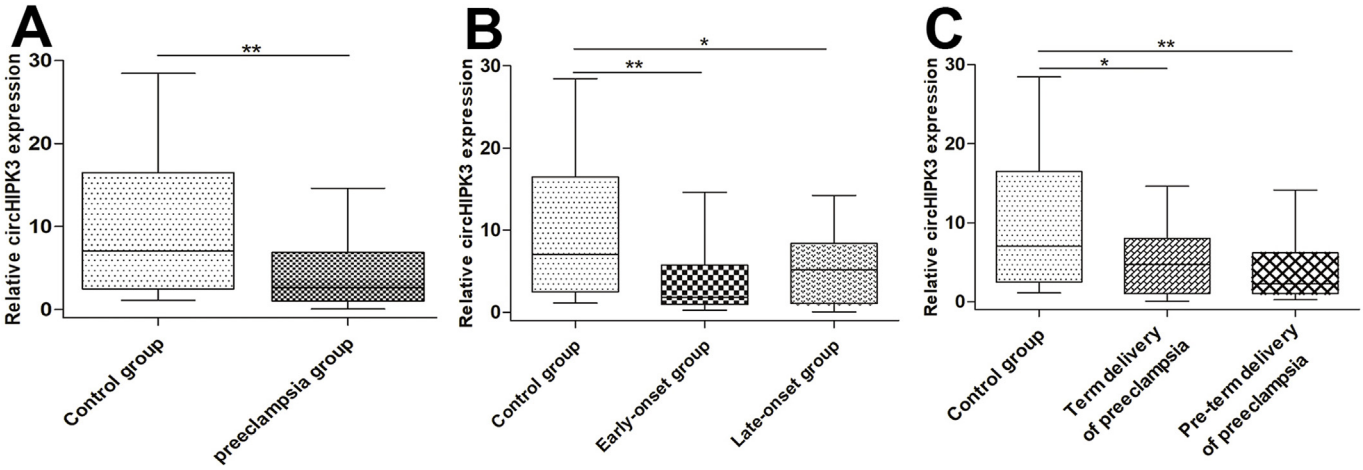


**Fig. 1.** CircHIPK3 expression in placenta from pregnant women with preeclampsia and healthy pregnancy controls. (A) CircHIPK3 was decreased in severe preeclampsia group compared with control group. (B)The expression levels of circHIPK3 were lower in late-onset group and early-onset group than control group. (C) Down-regulated circHIPK3 was detected in preterm severe preeclampsia delivery and term severe preeclampsia delivery compared with control group. (Values are median and interquartile range; **: P < 0.01, *: P < 0.05).

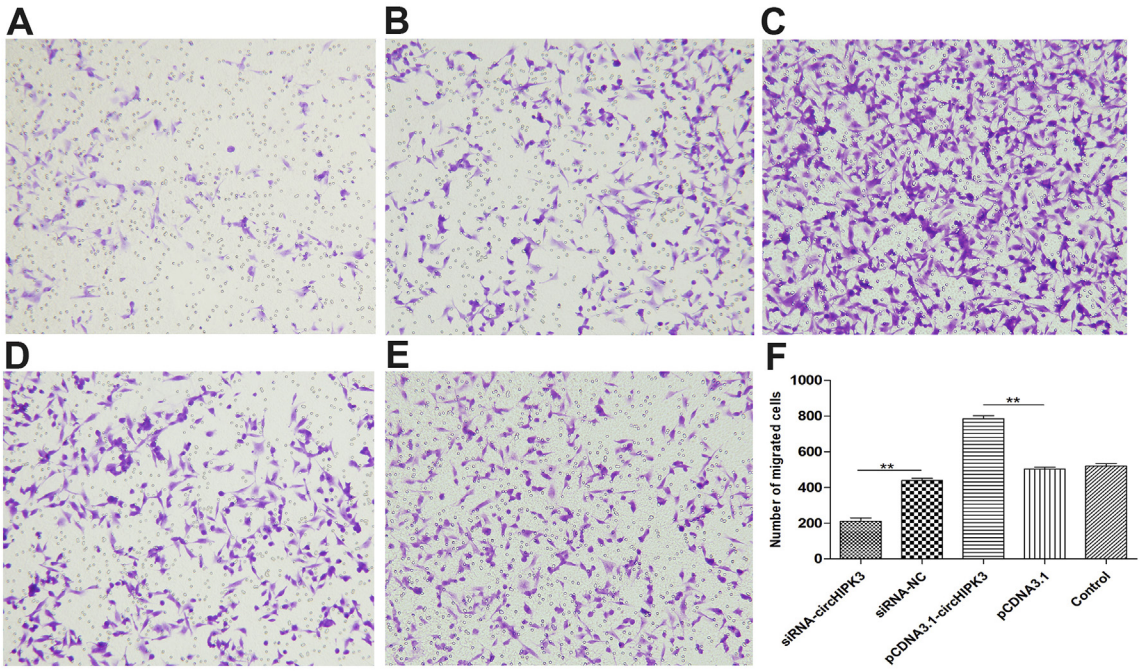


**Fig. 2.** Effect of circHIPK3 on trophoblast cell migration. $5 \times 10^4$ transfected cells suspended in 200 μl of FBS-free RPMI-1640 medium and seeded into the upper chamber. After incubation for 24 h, the migrated cells were fixed, dyed, and counted. (A–E) Representative images of transwell migration assay. (A) siRNA-circHIPK3, (B) siRNA-NC, (C) pCDNA3.1-circHIPK3, (D) pCDNA3.1, and (E) control. Magnification: 100 × . (F) Quantification of the number of migrated cells. (Values are mean ± SD; **: $P < 0.01$).

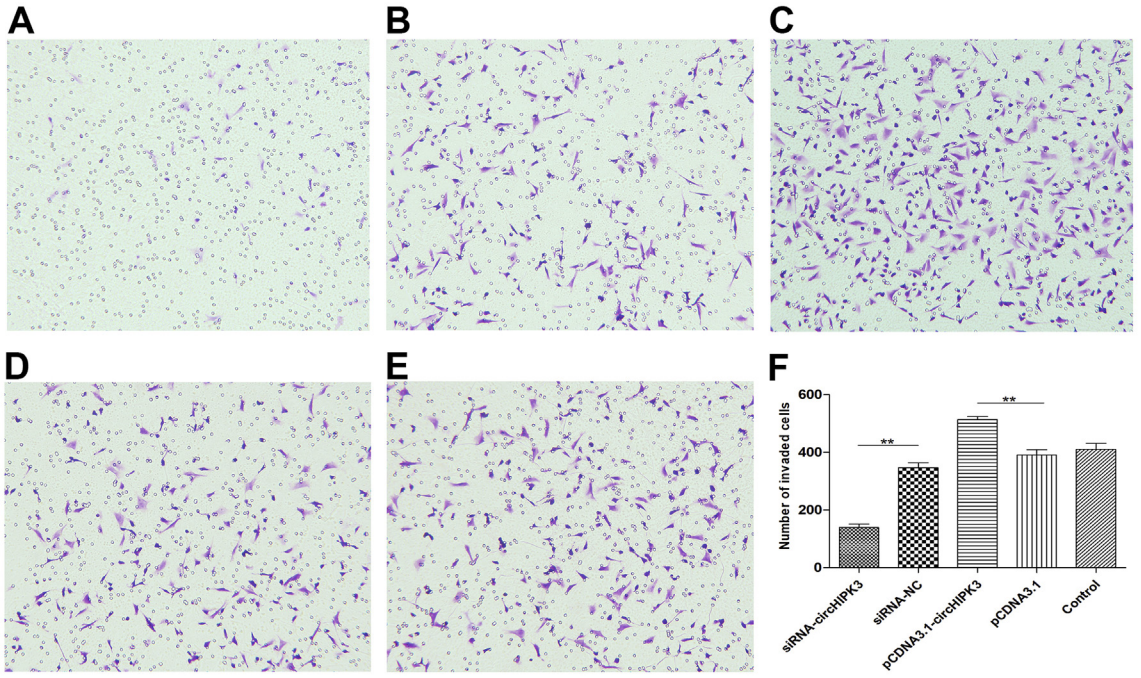


**Fig. 3.** Effect of circHIPK3 on trophoblast cell invasion. $5 \times 10^4$ transfected cells suspended in 200 μl of FBS-free RPMI-1640 medium and seeded into the upper chamber precoated with Matrigel matrix. After cultured for 24 h, the invaded cells were fixed, stained, and counted. (A–E) Representative pictures of transwell invasion assay. (A) siRNA-circHIPK3, (B) siRNA-NC, (C) pCDNA3.1-circHIPK3, (D) pCDNA3.1, and (E) control. Magnification: 100 × . (F) Comparison of the amount of invaded cells. (Values are mean ± SD; **: $P < 0.01$).

(513.60 ± 10.81 cells) compared with the pCDNA3.1 group (391.20 ± 17.98 cells) and the control group (410.40 ± 21.47 cells) ($P < 0.01$) (Fig. 3).

### *4.4. Knockdown of circHIPK3 inhibits the proliferation of HTR-8/SVneo cells*

Cell proliferation in the siRNA-circHIPK3 group was slower than that in the control and siRNA-NC groups at 48 h, 72 h and 96 h after transfection ($P < 0.01$), while overexpression of circHIPK3 had no

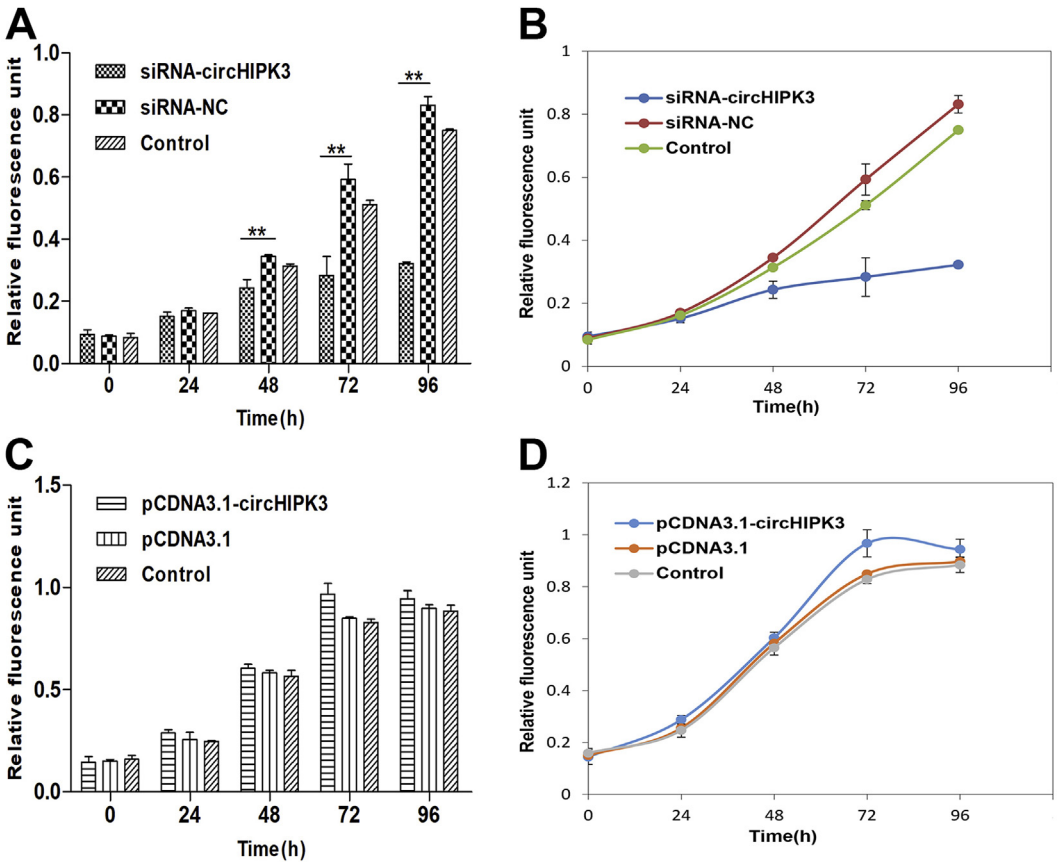


**Fig. 4.** Knockdown of circHIPK3 suppressed trophoblast cell proliferation. Cell proliferation at 0 h, 24 h, 48 h, 72 h and 96 h after transfection was detected using CCK-8 assays. Proliferative capacity of siRNA-circHIPK3 group at 48 h, 72 h and 96 h was significantly inhibited compared with that in control and siRNA-NC group (A,B), but overexpression circHIPK3 have no effect on cell proliferation (C, D). (**: $P < 0.01$).

significant effect on cell proliferation compared with the pCDNA3.1 group and control group($P > 0.05$) (Fig. 4).

### *4.5. The effect of circHIPK3 on trophoblast cell tube formation*

A decrease in branching points per field was noted in the siRNA-circHIPK3 group (26.20 ± 3.96) compared with the siRNA-NC group (37.80 ± 2.17) and the control group (38.60 ± 2.30) ($P < 0.01$). In contrast, overexpression of circHIPK3 (55.80 ± 3.49) contributed to a significant increase in branching points per field compared with the pCDNA3.1 group (35.60 ± 2.97) and the control group (38.60 ± 2.30) ($P < 0.01$) (Fig. 5).

### *4.6. The effect of circHIPK3 on trophoblast cell apoptosis*

No significant difference was detected in apoptosis among the siRNA silencing group (12.27 ± 4.53), siRNA-NC group (10.35 ± 3.07), and control group (7.12 ± 1.74) ($P > 0.05$). Similarly, cell apoptosis in the overexpression plasmid group (11.49 ± 2.93) showed no significant difference when compared with the pCDNA3.1 group (12.34 ± 2.45) and the control group (7.12 ± 1.74) ($P > 0.05$) (Fig. 6).

## 5. Discussion

Preeclampsia is a pregnancy-specific syndrome that poses a great threat to women's health worldwide. Although its etiology remains unclear, the placenta is indispensable in the development of preeclampsia [2]. Since removal of the placenta remains the only treatment, the placenta has always been a central figure in the pathogenesis of preeclampsia [18]. Normal placental development is characterized by adequate trophoblast invasion and remodeling of uterine spiral arteries [3]. Abnormal placentation is related to preeclampsia [1,2]. Therefore, to explore the pathogenesis of preeclampsia, it is crucial to investigate the differentially expressed factors in placenta with and without preeclampsia. In this study, we found that circHIPK3 expression levels were downregulated in the placenta of preeclampsia. Furthermore, its expression levels were not affected by gestational age because no significant difference was observed between term delivery and pre-term delivery of preeclampsia. To the best of our knowledge, this is the first study to report that circHIPK3 is significantly decreased in preeclampsia.

CircHIPK3, dysregulated in multiple cancers, has emerged as a critical regulator of tumor cell invasion, metastasis, proliferation, and apoptosis [9–13]. EVTs, which behave like malignant cells, are responsible for endometrium invasion and vascular transformation [3,14]. Undeniably, EVT migration and invasion into the first trimester decidua are fundamental events in the initiation of placentation [19,20]. To further investigate the exact effect of circHIPK3 in the development of preeclampsia, we cultured the human first-trimester extravillous trophoblast cell line HTR8/SVneo cells in vitro and changed their expression levels of circHIPK3. Similar to the impact of circHIPK3 on most malignant cells, we found that overexpression of circHIPK3 significantly promoted human trophoblast migration and invasion, whereas downregulation of circHIPK3 markedly suppressed these capacities. Herein, our results suggest that circHIPK3 may contribute to regulating the migration and invasion of EVTs and thereby participate in the development of preeclampsia. However, limited studies have suggested that upregulated circHIPK3 may repress cell migration and invasion in osteosarcoma [21] and bladder cancer [10]. Different

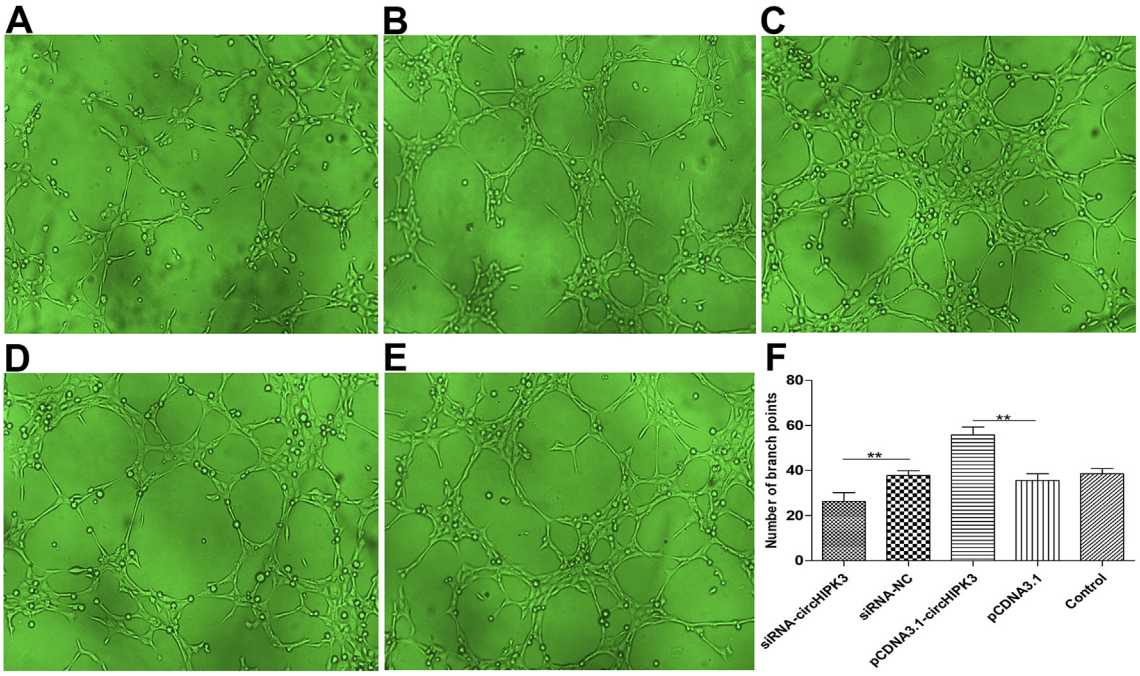


**Fig. 5.** Effect of circHIPK3 on trophoblast cell tube formation. $5 \times 10^4$ transfected cells were planted into 96-well flat-bottom plate coated with 50 μl of Matrigel matrix. After 4 h of incubation, the tube formation was observed and photographed. (A) siRNA-circHIPK3, (B) siRNA-NC, (C) pCDNA3.1-circHIPK3, (D) pCDNA3.1, and (E) control. Magnification: 50 × . (F) The number of branching points obtained from five random microscopic fields. (Values are mean ± SD; **: P < 0.01).

disease types may lead to these inconsistent results.

Early placental development largely depends on the coordinated action of trophoblast proliferation, differentiation, migration, and invasion [22]. In the current study, proliferation of HTR8/SVneo cells was inhibited by knockdown of circHIPK3, while there was no significant effect on growth when circHIPK3 was overexpressed.

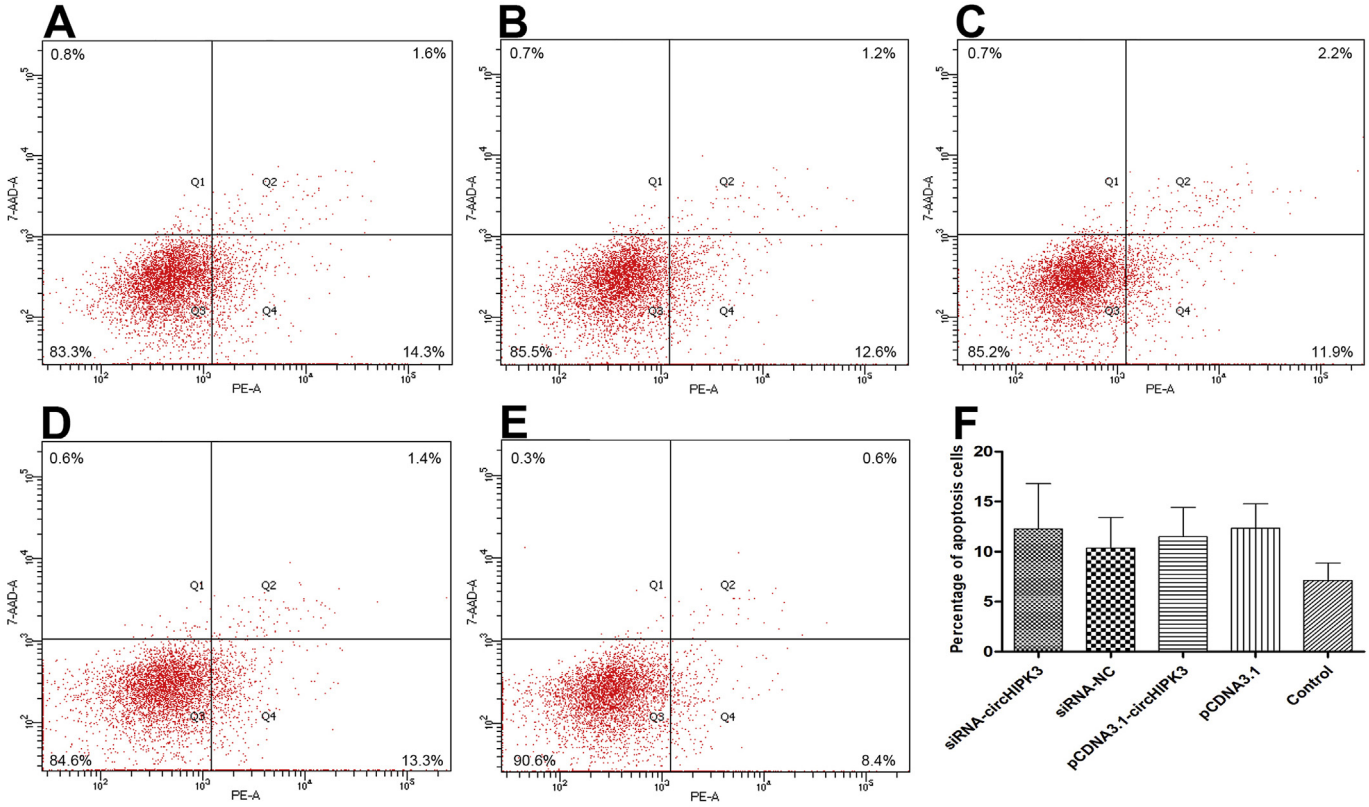


**Fig. 6.** Effect of circHIPK3 on trophoblast cell apoptosis. Transfected cells harvested with 0.25% trypsin without EDTA, and double-stained with annexin V-APC/7-AAD for flow analysis. No significant difference was observed neither in knockdown group nor in overexpression group. (A) siRNA-circHIPK3, (B) siRNA-NC, (C) pCDNA3.1-circHIPK3, (D) pCDNA3.1, and (E) control. (F) The percentage of apoptosis in every group. (Data were represented as the mean ± SD).

Consistent with our results from the siRNA knockdown experiments, previous studies revealed that the proliferation of various human tumor cells was significantly suppressed by silencing circHIPK3 [11,12,23–25]. Additionally, the repressive effect was reversed in colorectal cancer [9], gastric cancer cells [12] and gallbladder cancer [25] by overexpressing circHIPK3. Given that the proliferation of HTR8/SVneo cells is modulated by diversified factors and circHIPK3 shows effect when it is downregulated, circHIPK3 may serve as one of the proliferative regulators in a controlled manner. The potential mechanism requires further study.

Placental vascular transformation, induced by EVTs differentiating into endovascular trophoblasts, results in vessels with low resistance and high blood flow to better nourish the developing fetus [26]. Our data revealed that the tubule formation capacity of HTR8/SVneo cells was enhanced via circHIPK3 overexpression and inhibited by circHIPK3 silencing. Thus, we speculate that the angiogenesis inhibitory effect of downregulation of circHIPK3 may lead to inadequate spiral artery remodeling, involving the pathogenesis of preeclampsia related to poor placentation angiogenesis. Reportedly, several studies have discussed its role in the vascular function of diabetes mellitus. Shan et al. showed that circHIPK3 expression was significantly upregulated in diabetic retinas and retinal endothelial cells after high-glucose treatment [27]. Consistent with our findings, circHIPK3 overexpression significantly increased tube formation in retinal endothelial cells, but neovascularization always indicated retinal microvascular dysfunction in diabetes mellitus [27]. In contrast, Cao et al. found that circHIPK3 was downregulated in high glucose-treated human umbilical vein endothelial cells and primary aortic endothelial cells from diabetic patients, and circHIPK3 downregulation contributed to endothelial cell injury, suggesting a benefit of circHIPK3 on vascular protection [28]. Combined with these studies, circHIPK3's role in vascular function is not clear, but we found evidence supporting its angiogenesis promotion. Additionally, circHIPK3 exerting its biological roles with tissue and cell specificity may explain these conflicting results.

In present study, we found that circHIPK3 expression was decreased in the preeclampsia. Furthermore, the downregulation of circHIPK3 suppressed the migration, invasion, proliferation, and tube formation abilities of HTR-8/SVneo. Our study suggested that circHIPK3 might be a regulator of trophoblast behaviors and contribute to the pathogenesis of preeclampsia, but the potential mechanisms were not discussed. In contrast to cancer models, circHIPK3 showed no changes in apoptosis and decreased proliferation only in silenced circHIPK3, without an inverse correlation with overexpression. The results of our study seem to imply other associated pathways, which may help to reveal the pathogenesis of pre-eclampsia. Zheng et al. reported that circHIPK3, but not HIPK3 mRNA, significantly affected cell proliferation in different human cells [11]. Thus, it is worth discussing whether HIPK3 mRNA or the balance of circHIPK3 RNA/HIPK3 mRNA functions as a cell modulator in placental/HTR8/SVneo activities. In addition, circHIPK3 was observed to sponge 9 miRNAs with 18 potential binding sites, exerting regulatory functions in different cell models [11]. The induced effect of circHIPK3 through the modulation of miRNAs in preeclampsia deserves further study.

In conclusion, our study provides new insight into the role of circHIPK3 in the pathogenesis of preeclampsia. However, it is worth noting that HTR-8/SVneo cells cultured in vitro cannot completely simulate the in vivo environment with continuous interactions among multiple cells and cytokines. Primary human trophoblasts are needed to verify our results in future studies. The underlying mechanisms of circHIPK3 involved in the pathogenesis of preeclampsia remain unexplored and require further research.

## Author's contributions

Conceived and designed the experiments: Rong Zhou. Performed the experiments: Yanping Zhang and Liyuan Cao. Analyzed the data: Yanping Zhang, Liyuan Cao, Lei Ye, and Jin Jia. Contributed reagents/materials/analysis tools: Yanyun Wang and Bin Zhou. Wrote the paper: Yanping Zhang.

## Conflicts of interest

No competing interests exist.

## Conflicts of interest

The authors have no conflicts of interests to declare.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2019.07.010.

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