



Circ_FURIN promotes trophoblast cell proliferation, migration and invasion in preeclampsia by regulating miR-34a-5p and TFAP2A

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Abstract

Emerging evidence has shown that circular RNAs (circRNAs) play vital roles in the progression of diverse human diseases. However, the functions of circRNAs in preeclampsia (PE) are largely unknown. In this study, we aimed to explore the functions of the circRNA furin, paired basic amino acid cleaving enzyme (circ_FURIN) in PE development. qRT-PCR and western blot analyses were conducted to determine the levels of circ_FURIN, miR-34a-5p and transcription factor AP-2 alpha (TFAP2A). A Cell Counting Kit-8 (CCK-8) assay and a 5'-ethynyl-2'-deoxyuridine (EdU) incorporation assay were utilized to evaluate the cell proliferation ability. Transwell assays were adopted to estimate cell migration and invasion. A dual-luciferase reporter assay and an RNA pulldown assay were utilized to analyze the relationships among circ_FURIN, miR-34a-5p and TFAP2A. It was found that circ_FURIN was downregulated in PE placental tissues and hypoxia-treated placental trophoblast cells. Overexpression of circ_FURIN promoted trophoblast cell proliferation, migration and invasion under hypoxic conditions. Circ_FURIN functioned as the sponge for miR-34a-5p. MiR-34a-5p overexpression abrogated the effects of circ_FURIN on the proliferation, migration and invasion of trophoblast cells under hypoxic conditions. In addition, TFAP2A was demonstrated to be the target gene of miR-34a-5p. TFAP2A silencing ameliorated the promotive effects of miR-34a-5p inhibition on trophoblast cell proliferation, migration and invasion under hypoxic conditions. In conclusion, circ_FURIN enhanced trophoblast cell proliferation, migration and invasion under hypoxic conditions by elevating TFAP2A expression through sponging miR-34a-5p.

Keywords Preeclampsia · Trophoblast cells · circ_FURIN · miR-34a-5p · TFAP2A

Introduction

Preeclampsia (PE) is a complication of pregnancy and is a major cause of increased fetal morbidity and maternal mortality [1, 2]. However, the pathogenesis of PE remains unclear [3, 4]. Increasing evidence has determined that placental dysfunction, impaired spiral artery remodeling, dysregulation of oxygen and inadequate trophoblast infiltration may be involved in PE occurrence and development [5, 6]. Extravasated trophoblast cells (EVTs) play a vital role in placental progression, and reduced trophoblast infiltration and abnormal vascular remodeling can lead to prolonged placental oxidative stress, thus leading to trophoblast dysfunction, which is a

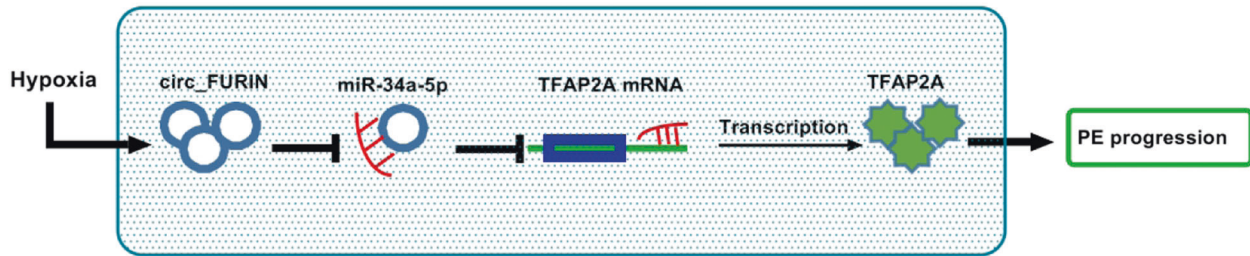
hallmark of PE [7]. Therefore, revealing the pathophysiological mechanism underlying the abnormal migration and invasion of EVT is crucial for PE therapy.

Circular RNAs (circRNAs) are a class of noncoding RNAs (ncRNAs) that possess covalently closed loop structures [8]. CircRNAs have attracted considerable attention for their actions in diverse human disorders [9]. CircRNAs perform their functions in various ways, such as by acting as microRNA (miRNA) sponges and altering the levels of miRNAs that bind to target mRNAs [10]. Previously, some studies indicated that circRNAs are related to PE pathogenesis. For instance, circSFXN1 suppresses trophoblast invasion and might be related to PE development [11]. Circ_0085296 inhibits the growth and metastasis of trophoblasts by elevating E-cadherin expression through sponging miR-144 [12]. These reports suggest that circRNAs participate in PE development. Moreover, the circRNA furin, paired basic amino acid cleaving enzyme (circ_FURIN, also named hsa_circ_0036877), was discovered to exhibit elevated expression and might be a blood

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Graphical Abstract



biomarker for PE [13]. However, the roles of circ_FURIN in PE have rarely been investigated.

MiRNAs are short ncRNAs and are associated with placental development [14]. The miRNA miR-34a-5p has emerged as a vital regulator in human diseases such as gastric cancer [15], Hirschsprung's disease [16], multiple myeloma [17], and ovarian cancer [18]. Moreover, Xue et al. reported that miR-34a-5p blocks the invasion and migration of trophoblast cells via Smad4 [19]. However, the related mechanisms of miR-34a-5p in PE need to be studied.

In this paper, our goal was to explore the functions and mechanisms of circ_FURIN in trophoblast cell behaviors, attempting to discover a therapeutic target for PE.

Materials and methods

Tissue acquisition

A total of 19 PE patients and 13 women with normal pregnancies at Shanxi Provincial People's Hospital were enrolled in this study. PE was defined according to the criteria of the ACOG Clinical Guidelines. Placental tissues were harvested from the umbilical cord in the central region of the maternal surface of placentas after delivery of the placenta. The samples were preserved at -80°C until further use. The research was approved by the Ethics Committee of Shanxi Provincial People's Hospital. Written informed consent was provided by the patients. The clinical characteristics of the normal controls and PE patients are shown in Table 1.

Cell culture and treatment

The human placental trophoblast cell line HTR-8/SVneo was acquired from Procell (Wuhan, China), and TEV-1 cells were purchased from Shanghai QinCheng Bio (Shanghai, China). These cells were cultured in RPMI 1640 medium (Procell) supplemented with 5% FBS (Procell) and 1% penicillin–streptomycin (Procell) at 37°C in a humidified incubator with 5% CO_2 .

To induce hypoxic conditions, cells were maintained in an incubator with 93% N_2 , 5% CO_2 and 2% O_2 for 2 h and then maintained for 6 h under normoxic conditions.

Quantitative real-time polymerase chain reaction (qRT–PCR)

After total RNA was extracted using TRIzol (Beyotime, Shanghai, China), M-MLV Reverse Transcriptase Reagent (Promega, Madison, WI, USA) or TaqMan MicroRNA Reverse Transcription Reagent (Applied Biosystems, Foster City, CA, USA) was employed for the synthesis of cDNAs. Next, cDNA amplification was conducted using SYBR Green Master Mix (Takara, Dalian, China). Expression values were calculated using the $2^{-\Delta\Delta\text{Ct}}$ method with GAPDH or U6 as the internal control. The primers used are listed in Table 2.

Subcellular fractionation analysis

Nuclear and cytoplasmic RNAs in HTR-8/SVneo and TEV-1 cells were separated by using Cytoplasmic & Nuclear RNA Purification Reagent (Norgen Biotek, Thorold, ON, Canada) based on the manufacturer's protocols. GAPDH and U6 were utilized as the controls for cytoplasmic and nuclear transcripts, respectively. The enrichment of circ_FURIN in the cytoplasm and nucleus was determined.

RNase R treatment

To analyze the circular structure of circ_FURIN, total RNA in HTR-8/SVneo and TEV-1 cells was treated with or without RNase R (Epicentre Biotechnologies, Madison, WI, USA). Next, circ_FURIN and FURIN levels were examined.

Cell transfection

The circ_FURIN overexpression vector (circ_FURIN) and empty control vector (Vector), miR-34a-5p mimic (miR-34a-5p) and negative control miR-NC, miR-34a-5p inhibitor (anti-miR-34a-5p) and related control anti-miR-NC, and transcription factor AP-2 alpha (TFAP2A) small

Table 1 Clinical characteristics of normal and PE patients

Parameters	Control (<i>n</i> = 13)	PE (<i>n</i> = 19)	<i>P</i> value
Maternal age at delivery(years)	28.5 ± 5.2	29.82 ± 4.1	0.8068
Gestational weeks	38.7 ± 0.8	35.2 ± 0.9	<0.01
Systolic blood pressure (mmHg)	117.6 ± 8.9	168.8 ± 7.9	<0.01
Diastolic blood pressure (mmHg)	75.6 ± 6.3	105.5 ± 8.9	<0.01
Fetal birth weight(g)	3592.3 ± 387.8	2573.7 ± 452.6	<0.05

Table 2 Primers sequences used for qRT-PCR

Name		Primers (5'–3')
Circ_FURIN (hsa_circ_0036877)	Forward	TGAGATAATGTTAGAGGTTTT
	Reverse	CTGTAGATGTGGATGTGGTTG
FURIN	Forward	ACGGTGTCTGTGGTGTAGGTGTG
	Reverse	TAGATGTGGATGTGGTTGGGGTT
TFAP2A	Forward	GGCGAAGTCTAAAAATGGAGGAA
	Reverse	CTAGTGATGTGAGCAGGGTAACG
miR-34a-5p	Forward	GTATGATGGCAGTGTCTTAG
	Reverse	TGGTTCGTGGAGTCGT
GAPDH	Forward	AAGGCTGTGGGCAAGGTCATC
	Reverse	GCGTCAAAGGTGGAGGAGTGG
U6	Forward	CTCGCTTCGGCAGCACATA
	Reverse	CGAATTTGCGTGTATCCT

interfering RNA (si-TFAP2A) and scrambled control (si-NC) were provided by GenePharma Co., Ltd. (Shanghai, China). Cell transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Cell proliferation assay

Cell proliferation was examined with a CCK-8 assay kit (Beyotime) and 5'-ethynyl-2'-deoxyuridine (EdU) detection reagent (RiboBio, Guangzhou, China).

For the CCK-8 assay, HTR-8/SVneo and TEV-1 cells were aliquoted into 96-well plates and incubated for 24 h, and then 10 µL of CCK-8 reagent was added. The absorbance at 450 nm was measured using a microplate reader (Olympus, Tokyo, Japan) after 4 h of incubation.

For the EdU incorporation assay, HTR-8/SVneo and TEV-1 cells were seeded into 24-well plates and maintained with EdU for 12 h. Next, the cells were fixed with 4% paraformaldehyde (Sigma–Aldrich, St. Louis, MO, USA) and incubated with 0.5% Triton-X-100 (Sigma–Aldrich). Nuclei were then stained with DAPI, and EdU-positive cells were observed under a fluorescence microscope (Olympus).

Transwell assays

To analyze the cell migration ability, trophoblast cells were suspended in serum-free medium and plated into the upper compartments of Transwell chambers (BD Biosciences, San Jose, CA, USA). The lower chambers were filled with complete culture medium. After culture for 24 h, the cells

that adhered to the bottom surface of the insert membranes in the chambers were stained with crystal violet (Sigma–Aldrich) and estimated under a microscope (Olympus) at 100×. For assessment of the cell invasion ability, the upper chambers were precoated with Matrigel (BD Biosciences).

Western blot analysis

Total protein was isolated using RIPA buffer (Beyotime) and then separated by SDS–PAGE. Next, proteins were transferred to PVDF membranes and blocked for 2 h in 5% skim milk. Subsequently, the membranes were incubated overnight with primary antibodies against MMP2 (ab97779), MMP9 (ab38898), TFAP2A (ab52222) or GAPDH (ab9485) and then for 2 h with a secondary antibody (ab2302). Protein bands were imaged via an ECL system (Beyotime). All antibodies were purchased from Abcam (Cambridge, MA, USA).

Dual-luciferase reporter assay

The sequences of circ_FURIN and TFAP2A 3'UTR containing the wild-type (WT) or mutant (MUT) binding sites for miR-34a-5p were cloned and inserted into pmirGLO (Promega, Fitchburg, WI, USA) to generate the dual-luciferase reporter vectors, which were named circ_FURIN-WT, circ_FURIN-MUT, TFAP2A-WT and TFAP2A-MUT. Next, the vectors were transfected into HTR-8/SVneo and TEV-1 cells together with miR-34a-5p/miR-NC. Luciferase intensity was examined using dual-luciferase reporter assay reagent (Promega).

RNA pulldown assay

Biotin-labeled probes for WT and mutated circ_FURIN/TRAF2A (Bio-circ_FURIN-WT/Bio-circ_FURIN-MUT and Bio-TRAF2A-WT/Bio-TRAF2A-MUT) were incubated with streptavidin-coated magnetic beads to generate probe-coated beads. Then, HTR-8/SVneo and TEV-1 cells were incubated with the beads, and RNA was isolated from the beads. The expression of miR-34a-5p was detected.

Statistical analysis

Experiments were conducted three times. The obtained data were analyzed using GraphPad Prism 7 and are presented as the mean \pm SD values. Linear correlations were estimated with the Spearman correlation coefficients. Difference analysis was performed via Student's *t* test or one-way ANOVA. Differences with $P < 0.05$ were considered significant.

Results

Circ_FURIN was downregulated in PE placental specimens and trophoblast cells under hypoxic conditions

To explore the function of circFURIN in PE, the expression of circ_FURIN in the placentas of PE patients and healthy controls was determined by qRT-PCR. The results showed that circ_FURIN was aberrantly downregulated in PE placentas compared to normal control placentas (Fig. 1A). Moreover, circ_FURIN was expressed at low levels in HTR-8/SVneo and TEV-1 cells after hypoxia exposure (Fig. 1B). Circ_FURIN, also named hsa_circ_0036877, is derived from exons 8-16 of the FURIN gene (Fig. 1C). As indicated by subcellular fractionation analysis, circ_FURIN was mainly enriched in the cytoplasm and not the nucleus of HTR-8/SVneo and TEV-1 cells (Fig. 1D, E). In addition, the RNase R digestion assay indicated that circ_FURIN was resistant to RNase R treatment but that FURIN was digested

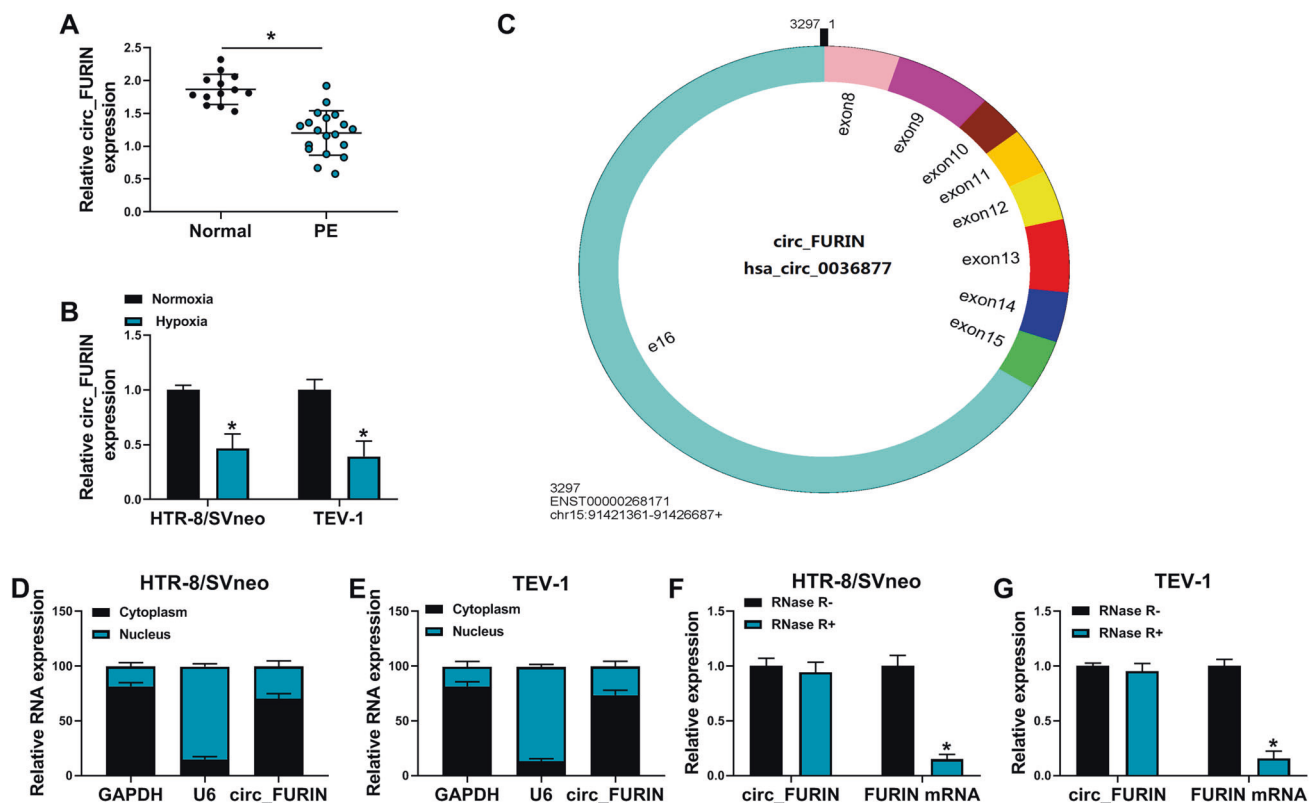


Fig. 1 Circ_FURIN was downregulated in PE patients and hypoxia-treated trophoblast cells. **A** The expression of circ_FURIN in placental specimens of PE patients and normal controls was detected by qRT-PCR. **B** The expression of circ_FURIN in HTR-8/SVneo and TEV-1 cells under normoxic and hypoxic conditions was detected by

qRT-PCR. **C** Circ_FURIN is derived from the FURIN gene. **D, E** The expression of circ_FURIN in the cytoplasm and nucleus of HTR-8/SVneo and TEV-1 cells was determined by qRT-PCR. **F, G** After RNase R treatment, the expression of circ_FURIN and FURIN in HTR-8/SVneo and TEV-1 cells was detected by qRT-PCR. * $P < 0.05$

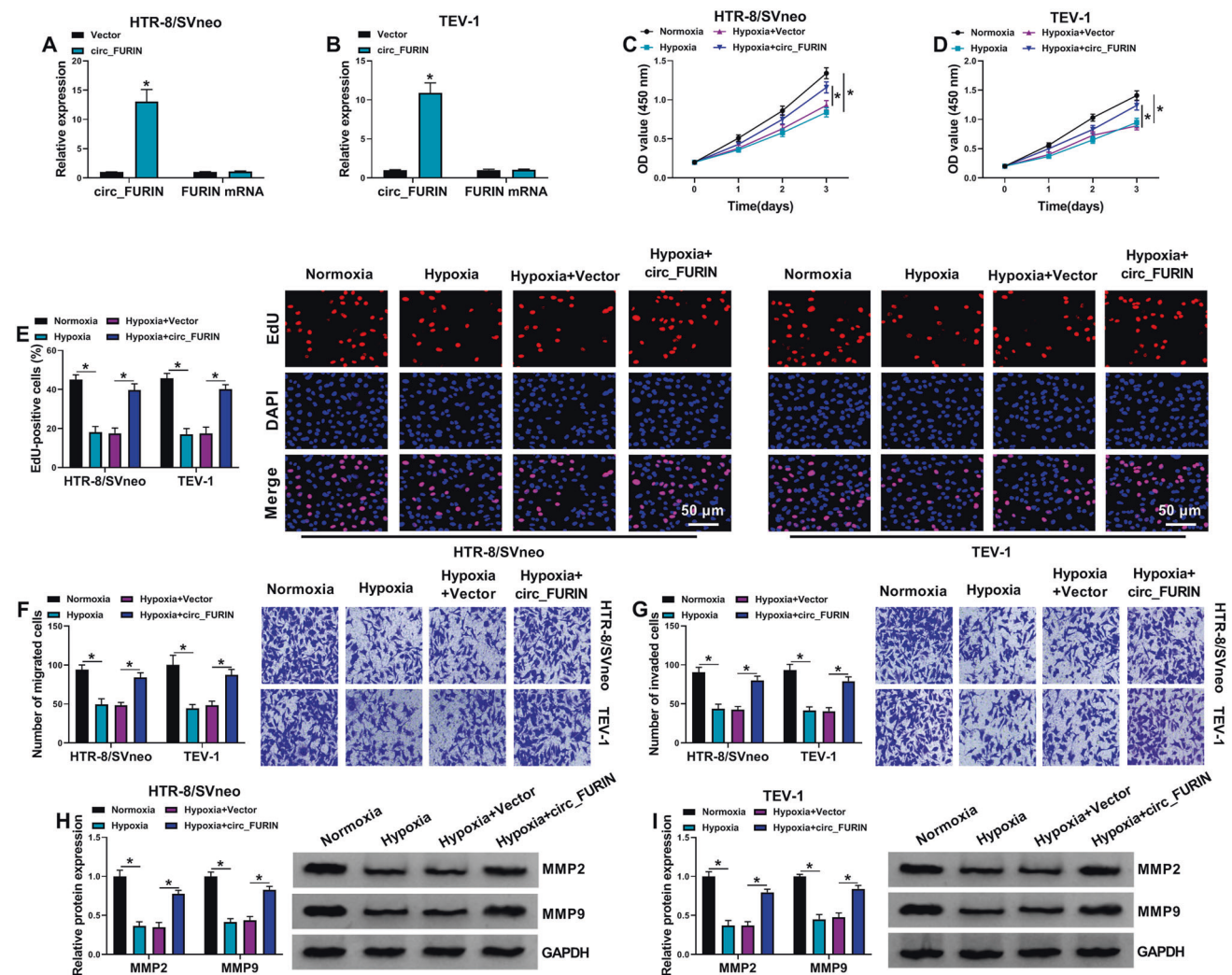


Fig. 2 Overexpression of circ_FURIN promoted hypoxia-mediated effects on trophoblast cell proliferation, migration and invasion. **A, B** The levels of circ_FURIN and FURIN in HTR-8/SVneo and TEV-1 cells transfected with Vector or circ_FURIN were measured by qRT-PCR. **C–E** HTR-8/SVneo and TEV-1 cells were treated with normoxia, hypoxia, hypoxia+Vector or hypoxia+circ_FURIN.

by RNase R (Fig. 1F, G). These findings indicated that the abnormal expression of circ_FURIN might be related to PE.

Circ_FURIN overexpression promoted the proliferation, migration and invasion of trophoblast cells under hypoxic conditions

To explore the exact roles of circ_FURIN in PE development, HTR-8/SVneo and TEV-1 cells were transfected with the circ_FURIN overexpression vector to elevate circ_FURIN expression. As shown in Fig. 2A, B, circ_FURIN overexpression vector transfection led to marked elevation of circ_FURIN expression but did not affect FURIN expression in HTR-8/SVneo and TEV-1 cells. The CCK-8 and EdU incorporation assays showed that the proliferation ability of

HTR-8/SVneo and TEV-1 cells was reduced after hypoxia exposure, while this effect was abrogated by circ_FURIN overexpression (Fig. 2C–E). The Transwell assays suggested that hypoxia exposure markedly inhibited the migration and invasion of HTR-8/SVneo and TEV-1 cells, while circ_FURIN enhancement ameliorated these effects (Fig. 2F, G). In addition, we measured the protein levels of EMT markers (including MMP2 and MMP9) via western blot analysis. The results showed that the protein levels of MMP2 and MMP9 were decreased in HTR-8/SVneo and TEV-1 cells exposed to hypoxia, whereas these impacts were reversed by upregulation of circ_FURIN (Fig. 2H, I). Taken together, these findings indicated that circ_FURIN promoted trophoblast cell proliferation, migration and invasion under hypoxic conditions.

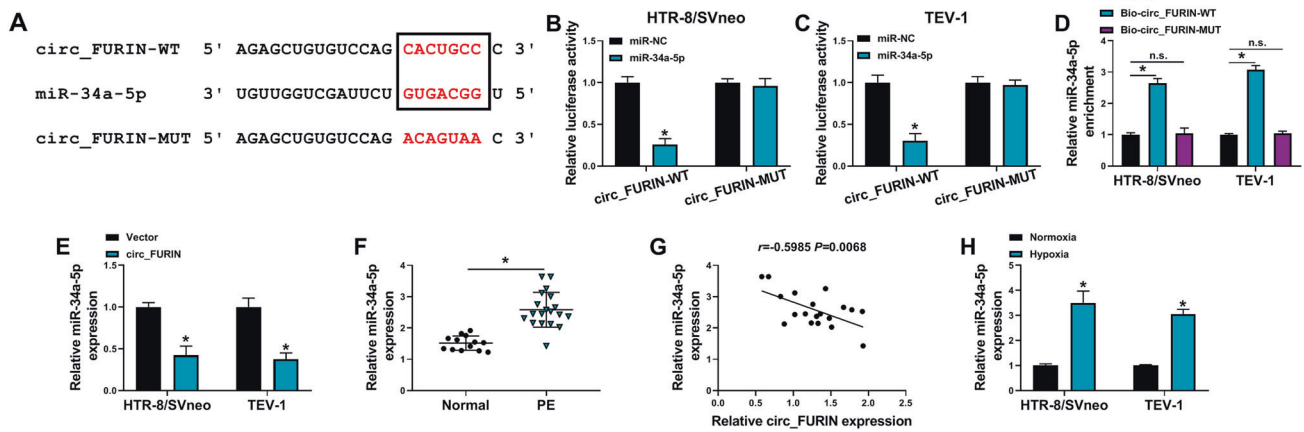


Fig. 3 MiR-34a-5p was identified as the target of circ_FURIN. **A** The complementary sequences in circ_FURIN and miR-34a-5p. **B–D** The binding relationship between circ_FURIN and miR-34a-5p was analyzed by dual-luciferase reporter and RNA pull-down assays. **E** The expression of miR-34a-5p in HTR-8/SVneo and TEV-1 cells transfected with circ_FURIN or Vector was determined by qRT-PCR.

F The expression of miR-34a-5p in the placental specimens of PE patients and healthy controls was examined by qRT-PCR. **G** MiR-34a-5p expression was negatively correlated with circ_FURIN expression in PE patients. **H** The expression of miR-34a-5p in HTR-8/SVneo and TEV-1 cells after normoxia or hypoxia exposure was determined by qRT-PCR. * $P < 0.05$

Circ_FURIN directly targeted miR-34a-5p

To validate the underlying mechanism of circ_FURIN in trophoblast cell progression in PE, the online prediction website StarBase (<https://starbase.sysu.edu.cn/starbase2/>) was used to analyze the potential miRNA targets of circ_FURIN. As shown in Fig. 3A, miR-34a-5p contains binding sites for circ_FURIN. The dual-luciferase reporter assay showed that luciferase activity in HTR-8/SVneo and TEV-1 cells was markedly inhibited by miR-34a-5p and circ_FURIN-WT cotransfection but was not affected by miR-34a-5p and circ_FURIN-MUT cotransfection (Fig. 3B, C). The RNA pull-down assay showed that the Bio-circ_FURIN-WT probe pulled down more miR-34a-5p than the Bio-NC control probe (Fig. 3D). Overexpression of circ_FURIN drastically reduced the miR-34a-5p level in HTR-8/SVneo and TEV-1 cells compared to the corresponding vector-transfected cells (Fig. 3E). Indeed, miR-34a-5p was upregulated in PE placental specimens compared to normal controls (Fig. 3F). Moreover, there was an inverse correlation between the levels of miR-34a-5p and circ_FURIN in PE patients (Fig. 3G). After hypoxia exposure, the miR-34a-5p level was notably increased in HTR-8/SVneo and TEV-1 cells (Fig. 3H). Collectively, these results indicated that circ_FURIN directly bound to miR-34a-5p to regulate miR-34a-5p expression.

Circ_FURIN overexpression promoted the proliferation, migration, and invasion of trophoblast cells under hypoxic conditions by targeting miR-34a-5p

Subsequently, the relationship between circ_FURIN and miR-34a-5p in the regulation of trophoblast cell progression was

explored. As shown in Fig. 4A, circ_FURIN overexpression markedly decreased miR-34a-5p expression in HTR-8/SVneo and TEV-1 cells, while miR-34a-5p mimic transfection abrogated this effect. The CCK-8 and EdU incorporation assays indicated that overexpression of circ_FURIN promoted hypoxia-mediated effects on HTR-8/SVneo and TEV-1 cell proliferation, while miR-34a-5p upregulation inhibited this promotion (Fig. 4B–D). The Transwell assays showed that circ_FURIN overexpression contributed to the migration and invasion of HTR-8/SVneo and TEV-1 cells under hypoxic conditions, while these effects were weakened by increasing miR-34a-5p expression (Fig. 4E, F). In addition, miR-34a-5p overexpression reversed the effects of circ_FURIN on the MMP2 and MMP9 protein levels in HTR-8/SVneo and TEV-1 cells under hypoxic conditions (Fig. 4G, H). Collectively, these findings indicated that circ_FURIN overexpression promoted hypoxia-mediated effects on trophoblast cell proliferation, migration and invasion by targeting miR-34a-5p.

TFAP2A was the target gene of miR-34a-5p

By a further search of StarBase, TFAP2A was found to be the target gene of miR-34a-5p, and its binding sites are shown in Fig. 5A. The dual-luciferase reporter assay showed that miR-34a-5p mimic transfection suppressed the luciferase activity of TFAP2A-WT but did not affect the luciferase activity of TFAP2A-MUT in HTR-8/SVneo and TEV-1 cells (Fig. 5B, C). The RNA pull-down assay showed that the enrichment of miR-34a-5p was increased by the Bio-TFAP2A-WT probe compared to the Bio-NC control probe (Fig. 5D). These results suggested the interaction between miR-34a-5p and TFAP2A. Then, the miR-34a-5p mimic or anti-miR-34a-5p was transfected into HTR-8/SVneo and TEV-1 cells to elevate or reduce

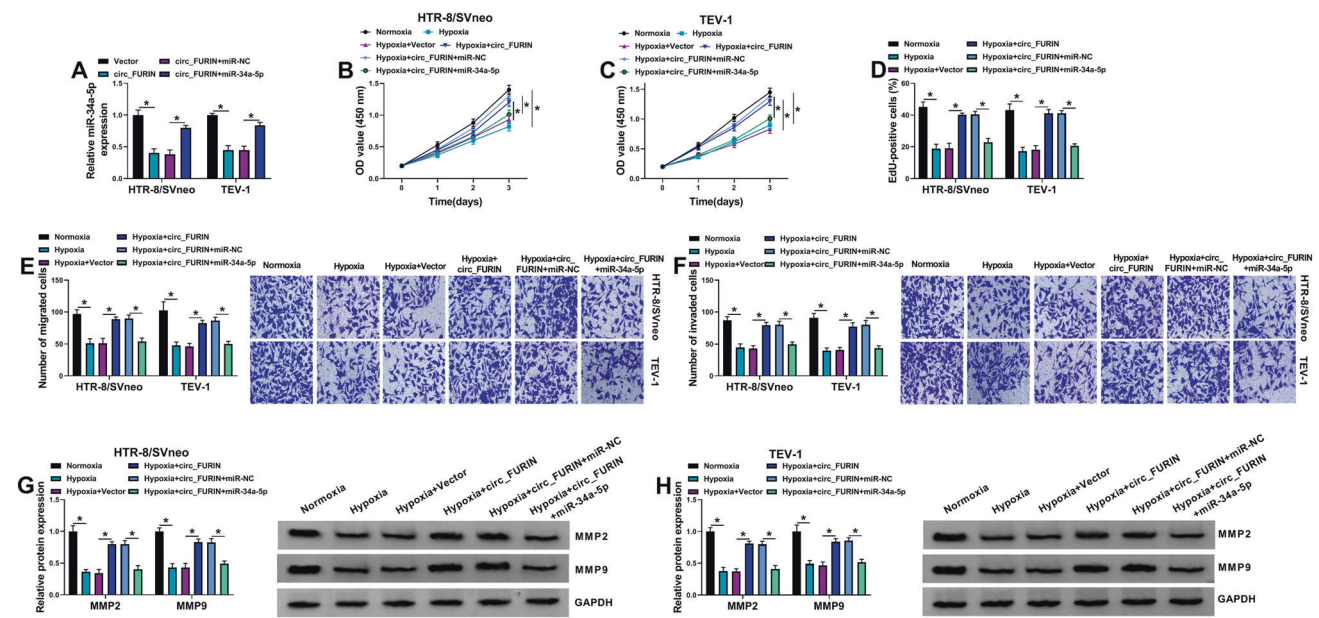


Fig. 4 Circ_FURIN regulated trophoblast cell growth, migration and invasion under hypoxic conditions by targeting miR-34a-5p. **A** The expression of miR-34a-5p in HTR-8/SVneo and TEV-1 cells transfected with Vector, circ_FURIN, circ_FURIN + miR-NC or circ_FURIN + miR-34a-5p was determined by qRT-PCR. **B–H** HTR-8/SVneo and TEV-1 cells were treated with normoxia, hypoxia, hypoxia + Vector, hypoxia + circ_FURIN, hypoxia + circ_FURIN + miR-NC or

hypoxia + circ_FURIN + miR-34a-5p. **B–D** The proliferation of HTR-8/SVneo and TEV-1 cells was assessed by CCK-8 and EdU incorporation assays. **E, F** The migration and invasion of HTR-8/SVneo and TEV-1 cells were estimated by Transwell assays. **G, H** The protein levels of MMP2 and MMP9 in HTR-8/SVneo and TEV-1 cells were measured by western blot analysis. * $P < 0.05$

miR-34a-5p expression, respectively, which was demonstrated by qRT-PCR (Fig. 5E). Our results showed that miR-34a-5p overexpression reduced the TFAP2A mRNA and protein levels and that miR-34a-5p inhibition increased the TFAP2A mRNA and protein levels in HTR-8/SVneo and TEV-1 cells (Fig. 5F, G). As expected, TFAP2A mRNA and protein expression was downregulated in PE placental specimens in comparison with normal controls (Fig. 5H, I). The results of Spearman correlation analysis showed that in PE patients, the TFAP2A mRNA level was negatively correlated with the miR-34a-5p level and positively correlated with the circ_FURIN level (Fig. 5J, K). Furthermore, we observed that hypoxia exposure led to reductions in the TFAP2A mRNA and protein levels in HTR-8/SVneo and TEV-1 cells (Fig. 5L, M). Notably, circ_FURIN overexpression increased the mRNA and protein levels of TFAP2A in HTR-8/SVneo and TEV-1 cells, while miR-34a-5p mimic transfection abolished these effects (Fig. 5N, O). Taken together, these results indicated that circ_FURIN positively modulated TFAP2A expression by targeting miR-34a-5p.

MiR-34a-5p inhibition promoted trophoblast cell proliferation, migration, and invasion under hypoxic conditions by targeting TFAP2A

The results in Fig. 6A, B show that anti-miR-34a-5p transfection increased the mRNA and protein levels of TFAP2A in HTR-8/SVneo and TEV-1 cells, while these

effects were suppressed by silencing TFAP2A. Thereafter, the effect of miR-34a-5p and TFAP2A on trophoblast cell development was explored. The CCK-8 and EdU incorporation assays indicated that miR-34a-5p inhibition contributed to the proliferation of HTR-8/SVneo and TEV-1 cells under hypoxic conditions, while this effect was weakened by downregulating TFAP2A (Fig. 6C–E). The Transwell assays showed that the migration and invasion of HTR-8/SVneo and TEV-1 cells under hypoxic conditions were promoted by miR-34a-5p inhibition and that TFAP2A knockdown alleviated these effects (Fig. 6F, G). In addition, miR-34a-5p inhibition increased the levels of MMP2 and MMP9 in HTR-8/SVneo and TEV-1 cells under hypoxic conditions, whereas TFAP2A silencing abolished these increases (Fig. 6H, I). In summary, miR-34a-5p inhibition promoted trophoblast cell progression under hypoxic conditions by binding to TFAP2A.

Discussion

Poor migration and invasion of trophoblast cells are thought to be the main causes of PE [20]. Damage to and deformation of placental trophoblast cells and inadequate remodeling of the maternal spiral artery lead to placental ischemia and hypoxia, which are the first steps in PE development [6]. Thus, exploring the underlying mechanism of placental trophoblast cell invasion is crucial for PE

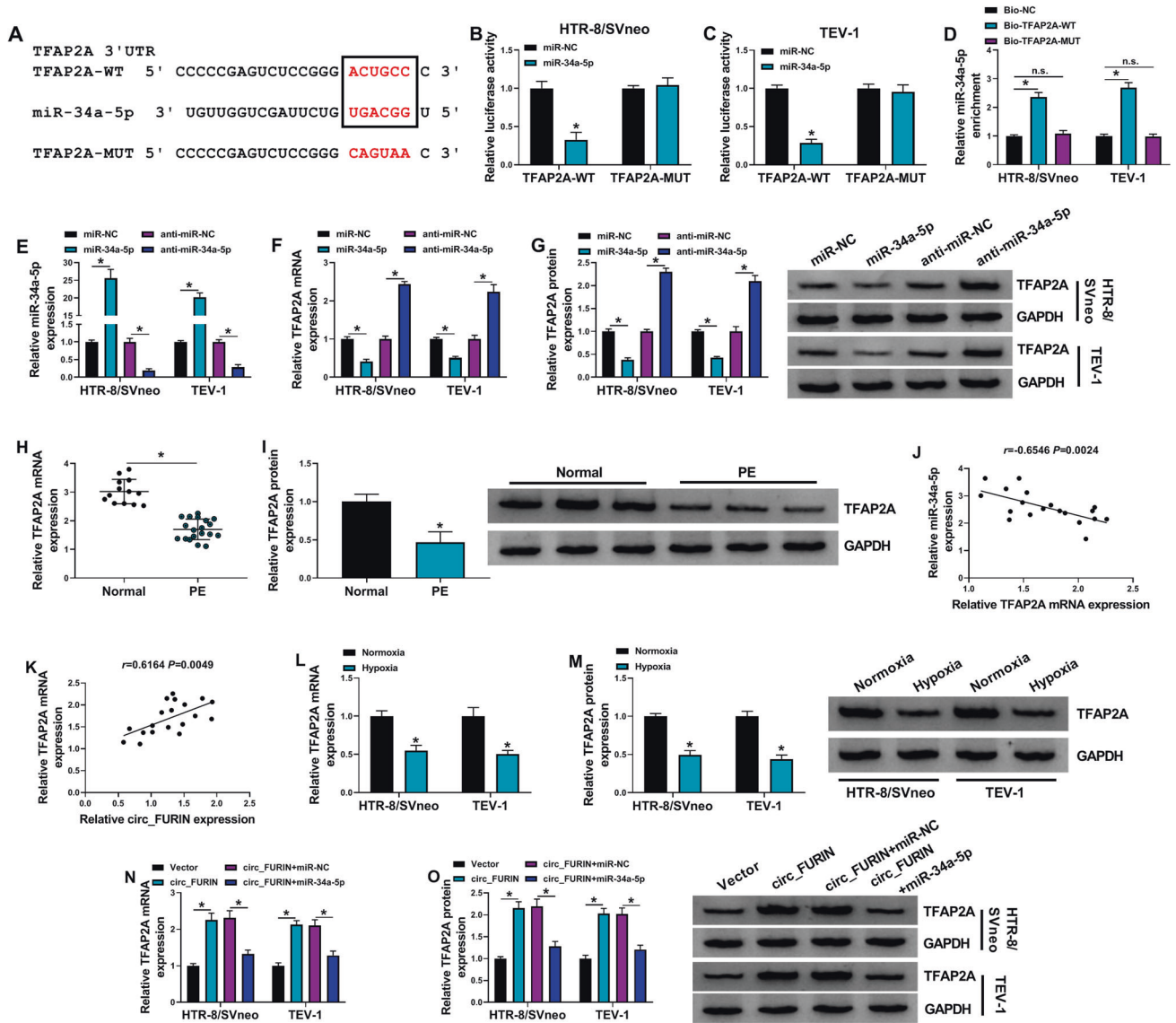


Fig. 5 MiR-34a-5p interacted with TFAP2A. **A** TFAP2A contains the binding sites for miR-34a-5p. **B–D** The association between miR-34a-5p and TFAP2A expression was analyzed by dual-luciferase reporter and RNA pull-down assays. **E–G** After HTR-8/SVneo and TEV-1 cells were transfected with miR-NC, miR-34a-5p, anti-miR-NC or anti-miR-34a-5p, the expression of miR-34a-5p, TFAP2A mRNA and TFAP2A protein was evaluated by qRT-PCR or western blot analysis. **H, I** The mRNA and protein levels of TFAP2A in PE placental specimens and normal placental specimens were measured by qRT-PCR

or western blot analysis. **J, K** The linear correlations of TFAP2A mRNA expression with miR-34a-5p and circ_FURIN expression were estimated. **L, M** The mRNA and protein levels of TFAP2A in HTR-8/SVneo and TEV-1 cells under normoxic and hypoxic conditions were quantified. **N, O** After HTR-8/SVneo and TEV-1 cells were transfected with Vector, circ_FURIN, circ_FURIN + miR-NC or circ_FURIN + miR-34a-5p, the mRNA and protein levels of TFAP2A were measured. $*P < 0.05$

treatment. To date, relations between circRNAs and PE have been gradually identified [21]. In this study, we found using hypoxia-induced trophoblast cell models that circ_FURIN contributed to PE progression via the miR-34a-5p/TFAP2A pathway.

CircRNAs participate in cell migration and invasion in multiple diseases [22, 23]. Moreover, in PE, several circRNAs, such as circPAPPA [24], CircHIPK3 [25], circTRNC18 [26] and circ_0111277 [27], are involved in trophoblast cell proliferation, invasion and migration. In this

study, circ_FURIN expression was found to be decreased in PE patients and trophoblast cells under hypoxic conditions. Therefore, we further investigated the exact roles of circ_FURIN in PE. Our findings demonstrated that enhancement of circ_FURIN expression promoted the growth, migration and invasion of PE trophoblast cells under hypoxic conditions.

To date, circRNA/miRNA/mRNA regulatory axes in biological processes have gradually been reported. For example, circ-ITCH decelerates the development of

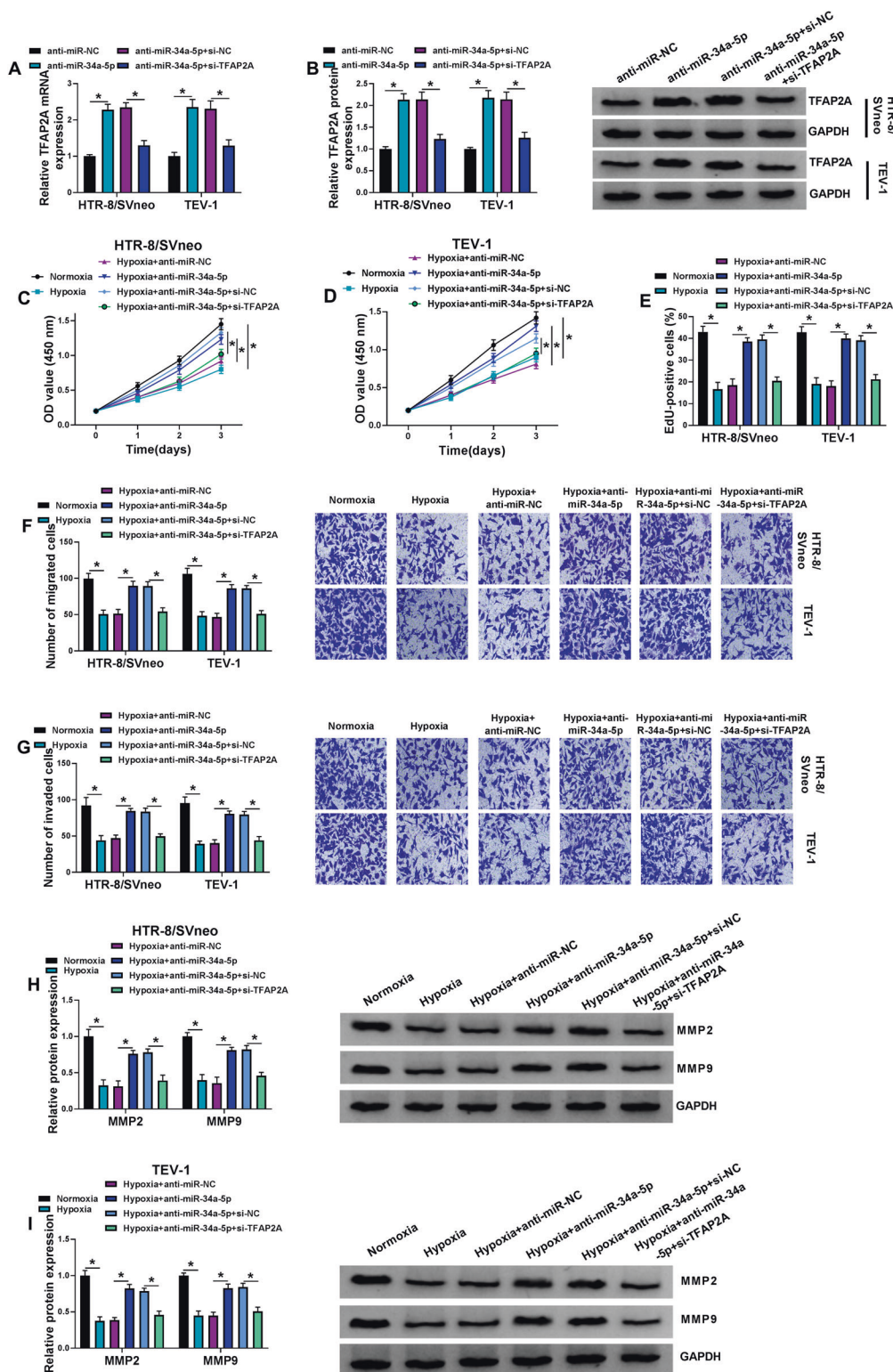
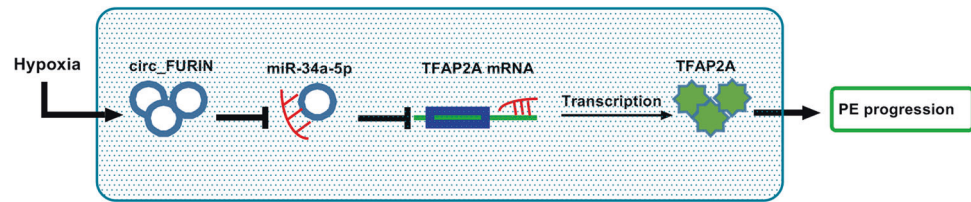


Fig. 6 MiR-34a-5p inhibition promoted the proliferation, migration and invasion of trophoblast cells under hypoxic conditions by targeting TFAP2A. **A, B** After HTR-8/SVneo and TEV-1 cells were transfected with anti-miR-NC, anti-miR-34a-5p, anti-miR-34a-5p+si-NC or anti-miR-34a-5p+si-TFAP2A, TFAP2A mRNA and protein levels were determined. **C–E** HTR-8/SVneo and TEV-1 cells were treated with normoxia, hypoxia, hypoxia+anti-miR-NC, hypoxia+anti-miR-34a-5p,

hypoxia+anti-miR-34a-5p+si-NC or hypoxia+anti-miR-34a-5p+si-TFAP2A. **C–E** The proliferation of HTR-8/SVneo and TEV-1 cells was assessed by CCK-8 and EdU incorporation assays. **F, G** The migration and invasion of HTR-8/SVneo and TEV-1 cells were assessed by Transwell assays. **H, I** The protein levels of MMP2 and MMP9 in HTR-8/SVneo and TEV-1 cells were measured via western blot analysis. * $P < 0.05$

Fig. 7 Diagram showing that hypoxia-induced circ_FURIN promoted PE progression via the miR-34a-5p/TFAP2A axis



nasopharyngeal carcinoma by altering miR-214 and PTEN [28]. Circ_0007534 exacerbates glioma malignancy via the miR-22-3p/PROX1 axis [29]. Herein, the underlying mechanism of circ_FURIN in PE was further investigated. It was discovered that circ_FURIN sponged miR-34a-5p. MiR-34a-5p was upregulated in PE, and miR-34a-5p inhibition facilitated trophoblast cell proliferation, migration and invasion under hypoxic conditions, consistent with the results in a previous report [19]. Moreover, we demonstrated that circ_FURIN overexpression facilitated trophoblast cell progression by sponging miR-34a-5p.

TFAP2A is a bifunctional Ra-induced transcription factor and is a member of the AP-2-alpha family [30]. The involvement of TFAP2A in PE has been demonstrated. Previous studies have indicated that TFAP2A (also called AP-2 and AP-2alpha) inhibits the proliferation, invasion and migration of trophoblast cells in PE [31–33]. In this work, TFAP2A was verified to be the target gene of miR-34a-5p. Of note, TFAP2A silencing reversed the impacts of miR-34a-5p inhibition on trophoblast cell behaviors.

Taken together, these findings suggest that circ_FURIN contributes to trophoblast cell proliferation, migration and invasion by sponging miR-34a-5p and elevating TFAP2A expression, thereby inhibiting PE progression (Fig. 7). This research provides novel insight into PE pathogenesis and might identify a novel therapeutic target for PE.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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