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GATA1-mediated macrophage polarization via TrkB/cGMP–PKG signaling pathway to promote the development of preeclampsia

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Abstract

Background Preeclampsia (PE) is a severe pregnancy complication characterized by hypertension and proteinuria. PE poses a substantial threat to the health of both mothers and fetuses, and currently, there is no definitive treatment available. Recent studies have indicated that the transcription factor GATA1 may be implicated in the pathological processes of PE, but the underlying mechanism remains elusive. NTRK2/cGMP–PKG signaling pathway plays a crucial role in regulating the function and polarization of macrophages, which are key immune cells at the maternal–fetal interface. This study aims to investigate the role of GATA1 in the pathogenesis of PE, with a specific focus on how GATA1-regulated TrkB/cGMP–PKG signaling in macrophages and its dysregulation contribute to the development of preeclampsia.

Methods By employing THP-1 cells, co-culture systems of THP-1 cells and HTR-8/Svneo, HPVECs and Sprague–Dawley (SD) rats, in conjunction with gene knockdown and overexpression techniques, we explored the effects of GATA1 on the TrkB/cGMP–PKG signaling pathway. Transcriptomic sequencing, bioinformatics analysis, animal experiments, and clinical sample collection were conducted to validate the role of GATA1 in PE.

Results Knockdown of GATA1 mitigated the symptoms of PE, and this effect was reversed by overexpression of TrkB. In comparison with the control group, the proportion of M2 cells elevated significantly in the sh-GATA1 group ($P < 0.001$). In addition, the protein expressions levels of TrkB, cGMP, and PKG were significantly decreased in the sh-GATA1 group were significantly decreased compared with those in the control group ($P < 0.001$, $P < 0.001$, $P < 0.001$, $P < 0.05$, respectively). Moreover, knockdown of GATA1 significantly promoted the migration rate and blood vessel formation of HTR-8/Svneo cells ($P < 0.001$, $P < 0.05$, respectively) which inhibited by overexpression of NTRK2 ($P < 0.05$, $P < 0.01$, respectively).

Conclusions The study demonstrated that knockdown of GATA1 modulates M2 polarization of macrophage through the TrkB/cGMP–PKG signaling pathway, influencing the progression of PE. In addition, significant associations between GATA1 and the TrkB/cGMP–PKG signaling pathway were identified in the transcriptomic data from PE patient placentas.

Keywords GATA1, Knockdown, NTRK2, TrkB, cGMP–PKG, Macrophage, Vascularization

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Introduction

Preeclampsia (PE), a prevalent pregnancy complication affecting 2–8% of pregnant women, is manifested by hypertension, proteinuria, and edema, posing a serious threat to maternal and fetal well-being [1, 2]. The pathogenesis of PE is complex, involving genetic, immune, and environmental factors. Uncovering the molecular and cellular mechanisms underlying PE has become a focal point and challenge in obstetric research [3].

Macrophage polarization has emerged as a crucial factor in the progression of various diseases, particularly in the context of inflammatory and immune responses during pregnancy. Macrophages are multifunctional immune cells that maintain internal homeostasis and defend against pathogens, thereby playing a crucial role in immune responses and tissue repair. They can be polarized into M1 (pro-inflammatory) and M2 (anti-inflammatory and tissue-repairing) subtypes [4]. Studies have demonstrated that dysregulation of macrophage polarization, with a novel macrophage subgroup identified in PE patients that is enriched around the spiral arteries and exhibits a pro-inflammatory phenotype, plays a pivotal role in the initiation of PE [5, 6].

Trophoblastic cells are smaller cells that start to differentiate, extend, and arrange along the inner wall of the zona pellucida as the morula further develops from the embryonic stage [7]. In PE, macrophage polarization shows a proinflammatory signature and a disturbed crosstalk with trophoblasts [8, 9]. However, the specific molecular pathways and key genes involved in and regulating this process remain unclear.

GATA Binding Protein 1 (GATA1), initially studied for its role in red blood cell maturation and the hematopoietic system, has relationship with macrophages has gradually been revealed [10]. Studies have shown that GATA1 is a key transcription factor controlling the expression of the macrophage colony-stimulating factor receptor gene locus, and by expressing the corresponding receptor, macrophages can perform different functions [11]. In addition, GATA1 can activated transcription of Neurokinin-B (NK-B), and the activation of NK-B is closely related to PE [12]. Thus, GATA1 may be a new target of PE, but there are currently few studies relating to GATA1 and PE.

Tyrosine kinase receptor B (TrkB), also known as neurotrophic tyrosine receptor kinase 2 (NTRK2), is a member of the tyrosine protein kinase receptor family and plays an important role in the growth, survival, and differentiation of nerve cells. Recent studies have shown that the expression of TrkB is significantly elevated in placental tissue in PE patients, which suggests that TrkB may have a positive regulatory role playing in the process of PE [13]. TrkB, mainly expressed in the nervous system but also significant in macrophage polarization in peripheral organs, can inhibit M2 polarization and modulate cGMP levels, which in turn activates PKG and affects macrophage function [6, 14, 15]. However, in macrophages of PE patients, it is unclear whether GATA1 can affect the polarization of macrophages and the development of PE by regulating TrkB and its specific mechanisms. The cGMP–PKG signaling pathway is a classical inflammatory pathway that promotes M1 polarization of macrophages when it is overactivated [16]. We speculate that knockdown of GATA1 downregulates TrkB, which in turn promote M2 polarization of macrophages via inhibiting cGMP–PKG signaling pathway. The cGMP–PKG signaling pathway is a classical inflammatory pathway that promotes M1 polarization of macrophages when it is overactivated [16].

In this study, we constructed in vitro and in vivo models of PE utilizing SD rats, THP-1 cells, HTR-8/Svneo cells, and HPVECs. Our aim was to explore the underlying mechanisms by knocking down GATA1 and overexpressing NTRK2 in macrophages, utilizing a combination of transcriptome sequencing, bioinformatics analysis, and molecular biology experiments. Through a combination of transcriptome sequencing, bioinformatics analysis, and molecular biology experiments, we demonstrated that knockdown of GATA1 in macrophages can promote M2 polarization of macrophages by downregulating TrkB/cGMP–PKG signaling pathway, thereby inhibiting PE. This study aims to provide new therapeutic targets and data support for the treatment of PE.

Materials and methods

Cells cultivation and treatment

THP-1, HTR-8/Svneo, HPVECs, and HEK-293 T cell lines were procured from Wuhan Punoax Biotechnology Co., Ltd. (Catalog numbers: CL-0233, CL-0765, CP-H082, and CL-0005, respectively). For HTR-8/Svneo cells, HPVEC cells, and 293 T cells, which are all cultured in an adherent manner, the culture medium composition comprises 89% DMEM (11,965,118, Gibco, America), 10% FBS (FSD500, Excell Bio, China), and 1% PS (C0222, Beyotime, China). At 80% confluence, the medium was replaced, and cells were washed twice with 2 ml phosphate-buffered saline (PBS, C0221A, Beyotime, China). Add 2 ml 0.25% trypsin-0.02% EDTA mixture (R001100, Gibco, China), observe cell rounding for ~ 1 min. Add 4 ml complete medium to stop digestion, pipette cells gently. Centrifuge at 800 rpm for 5 min at 4 °C, discard supernatant, resuspend in medium. Subculture or seed cells for further use.

For THP-1 cells (grown in suspension culture), the culture medium consisted of 90% RPMI 1640 (11,875,093, Gibco, China), 10% of FBS, 1% of PS, and 0.05 mM

2-mercaptoethanol (HY-Y0326, MCE, China). At 80% confluence, 100 nM of phorbol 12-myristate 13-acetate (PMA, HY-18739, MCE, China) was added to culture medium to treat THP-1 cells for 24 h. Following this, cells were observed under a microscope. Through morphological observation, we found that THP-1 has been successfully transformed into M0 type macrophages, whose morphological features showed enlarged cell size, abundant cytoplasm and irregular edges. Subsequently, 10 ng/mL of LPS (L2630, Merck, Germany) was employed to promote M1 type polarization in M0 THP-1 cells. In subsequent experiments of co-culture system, THP-1 cells after LPS treatment were used to investigate the effect of GATA1 knockdown on PE.

In co-culture experiments where the target for detection is THP-1 cells, transwell inserts with a 3 μm pore size (model: 3415, CORNING, USA) were used. Activated THP-1 in lower chamber, HTR-8/Svneo in upper. For HTR-8/Svneo or HPVECs as target, activated THP-1 in upper, target cells in lower, utilizing same Transwell insert for co-culture. For Transwell assays, Transwell inserts with an 8 μm pore size (model: 3422, CORNING, USA) are employed, with activated THP-1 cells in the lower chamber and HTR-8/Svneo cells in the upper chamber.

Activated THP-1 cells by LPS were divided into Control and sh-GATA1 groups. The Control group was left, while the sh-GATA1 group had GATA1 expression knocked down through RNAi technology.

A lentiviral vector was utilized to introduce a mutated NTRK2 gene into the HEK 293 T cells, thereby generating HEK 293 T cells with NTRK2–MUT genotype. Wild-type and NTRK2–MUT HEK 293 T cells were then divided into sh-NC and sh-GATA1 groups; the sh-NC group was transfected with an empty lentiviral vector, while the sh-GATA1 group was GATA1 expression knocked down utilizing RNAi technology.

THP-1 and HTR-8/Svneo cells co-cultured were divided into three groups: Control, Hyp + sh-NC, and Hyp + sh-GATA1. The Control group co-cultured normal THP-1 cells with HTR-8/Svneo cells. The Hyp + sh-NC group co-cultured regular THP-1 cells with HTR-8/Svneo cells in a hypoxic chamber with an O2 concentration of 2%. The Hyp + sh-GATA1 group co-cultured GATA1-knockdown THP-1 cells with HTR-8/Svneo cells in the same hypoxic conditions.

THP-1 and HPVECs cells co-cultured were also divided into Control, Hyp + sh-NC, and Hyp + sh-GATA1 groups. The Control group co-cultured normal THP-1 cells with HPVECs cells. The Hyp + sh-NC group co-cultured regular THP-1 cells with HPVECs cells in a hypoxic chamber with an O2 concentration of 2%. The Hyp + sh-GATA1 group co-cultured GATA1-knockdown THP-1 cells with HPVECs cells under the same hypoxic conditions.

The co-cultured THP-1 and HTR-8/Svneo cells were allocated into three groups: sh-NC + oe-NC, sh-GATA1 + oe-NC, and sh-GATA1 + oe-NTRK2. The sh-NC + oe-NC group co-cultured HTR-8/Svneo cells and THP-1 cells transfected with an empty lentiviral vector. The sh-GATA1 + oe-NC group co-cultured GATA1-knockdown THP-1 cells with HTR-8/Svneo cells. The sh-GATA1 + oe-NTRK2 group co-cultured GATA1-knockdown and NTRK2-overexpressing THP-1 cells with HTR-8/Svneo cells.

The co-cultured THP-1 and HPVECs cells were also divided into three groups: sh-NC + oe-NC, sh-GATA1 + oe-NC, and sh-GATA1 + oe-NTRK2. The sh-NC + oe-NC group co-cultured THP-1 cells and HPVECs cells transfected with an empty lentiviral vector. The sh-GATA1 + oe-NC group co-cultured GATA1-knockdown THP-1 cells with HPVECs cells. The sh-GATA1 + oe-NTRK2 group co-cultured GATA1-knockdown and NTRK2-overexpressing THP-1 cells with HPVECs cells (Table 1).

Animals cultivation and treatment

Sprague–Dawley (SD) rats were bought from Guangdong Laixi Biomedical Research Institute Co., LTD.

Table 1 shRNA sequences designed by the GATA1 gene sequence

shRNA	shRNA order
GATA1 shRNA-1	5′-AAATCATGTCTACATAAACCTTTCAAGAGAAGGTTTATGTAGACATGATTT-3′ 3′-GTTTATGTAGACATGATTTTAAAGTTCTCTTAAAATCATGTCTACATAAAC-5′
GATA1 shRNA-2	5′-TTGTACATAAAATCATGTCTATTCAAGAGATAGACATGATTTTATGTACAA-3′ 3′-GACATGATTTTATGTACAATGAAGTTCTCTCATTGTACATAAAATCATGTC-5′
GATA1 shRNA-3	5′-AGTAAGAACCGATAATCTCACTTCAAGAGAGTGAGATTATCGGTTCTTACT-3′ 3′-GAGATTATCGGTTCTTACTCAAAGTTCTCTTGAGTAAGAACCGATAATCTC-5′
sh-NC	5′-CACCGTTCTCCGAACGTGTCACGTTTCAAGAGAACGTGACACGTTCGGAGA ATTTTTT G-3′ 5′-GATCCAAAAAATTCTCCGAACGTGTCACGTTCTCTTGAAACGTGACACGTT CGGAGAAC-3′

Ten-week-old healthy SD female rats (weighing 200–250 g) and male rats (weighing 250–300 g) are housed in standard breeding cages at a temperature of 20–25 °C and a relative humidity of 40–70%. After 1 week of acclimatization, the females are mated with healthy male Sprague–Dawley (SD) rats at a ratio of 2:1 during the night. Pregnancy is confirmed by the presence of sperm in the vaginal smear and is designated as E0 (Embryonic Day 0).

The SD rats were divided into four groups: Normal, PE, PE + sh-GATA1, and PE + shGATA1 + oe-NTRK2. The Normal group received no treatment. On Embryonic Day 5 (E5), the PE group was intravenously injected with LPS at a dose of 1.0 μg/kg body weight via the tail vein. The PE + sh-GATA1 group consisted of pregnant female SD rats with GATA1 knockdown in macrophages, and LPS (at a dose of 1.0 μg/kg body weight) was injected via the tail vein into these rats on E5. The PE + shGATA1 + oe-NTRK2 group comprised pregnant female SD rats with both GATA1 knockdown and NTRK2 overexpression in macrophages, and LPS (1.0 μg/kg body weight) was also injected into these rats via the tail vein on E5.

For the sh-GATA1 and oe-NTRK2 lentiviral injections, the lentiviral titer and volume were 1 × 10^9 PFU and 100 μL, respectively, and they were administered via tail vein injection on E6 and E15. On Embryonic Day 20 (E20), the rats were euthanized, and their abdomens were opened to expose the abdominal aorta. A disposable venous blood collection needle was used to collect 5 mL of blood, which was then mixed in a tube containing 3.2% sodium citrate anticoagulant. The blood was centrifuged, and the upper layer of serum was collected and maintained at − 80 °C for further detection of plasma factors. The abdominal aorta was clamped with hemostatic forceps, and an immediate cesarean section was performed to remove fetus and placenta, after which their lengths and weights were measured.

Clinical sample collection

3 cases of severely PE placentas from Jinan Maternity and Child Care Hospital Affiliated to Shandong First Medical University and 3 placental controls from normal pregnancies were selected. Collect baseline clinical data for both groups, including age, obstetric history, ensuring the data is comparable. After cesarean section, collect tissue samples within 10 min post-delivery, at approximately 5 cm from the central umbilical artery, with a depth of 1–2 cm, avoiding areas of infarction, bleeding, or calcification. All pregnant women provided written informed consent prior to surgery, and this study has been approved by the Ethics Committee of Jinan Maternity and Child Care Hospital Affiliated to Shandong First Medical University (Approval NO.2024-1-011).

RNA-seq

Perform RNA-Seq (using Illumina HiSeq) on the placentas of pregnant women with severe PE and the placental controls from normal pregnancies. The sequencing libraries are generated using the NEBNext®Ultra™RNA library Prep Kit for Illumina® (E7530, NEB, Beverly, MA). Quality control is performed using RNA-SeQC v1.1.8, and counting is done with HTSeqcounts v0.7.2. Differentially expressed genes (DEGs) between the two groups are identified using the limma software package in R language with $|\log\text{FoldChange}| > 2$ and P value < 0.05 as cut-off criteria. The "clusterProfiler" package, "org.Hs.eg.db" package, "enrichplot" package, and "ggplot2" package in R language are used to conduct KEGG pathway enrichment analysis on the obtained DEGs.

Overexpression of NTRK2 and knockdown of GATA1

An overexpression lentiviral vector for NTRK2 (OE-NTRK2) was constructed using a pLV-Puro vector targeted macrophages, which was synthesized by Shanghai GeneChem Co., Ltd. NTRK2 gene inserted into pLV-Puro vector, packaged into lentiviral particles, and used for transfection. Cells were grown to 80% confluence, then Lipofectamine 3000 and plasmid were mixed, incubated, and transfected into 293 T cells. Post-8 h, switch to complete DMEM; at 48 h, collect and filter viral supernatant, determine titer, store at − 80 °C. For cell infection, pLV-Puro-OE-NTRK2 vector was added with polybrene, remove supernatant after 8 h, switch to complete medium, continue for an additional 24 h. A sh-GATA1 knockdown lentiviral expression vector targeted macrophages were constructed (synthesized by Shanghai GeneChem Co., Ltd.) using the pLKO.1 with shRNA construct vector. For the construction of the sh-GATA1 knockdown plasmid vector, a synthetic GATA1 shRNA was selected. Based on the GATA1 gene sequence the following shRNA sequences were designed:

Three pairs of GATA1 shRNA were inserted into the pLKO.1 vector with shRNA construct to create shRNA plasmid vectors (synthesized by Suzhou GenScript Biotechnology Co., Ltd.). Vectors packaged into lentiviruses, cells seeded to 80% confluence, and plasmids mixed, incubated, and transfected into 293 T cells. Post-8 h, switch to complete DMEM; at 48 h, collect and filter viral supernatant, determine titer, store at − 80 °C. For infection, add lentiviral pLKO.1−shRNA vector with polybrene, remove supernatant after 8 h, switch to complete medium, and culture for 24 h.

Flow cytometry

Seed 1 × 10^6 cells/well in 6-well plates, treat, incubate 24 h at 37 °C, 5% CO_2. Harvest cells, centrifuge 800 rpm, 5 min, wash with 2 ml PBS, centrifuge again, discard

supernatant, resuspend in 500 μL PBS, count. The cell suspension was diluted, and 50 μL of a single-cell suspension (1 × 10^6 cells) was taken and mixed with 10 μL of antibodies labeled with anti-CD86 antibody (ab239075, Abcam, UK)、Anti-Mannose Receptor antibody (ab270647, Abcam, UK). Incubate mix at RT, dark for 60 min. Add 200 μL PBS, spin at 800 rpm for 5 min, discard supernatant. Wash cells with 2 ml PBS twice, resuspend in 300 μL PBS, and load for analysis.

Transwell

Digest log-phase cells, adjust to 5 × 10^5 cells/mL, add 100 μL to Transwell upper chamber, lower chamber with THP-1 medium + 10% FBS, incubate 24 h at 37 °C. Remove upper chamber medium, wash both chambers with PBS. The upper chamber was then fixed with 4% paraformaldehyde (ST506, Beyotime, China) for 10 min. After removal of the fixative, the chamber was stained with crystal violet (V5265, Sigma-Aldrich, USA) for 10 min. The upper chamber was then washed with distilled water, dried, and observed under a microscope with photography.

Western blot

Cell total protein extraction: Cells in the logarithmic growth phase were seeded at a density of 1 × 10^5 cells per well, treated, and then incubated at 37 °C. After washing with PBS, RIPA lysis buffer (P0013B, Beyotime, China) containing PMSF (ST506, Beyotime, China) was added to lyse the cells. The mixture was then centrifuged at high speed at 4 °C, and the supernatant obtained was the cell protein. After quantification with the BCA protein content assay kit (WB6501, NCM Biotech, China), the gel was prepared, and the cell proteins were separated by electoresis. Pre-stained protein markers (G2058, Servicebio, China) were used for positioning, followed by transfer to PVDF membranes. Primary and secondary antibodies were incubated with the membrane, and protein expression was detected using a mixed chemiluminescent reagent (P2100, NCM Biotech, China). The protein expression levels were analyzed using Image J software. Primary and secondary antibodies: anti-VEGFA antibody (ab46154, 1:1000), anti-TrkB antibody (ab134155, 1:1000), anti-PKG1 antibody (ab110124, 1:1000) were bought from Abcam, UK; GAPDH (14C10, 1:1000) Rabbit mAb #2118、GATA-1 (D52H6, 1:1000) XP® Rabbit mAb #3535 were bought from Cell Signaling Technology, USA.

Dual-luciferase assay

The dual-luciferase experiment was designed to assess the regulatory effects of GATA1 on NTRK2 promoter activity. For the transfection of reporter genes in HEK-293 T cells, lysate the cells by centrifugation at 12,000 g for 5 min. Collect the supernatant for subsequent analysis. Subsequently, dissolve and adjust the firefly luciferase assay reagent (RG027, Beyotime, China) to room temperature. Set the chemiluminescence instrument's (JP-K600, Shanghai Jiapeng Technology Co., LTD) interval to 2 s and duration to 10 s. For each sample, mix 100 μL with reporter lysate as blank control. Add 100 μL firefly luciferase reagent, mix, measure Flu values. Add 100 μL Renilla luciferase solution, mix, measure RLU in fluorescence instrument. Calculate relative RLU for target gene expression based on Flu and RLU values.

Vascularization

Thaw matrix gel at −20 °C–4 °C overnight, pre-cool materials at 4 °C. Dilute gel 1:2 with serum-free medium, add 300 μL to 12-well plate, incubate at 37 °C for 1 h. Digest HUVEC cells, adjust to 2 × 10^5 cells/well, group cells as designed. Afterward, add the processed cells to the surface the solidified matrix gel (354,230, CORNING, USA) according to their groups, and incubate at 37 °C. Observe and record the angiogenesis, and once the experiment is complete, take photographs.

ELISA

cGMP ELISA Kit (ABE10235) and cGMP ELISA Kit (E02339) were bought from wksubio, China. ET-1 ELISA Kit (E-EL-H0064) was bought from Elabscience, China; Human sFlt-1 ELISA Kit (JL13928-48 T) and sFlt-1 ELISA Kit (JL48077-48 T) were purchased from a Jianglai Biotechnology Co., Ltd; Rat ET-1 ELISA Kit (E-EL-R1458) was bought from Elabscience, China. To detect the levels of cGMP, ET-1, and sFlt-1 in samples using ELISA, the following procedures should be followed: Centrifuge supernatant at 1000 × g, 20 min, collect for examining. For co-culture, wash cells three times with PBS, suspend in 200 μL PBS + PMSF, lyse by sonication, centrifuge at 1500 × g, 10 min, 2–8 °C, collect supernatant. For tissue, cut into pieces, add RIPA lysis buffer (containing PMSF), homogenize, ice, repeat, supplement buffer, shake 20 min, centrifuge at 4 °C, 12,000 rpm, 15 min, store supernatant at −80 °C. Dilute standards per ELISA kit, add to microplate, incubate 30 min, wash, add enzyme conjugate, incubate, wash. After 15 min of color development, terminate the reaction and measure the absorbance (OD value) at 450 nm via Multiskan FC microplate reader (357–714,018, thermo scientific).

BCA assay

On the seventh day after 1.0 μg/kg/200ul of LPS (HY-D1056, medchemexpress, USA) (E5) injection (E13), urine protein testing was performed. Prepare BCA solution 50:1, dissolve and dilute standard to 0.5 mg/mL, add

standards/samples to 96-well plate with 200 μL BCA, incubate 30 min at 37 °C, measure absorbance at 562 nm, create standard curve, calculate sample protein content.

24 h SBP detection

On the seventh day (E13) after the LPS (E5) injection, the blood pressure of rats was measured using the tail-cuff method as follows: Place the sensor on the tail of the SD rat, then apply and release pressure on the tail artery by inflating and deflating the cuff while simultaneously monitoring the blood flow signal to obtain the blood pressure reading.

HE staining

Collect SD rat placenta, fix 12 h, rinse 1 h, store in 75% ethanol. Dehydrate in ethanol gradients (70%, 80%, 90%, 95%, 100%), clarify in xylene, embed in paraffin. Section at 4 μm, mount on slides, dry, bake. Dewax through xylene/ethanol, HE stain, rinse, dry, photograph.

Data analysis

The data were analyzed and graphed using GraphPad Prism 9 (Version 9.4.0). All data are presented as means ± SD. Statistical differences between groups were determined using *T* test or one-way ANOVA, with a *P* value less than 0.05 considered to indicate a significant difference.

Result

Transcriptome sequencing and bioinformatics analysis demonstrate the association of GATA1 with TrkB/cGMP–PKG signaling pathway in PE

To elucidate the potential mechanism by which GATA1 affects PE, we performed transcriptomic sequencing on clinical samples and employed bioinformatics methods to analyze the differential expression of proteins in PE. Based on the transcriptomic sequencing of placental tissue from PE patients, we identified 319 differentially expressed genes, of which 268 were upregulated and 51 were downregulated (Fig. 1a). KEGG pathway enrichment analysis indicated that these genes were primarily associated with systemic lupus erythematosus, viral carcinogenesis, and the IL17 signaling pathways (Fig. 1a). Immune cell infiltration analysis revealed that macrophages were the most significantly altered immune cell type in PE (Fig. 1b). Protein–protein interaction network analysis of differential genes highlighted NTRK2 as a core gene (Fig. 1c). Transcription factor analysis showed that GATA1 had the most pronounced expression changes (Fig. 1d). GSEA analysis further revealed significant alterations in the cGMP–PKG signaling pathway in PE, providing a direction for subsequent experimental investigation (Fig. 1e).

Knockdown of GATA1 increases the proportion of M2 macrophages

Knockdown of GATA1 in activated THP-1 cells to study the effect of GATA1 on macrophage polarization. As shown in Fig. 2, the flow cytometry results suggest that proportion of M1 macrophages was significantly reduced in the sh-GATA1 group compared to the control group ($P < 0.001$, Fig. 1a), whereas the proportion of M2 macrophages was significantly increased in the sh-GATA1 group compared to the control group ($P < 0.001$, Fig. 1a). In addition, results of western blot showed that expressions of GATA1, TrkB and PKG1 in sh-GATA1 group were significantly decreased ($P < 0.001$, $P < 0.05$, $P < 0.001$, respectively; compared with control group, Fig. 1b). Moreover, the result of ELISA hinted cGMP expression level of sh-GATA1 group was markedly lower than that in control group ($P < 0.001$, Fig. 1a).

Knockdown of GATA1 promotes trophoblast cell growth and angiogenesis in PE

Knockdown of GATA1 in THP-1 cells to investigate its role in PE. Results displayed that in wild-type HEK293T cells, the sh-GATA1 group had significantly increased fluorescence values compared to the sh-NC group ($P < 0.001$, Fig. 3a). However, in HEK 293 T cells of NTRK2–MUT, fluorescence values in sh-GATA1 group have no significant change compared with that in sh-NC group ($P > 0.05$, Fig. 3a). The invasion rate of the HTR-8/Svneo cell was significantly lower in the Hyp + sh-NC group compared to the control group, and the HTR-8/Svneo cell invasion rate was significantly higher in the Hyp + sh-GATA1 group compared to the Hyp + sh-NC group ($P < 0.001$, $P < 0.05$, respectively; Fig. 3b). In addition, the results of ELISA showed that the levels of ET-1 and sFlt-1 were significantly increased in HTR-8/Svneo cells in the Hyp + sh-NC group ($P < 0.001$, $P < 0.001$, respectively; Fig. 3c, d); ET-1 and sFlt-1 were significantly decreased in HTR-8/Hyp + sh-GATA1 cells compared with the Hyp + sh-NC group ($P < 0.001$, $P < 0.001$, respectively; Fig. 3c, d). In addition, the rate of blood vessel formation was significantly reduced in the Hyp + sh-NC group compared with the control group ($P < 0.001$, Fig. 3e), and the vessel formation rate was significantly higher in the Hyp + sh-GATA1 group as compared to the Hyp + sh-NC group ($P < 0.01$, Fig. 3e). Moreover, the expression of VEGFA in Hyp + sh-NC group's HPVECs was significantly lower than that in the control group, but VEGFA in the Hyp + sh-GATA1 group's HPVECs was increased compared with the Hyp + sh-NC group ($P < 0.001$, $P < 0.01$, respectively; Fig. 3f).

(See figure on next page.)

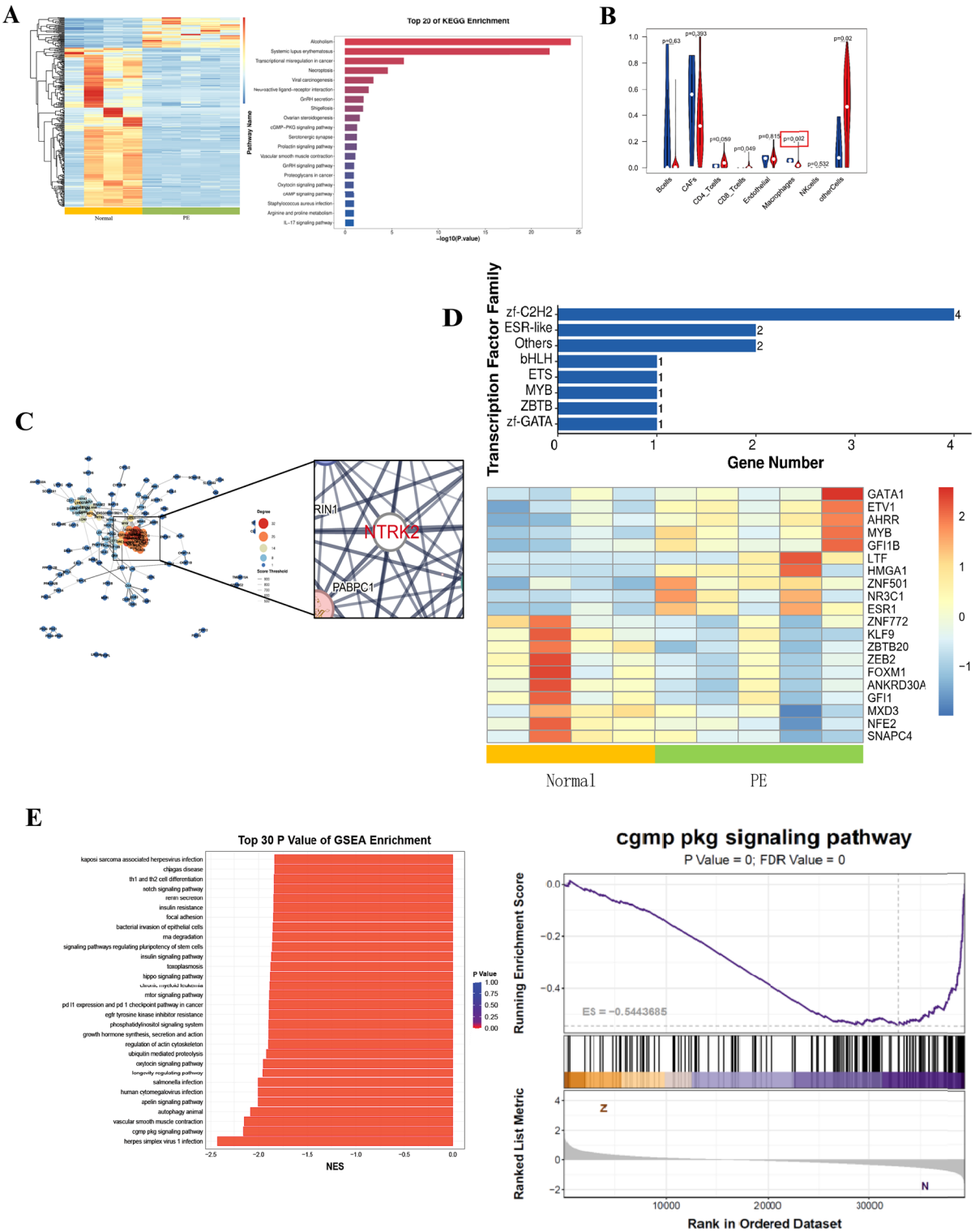
Fig. 1 Transcriptomic sequencing and bioinformatics analysis demonstrating the association of GATA1 with TrkB/cGMP–PKG signaling pathway in preeclampsia (PE). **a** Transcriptomic sequencing result of the placenta from PE patients. Transcriptomic sequencing of placental tissues from patients diagnosed with preeclampsia (PE) was performed to identify differentially expressed genes; **b** immune cell infiltration analysis. Bioinformatics analysis was used to identify the immune cell composition in PE placental tissues, with a focus on macrophage infiltration; **c** NTRK2 as a core gene in the protein–protein interaction network in PE. Protein–protein interaction analysis was conducted to identify key genes involved in PE, with NTRK2 emerging as a central hub; **d** GATA1 is the transcription factor with the most significant expression change in PE. Transcription factor analysis was performed to examine changes in GATA1 expression in PE placental tissues; **e** Significant changes in the cGMP–PKG signaling pathway in PE. Gene set enrichment analysis (GSEA) was conducted to examine the alteration of the cGMP–PKG signaling pathway in PE placental tissues. *$P < 0.05$, **$P < 0.01$, **$P < 0.001$

Knockdown of GATA1 promotes trophoblast cell growth and angiogenesis via promote the M2 polarization of the macrophages through inhibiting TrkB/cGMP–PKG signaling pathway in PE

To investigate whether the polarization of macrophages affected by GATA1 knockdown in vitro influences placental and angiogenic conditions in PE, we conducted subsequent experiments using co-cultures of THP-1 cells with HTR-8/Svneo cells or HPVECs. The proportion of M1 macrophages was significantly reduced, and the proportion of M2 macrophages was significantly increased in the sh-GATA + oe-NC group compared to the sh-NC + oe-NC group ($P < 0.001$, $P < 0.001$, respectively; Fig. 4a). Compared with the sh-GATA + oe-NC group, the proportion of M1 macrophages was significantly increased, and the proportion of M2 macrophages was significantly decreased in the sh-GATA + oe-NTRK2 group ($P < 0.001$, $P < 0.001$, respectively; Fig. 4a). Meanwhile, we used western blot to detect the expression levels of GATA1, TrkB, and PKG1 in THP-1 cells. The results showed that the expressions of GATA1, TrkB, and PKG1 proteins in THP-1 cells was significantly reduced in the sh-GATA + oe-NC group compared to the sh-NC + oe-NC group ($P < 0.001$, $P < 0.01$, $P < 0.01$, respectively; Fig. 4b). In the sh-GATA + oe-NTRK2 group, there was no significant change in GATA1 expression, while the expression of TrkB and PKG1 proteins was significantly increased compared to the sh-GATA + oe-NC group ($P > 0.05$, $P < 0.01$, $P < 0.01$, respectively; Fig. 4b). The ELISA results indicated that the cGMP content in THP-1 cells of the sh-GATA + oe-NC group was significantly reduced compared to the sh-NC + oe-NC group ($P < 0.001$, Fig. 4c), and the cGMP content in the sh-GATA + oe-NTRK2 group was significantly increased compared to the sh-GATA + oe-NC group ($P < 0.001$, Fig. 4c). In addition, we used the Transwell assay to detect the invasion rate of HTR-8/Svneo cells in the co-culture system, and ELISA was employed to measure the expression levels of ET-1 and sFlt-1 in HTR-8/Svneo cells. The results showed that the cell invasion rate in the sh-GATA + oe-NC group was significantly higher than that in the sh-NC + oe-NC group ($P < 0.05$, Fig. 4d). Compared with the sh-GATA + oe-NC group, the invasion rate of the sh-GATA + oe-NTRK2 group was significantly reduced ($P < 0.001$, Fig. 4d). The levels of ET-1 and sFlt-1 were significantly lower in the sh-GATA + oe-NC group compared to the sh-NC + oe-NC group ($P < 0.05$, $P < 0.001$, respectively; Fig. 4e). In contrast, the levels of ET-1 and sFlt-1 were significantly higher in the sh-GATA + oe-NTRK2 group compared to the sh-GATA + oe-NC group ($P < 0.001$, $P < 0.001$, respectively; Fig. 4e). As shown in Fig. 4f, g, the cellular blood vessel formation capacity was significantly increased in the sh-GATA + oe-NC group as compared to the sh-NC + oe-NC group ($P < 0.001$, Fig. 4f). The cellular blood vessel formation capacity was significantly reduced in the sh-GATA1 + oe-NTRK2 group as compared to that in sh-GATA + oe-NC group ($P < 0.001$, Fig. 4f). The results of Western blot showed that VEGFA expression in HPVECs was significantly increased in sh-GATA + oe-NC group compared with that in sh-NC + oe-NC group ($P < 0.01$, Fig. 4g), and VEGFA protein expression was significantly decreased in sh-GATA1 + oe-NTRK2 group compared with that in sh-GATA + oe-NC group ($P < 0.001$, Fig. 4g).

Knockdown of GATA1 promotes M2 polarization of macrophage to inhibits the development of PE through the TrkB/cGMP–PKG signaling pathway

In the subsequent experiments, we investigated the effects of GATA1 knockdown on pregnant SD rats and



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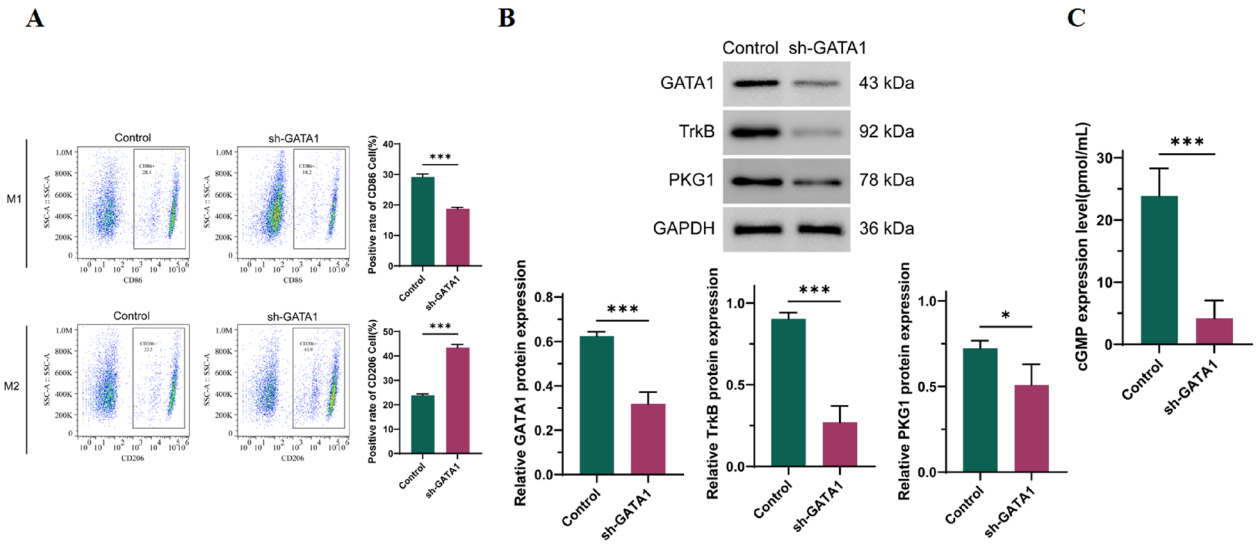
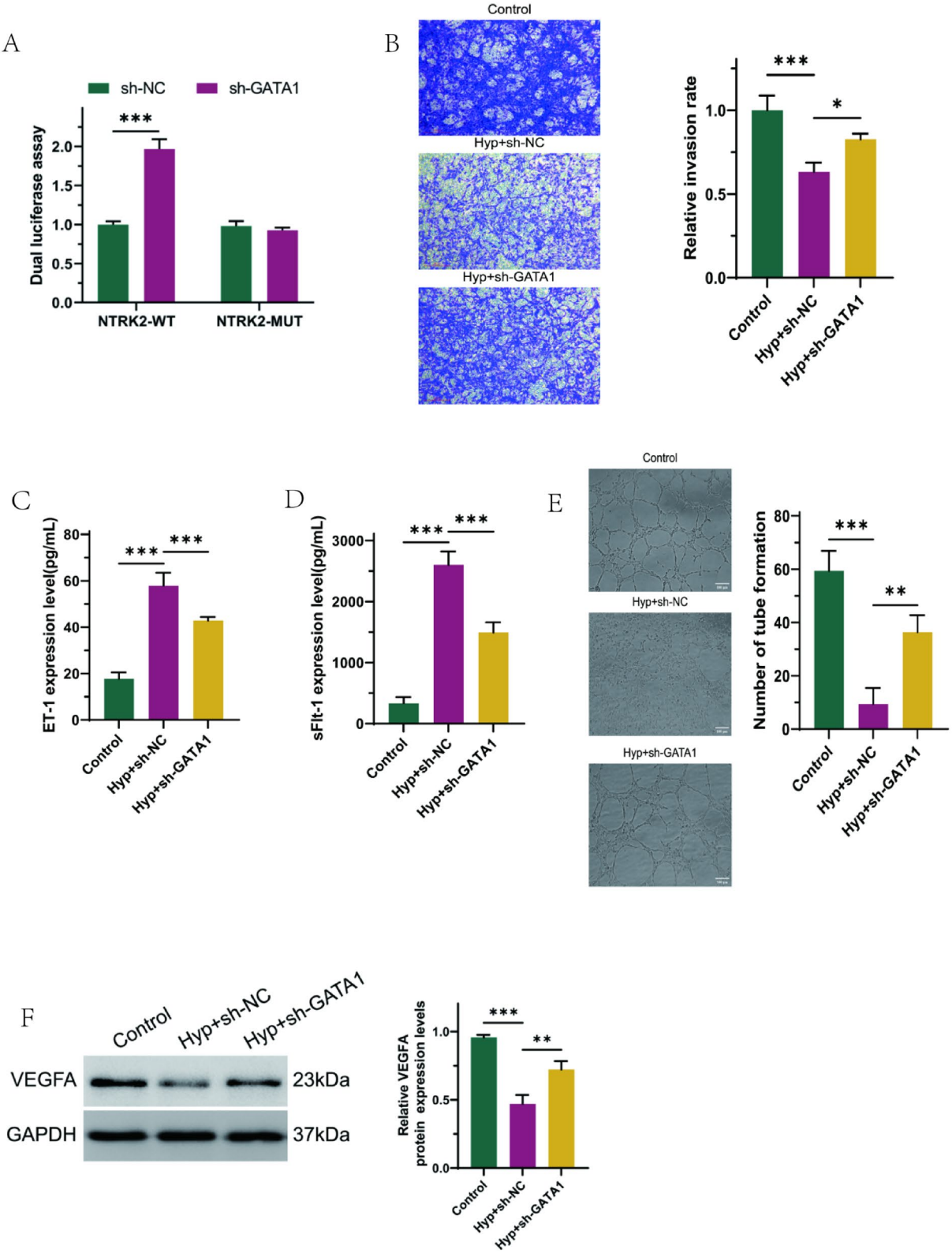


Fig. 2 Knockdown of GATA1 promoted M2 polarization of macrophages in PE. **a** Percentage of M1 and M2 determined by flow cytometry. Flow cytometry was used to assess the polarization of THP-1 cells into M1 and M2 macrophages following GATA1 knockdown. The cells were divided into control and sh-GATA1 groups, and analyzed for surface markers; **b** expression levels of GATA1, TrkB, and PKG1 in THP-1 cells. The protein expression levels of protein in THP-1 cells were analyzed using western blot; **c** content of cGMP in THP-1 cells. The content of cGMP in THP-1 cells was quantified using ELISA. *$P<0.05$, **$P<0.01$, **$P<0.001$, $n=3$

their fetuses. As shown in Fig. 5a, compared to the Normal group, the proteinuria and systolic blood pressure of pregnant rats in the PE group were significantly increased ($P<0.001$, $P<0.001$, respectively; Fig. 5a). Compared to the PE group, the proteinuria and systolic blood pressure of pregnant rats in the PE + sh-GATA1 group were significantly reduced ($P<0.001$, $P<0.001$, respectively; Fig. 5a). Compared to the PE + sh-GATA1 group, the proteinuria and systolic blood pressure of pregnant rats in the PE + shGATA1 + oe-NTRK2 group were significantly increased ($P<0.001$, $P<0.001$, respectively; Fig. 5a). Compared to the Normal group, the length and weight of the fetuses in the uterus of the PE group were reduced ($P<0.001$, $P<0.001$, respectively; Fig. 5b). Compared to the PE group, the length and weight of the fetuses in the uterus of the PE + sh-GATA1 group increased ($P<0.05$, $P<0.05$, respectively; Fig. 5b). Compared to the PE + sh-GATA1 group, the length and weight of the fetuses in the uterus of the PE + shGATA1 + oe-NTRK2 group decreased ($P<0.001$, $P<0.01$, respectively; Fig. 5b). Compared to the Normal group, the length and weight of the placenta in the uterus of the PE group decreased, but the decrease in the placental weight was not statistically significant ($P>0.05$, $P<0.01$, respectively; Fig. 5c). Compared to the PE group, the length and weight of the placenta in the uterus of the PE + sh-GATA1 group increased, but the decrease in the placental weight was not statistically significant ($P>0.05$, $P<0.001$, respectively; Fig. 5c). Compared to the PE + sh-GATA1 group, the length and weight of the placenta in the uterus of the PE + shGATA1 + oe-NTRK2 group decreased ($P<0.05$, $P<0.001$, respectively; Fig. 5c). Compared to the Normal group, the placental basilar layer of the PE group showed a large number of immune cell infiltrates, increased trophoblasts in the labyrinth zone, reduced blood vessels, and smaller pore size, and compared to the PE group, the immune cell infiltrates in the placental basilar layer of the PE + sh-GATA1 group were reduced, trophoblasts in the labyrinth zone decreased,

(See figure on next page.)

Fig. 3 Knockdown of GATA1 promotes trophoblast cell growth and angiogenesis in PE. **a** Fluorescence intensity in HEK293T cells. Fluorescence detection in HEK293T cells transfected with sh-GATA1 or control plasmids was performed to evaluate the expression of fluorescence markers; **b**. Invasions of HTR-8/Svneo cells were detected by transwell. The invasion capability of HTR-8/Svneo cells was assessed using a Transwell assay. The cells were co-cultured with control or sh-GATA1 THP-1 cells under hypoxic conditions; **c, d** Levels of ET-1 and sFlt-1 was detected by ELISA. ELISA was performed to measure ET-1 and sFlt-1 levels in HTR-8/Svneo cells after co-culture with sh-GATA1 or control THP-1 cells; **e** Angiogenesis assay of HPVECs. Angiogenesis was measured by culturing HPVECs on Matrigel and observing blood vessel formation after co-culture with HTR-8/Svneo cells; **f** western blot analysis of VEGFA protein in HPVECs. *$P<0.05$, **$P<0.01$, **$P<0.001$, $n=3$



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blood vessels increased, and pore size was larger (Fig. 5d). Compared to the PE + sh-GATA1 group, the placental basilar layer of the PE + shGATA1 + oe-NTRK2 group had a large number of immune cell infiltrates, increased trophoblasts in the labyrinth zone, reduced blood vessels, and smaller pore size (Fig. 5d). Compared to the Normal group, the quantity of macrophages in the placenta of the PE group significantly increased and the quantity of placental villous mesenchymal stem cells significantly reduced ($P < 0.001$, $P < 0.001$, respectively; Fig. 5e). Compared to the PE group, the quantity of macrophages in the placenta of the PE + sh-GATA1 group significantly decreased, and the quantity of placental villous mesenchymal stem cells significantly increased ($P < 0.001$, $P < 0.001$, respectively; Fig. 5e). Compared to the PE + sh-GATA1 group, the quantity of macrophages in the placenta of the PE + shGATA1 + oe-NTRK2 group significantly increased, and the quantity of placental villous mesenchymal stem cells significantly decreased ($P < 0.001$, $P < 0.001$, respectively; Fig. 5e). Compared to the Normal group, the expression levels of ET-1 and sFlt-1 in the placental tissue of the PE group increased ($P < 0.001$, $P < 0.001$, respectively; Fig. 5f). Compared to the PE group, the expression levels of ET-1 and sFlt-1 in the placental tissue of the PE + sh-GATA1 group decreased ($P < 0.05$, $P < 0.01$, respectively; Fig. 5f). Compared to the PE + sh-GATA1 group, the expression levels of ET-1 and sFlt-1 in the placental tissue of the PE + shGATA1 + oe-NTRK2 group increased ($P < 0.001$, $P < 0.001$, respectively; Fig. 5f). Compared to the Normal group, the expression level of VEGFA protein in the placenta of the PE group decreased ($P < 0.001$, Fig. 5g). Compared to the PE group, the expression level of VEGFA protein in the placenta of the PE + sh-GATA1 group increased ($P < 0.001$, Fig. 5g). Compared to the PE + sh-GATA1 group, the expression level of VEGFA protein in the placenta of the PE + shGATA1 + oe-NTRK2 group decreased ($P < 0.001$, Fig. 5g). Compared to the Normal group, the expression levels of GATA1, NTRK2, and PKG1 proteins in the placental tissue of the PE group increased ($P < 0.001$, $P < 0.01$, $P < 0.01$, respectively; Fig. 5h). Compared to the PE group, the expression levels of VEGFA, NTRK2, and PKG1 proteins in the placenta of the PE + sh-GATA1 group decreased ($P < 0.001$, $P < 0.01$, $P < 0.01$, respectively; Fig. 5h). Compared to the PE + sh-GATA1 group, the expression level of VEGFA protein in the placenta of the PE + shGATA1 + oe-NTRK2 group did not change significantly, while the expression of NTRK2 and PKG1 proteins significantly increased ($P > 0.05$, $P < 0.001$, $P < 0.001$, respectively; Fig. 5h). Compared to the Normal group, the expression level of cGMP in the placental tissue of the PE group increased, and compared to the PE group, the expression level of cGMP in the placental tissue of the PE + sh-GATA1 group decreased ($P < 0.001$, $P < 0.01$, respectively; Fig. 5i). Compared to the PE + sh-GATA1 group, the expression level of cGMP in the placental tissue of the PE + shGATA1 + oe-NTRK2 group significantly increased ($P < 0.001$, Fig. 5i).

Discussion

Our finding disclosed the pivotal role of GATA1 in the pathogenesis of PE, emphasizing its impact on macrophage polarization via the TrkB/cGMP–PKG signaling pathway. The most significant discovery is that the knockdown of GATA1 reduces the TrkB/cGMP–PKG signaling pathway, promoting M2 macrophage polarization and inhibiting the disease process. This aligns with our research hypothesis that GATA1 knockdown plays a significant role in PE. In addition, the impact of knockdown of GATA1 on promoting angiogenesis support our hypothesis.

In the context of the existing literature, our results on macrophage polarization in PE are consistent with previous studies highlighting the importance of macrophage function in this disease. For example, prior research has shown that macrophage polarization is disrupted in PE,

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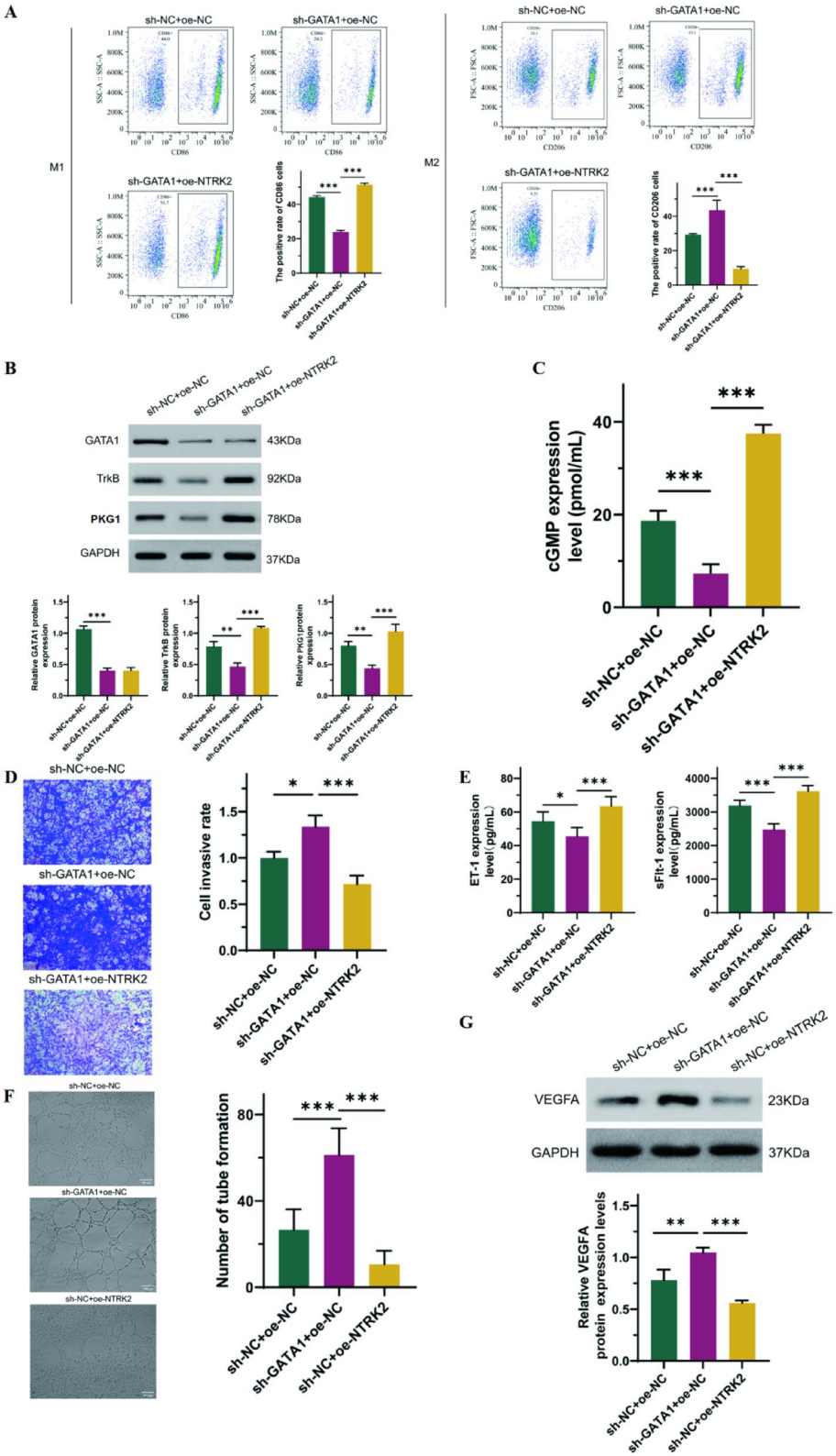
Fig. 4 Knockdown of GATA1 promotes trophoblast cell growth and angiogenesis via promote the M2 polarization of the macrophages through inhibiting TrkB/cGMP–PKG signaling pathway in PE. **a** Flow cytometry analysis of M1 and M2 macrophage polarization. THP-1 cells were co-cultured with HTR-8/Svneo cells and treated with sh-GATA1 and overexpressing NTRK2 plasmids. Flow cytometry was used to analyze macrophage polarization in the co-culture; **b** western blot analysis of GATA1, TrkB and PKG1 proteins expression in THP-1 cells. Western blotting was performed to assess the protein expression levels of GATA1, TrkB, and PKG1 in THP-1 cells after GATA1 knockdown and NTRK2 overexpression; **c** ELISA measurement of cGMP content in THP-1 cells. cGMP content in THP-1 cells was quantified by ELISA following GATA1 knockdown and NTRK2 overexpression; **d** transwell invasion assay of HTR-8/Svneo cells. Transwell assays were used to measure the invasion rate of HTR-8/Svneo cells in the presence of sh-GATA1 and NTRK2 overexpression; **e** ELISA measurement of ET-1 and sFlt-1 in HTR-8/Svneo cells. ELISA was performed to quantify ET-1 and sFlt-1 levels in HTR-8/Svneo cells following co-culture with sh-GATA1 and NTRK2 overexpressing THP-1 cells; **f** angiogenesis assay in HPVECs. HPVECs were cultured on Matrigel to assess angiogenesis, following co-culture with HTR-8/Svneo cells treated with sh-GATA1 and NTRK2 overexpressing THP-1 cells; **g** expression of VEGFA protein was detected by western blot. VEGFA protein expression in HPVECs was analyzed by Western blotting after co-culture with sh-GATA1 and NTRK2 overexpressing HTR-8/Svneo cells. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, $n = 3$



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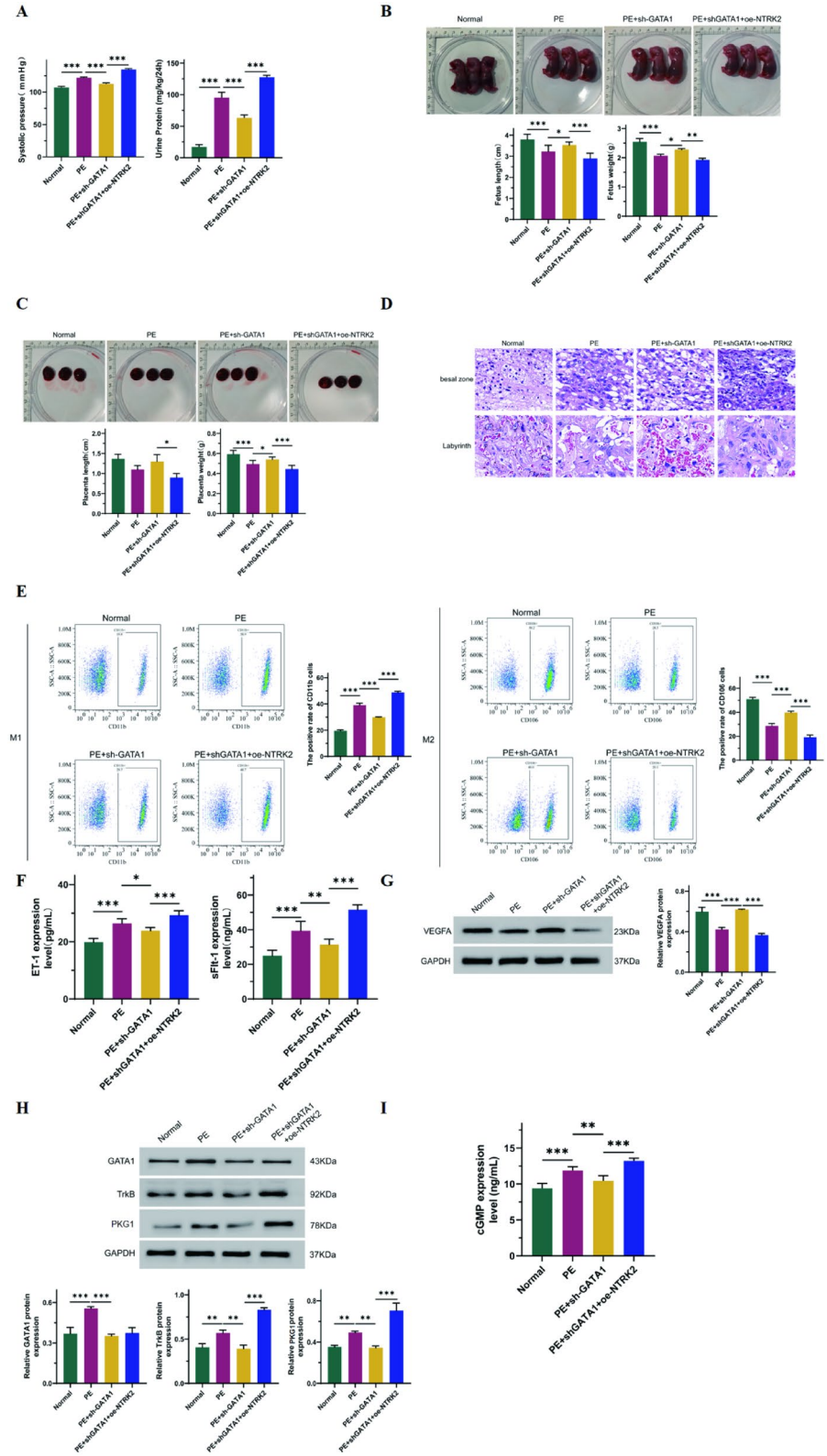
with an imbalance between M1 and M2 subtypes contributing to the exacerbation of inflammatory responses, placental damage, and the development of hypertension [17, 18]. Our study further elaborates on this by demonstrating the specific role of GATA1 in regulating macrophage polarization through the TrkB/cGMP–PKG signaling pathway. Based on our current understanding and previous literature, GATA1 may directly or indirectly regulate the transcription or post-transcriptional modification of NTRK2. This could potentially occur through interactions with other transcription factors or regulatory elements in the genome that control NTRK2 expression. In the context of our study, elucidating the effects of modulating GATA1 levels, both decreased and increased, has been instrumental in garnering a holistic understanding of its regulatory influence within the NTRK2/TrkB and cGMP–PKG signaling cascades, which are pivotal in the pathogenesis of preeclampsia. By modulating NTRK2 expression, GATA1 can affect its function in the context of macrophage polarization and the cGMP–PKG pathway. For instance, alterations in NTRK2 gene levels could affect the expression of TrkB, which, in turn, is known to modulate cGMP levels. The alteration of cGMP levels then affects the activation of PKG, ultimately regulating the function of downstream target proteins and thus macrophage polarization. In addition, there may be possible crosstalk between GATA1 and other factors within this pathway. For example, GATA1 could interact with proteins that are involved in the regulation of cGMP synthesis or degradation, or those that modulate the activity of PKG. These interactions might further contribute to the overall regulation of macrophage function in PE. Understanding these complex regulatory mechanisms in more detail could provide deeper insights into the pathogenesis of PE and potentially identify new targets for therapeutic intervention.

Angiogenesis between the placenta and the maternal body is a complex process during normal pregnancy; however, in PE patients, this process is aberrant, marked by dysregulation in the expression and function of angiogenic factors [19]. Observing the angiogenesis assay results, we found that GATA1 knockdown significantly enhanced the angiogenic potential in PE. This suggests that GATA1 may be involved in inhibiting the process of angiogenesis. Vascular Endothelial Growth Factor A (VEGFA) is a signaling protein that is crucial for angiogenesis, the formation of new blood vessels. VEGFA acts as a pro-angiogenic factor, stimulating the proliferation of vascular endothelial cells and thereby promoting the formation of new blood vessels [20]. This process is essential for various physiological functions in the body, including embryonic development, wound healing, and the repair of damaged tissues. VEGFA plays a complex and multifaceted role in the pathogenesis of PE, and its aberrant expression levels are closely correlated with the onset and progression of PE [21]. Further research revealed that knockdown of GATA1 led to a significant increase in VEGFA protein expression in vivo and in vitro, which is a key factor in promoting angiogenesis. Therefore, knockdown of GATA1 may promote neovascularization by upregulating VEGFA expression, which has important clinical implications for the treatment of vascular-related diseases.

The migratory ability of extravillous trophoblast cells is abnormal in PE [22]. These cells are responsible for invading the uterine wall and establishing the maternal–fetal blood circulation, which is crucial for maintaining a normal pregnancy [22]. In PE, the impaired migration of trophoblast cells can lead to poor placental development, thereby affecting the supply of oxygen and nutrients to the fetus while also causing maternal hypertension and other complications [22]. In this study, we used Transwell assays to detect the effect of GATA1 knockdown on cell invasion rates. The results showing that knockdown of GATA1 significantly increased invasion capabilities of trophocytes in PE suggest that GATA1 may affect development of PE by regulating cell invasion. In addition, we found that overexpression of NTRK2 in THP-1 cells could reverse the increase in invasion rates of HTR-8/Svneo cells and HPVECs caused by

(See figure on next page.)

Fig. 5 Knockdown of GATA1 inhibits PE development via promoting M2 polarization in macrophages mediated by TrkB/cGMP–PKG signaling pathway. **a** Urinary protein and SBP in pregnant rats. Sprague–Dawley (SD) rats were divided into Normal, PE, PE + sh-GATA1, and PE + shGATA1 + oe-NTRK2 groups. Proteinuria and systolic blood pressure were measured in the different experimental groups; **b** length and weight of the fetus. The length and weight of fetuses were measured on the day of euthanasia for pregnant rats in the various experimental groups; **c** placental length and weight of the placenta. Placental length and weight were measured after euthanization of the rats in the four experimental groups; **d** histopathological analysis of placenta by HE staining. Placental tissues from different experimental groups were stained with hematoxylin and eosin (HE) and examined for immune cell infiltration and trophoblast development; **e** flow cytometry analysis of macrophages content and villous mesenchymal stem cells in the placenta. Flow cytometry was used to quantify macrophages and placental villous mesenchymal stem cells in the placental tissues of rats from different experimental groups; **f** level of ET-1 and sFlt-1 was detected by ELISA. ELISA was performed to measure the levels of ET-1 and sFlt-1 in the placental tissue of rats from different experimental groups; **g** expression level of VEGFA protein was detected by western blot; **h** expression level of VEGFA protein was detected by western blot; **i** expression level of cGMP was detected by ELISA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $n = 3$



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knockdown of GATA1 in THP-1 cells, further confirming the important role of TrkB protein and NTRK2 gene in the cell invasion process. According to previous reports, hypoxia enhanced cell invasion, tube formation and the expression of VEGF, which is a paradox with our results. Our results, which suggest an alternative response to hypoxia, are likely influenced by the intricate co-culture systems we employed. These systems better reflect the in vivo maternal–fetal interface, where cellular crosstalk may significantly alter cellular responses. This microenvironmental complexity could account for the contrasting outcomes, highlighting the importance of context in cellular hypoxic studies.

ET-1 is a potent vasoconstrictor produced by vascular endothelial cells in the body, which can constrict blood vessels and increase blood pressure [23]. In PE patients, ET-1 levels are often elevated, possibly due to placental dysfunction leading to endothelial cell damage [23]. The increase in ET-1 can lead to vasoconstriction, further exacerbating blood pressure elevation and endothelial dysfunction, playing a significant role in the pathogenesis of PE. sFlt-1 is one of the receptors for vascular endothelial growth factor (VEGF), and its soluble form can bind to VEGF, preventing it from binding to its cell surface receptors, thus inhibiting angiogenesis. In PE, sFlt-1 levels also increase, which may be a compensatory response by the body to inhibit abnormal angiogenesis and reduce vascular permeability [24]. The increase in sFlt-1 helps to reduce VEGF activity, decrease vascular permeability, but may also lead to insufficient angiogenesis in the placenta, affecting placental function [24]. By employing ELISA, we examined the effect of knockdown of GATA1 in THP-1 cells on the expression's levels of ET-1 and sFlt-1 in HTR-8/Svneo cells. The results showed that knockdown of GATA1 significantly reduced the expressions of ET-1 and sFlt-1 in PE, which was related to the increased cell invasion rate and enhanced angiogenic capabilities. ET-1 is an important vasoconstrictor, and sFlt-1 is a receptor for VEGF, both of which play crucial roles in angiogenesis and cell invasion. Therefore, GATA1 knockdown in macrophage may influence angiogenesis and cell invasion through regulating the expression of ET-1 and sFlt-1. The results of this study not only reveal the important role of GATA1 in cellular signaling transduction and cell behavior but also provide novel insights for clinical treatment. For example, targeting the regulation of macrophage polarization may offer new therapeutic strategies for treating inflammatory diseases and cancer. Moreover, by regulating angiogenesis and cell invasion, GATA1 may become a potential target for treating vascular-related diseases and tumor metastasis. Targeting GATA1 or the TrkB/cGMP–PKG signaling pathway may offer significant therapeutic potential. By specifically inhibiting GATA1 activity or modulating the TrkB/cGMP–PKG pathway, it may be possible to correct the abnormal macrophage polarization, angiogenesis, and trophoblast cell behavior associated with PE, thereby intervening in the disease process. In addition, we have explored the possibility of using these findings to develop biomarkers for the early diagnosis and prediction of preeclampsia. Identifying specific molecular markers related to GATA1 or the TrkB/cGMP–PKG signaling pathway could potentially allow for earlier detection and more proactive management of the disease. Moreover, we have considered the implications of our study for understanding the pathophysiology of preeclampsia. The insights gained into the role of GATA1 and related pathways can help us better comprehend the complex mechanisms underlying the disease. This knowledge could then be translated into clinical practice, guiding the development of more personalized and effective treatment strategies.

Although this study has achieved certain results, there are still many questions that need to be further explored. First, the detailed molecular mechanism by which GATA1 regulates the TrkB/cGMP–PKG signaling pathway is not yet clear and requires further research to elucidate. Second, the physiological and pathological roles of GATA1 in the body also need to be investigated more deeply. In addition, the potential applications of GATA1 in clinical treatment need to be validated by more experiments and clinical trials. Third, in our in vivo experiment, the length of the placenta of the rats in the PE group did not change significantly, which may be related to the number of samples. In subsequent experiments, we will gradually explore these issues, and we will examine the phosphorylation activation level of TrkB to further confirm the molecular mechanism by which GATA 1 affects PE by regulating TrkB. Moreover, our study also has limitations. One limitation is the relatively small sample size in the clinical sample collection and animal experiments. With only 3 cases of severely PE placentas and a limited number of experimental animals in each group, this may affect the generalizability of our results. We suggest that future studies should include a larger sample size to validate our findings. Another limitation is the lack of detailed exploration of the downstream targets of the TrkB/cGMP–PKG signaling pathway. Future research could focus on identifying these downstream targets and further elucidating the molecular mechanism. In addition, although our in vivo studies using SD rats provided valuable insights, there is a need for in vivo studies using more advanced models that can better mimic the human pathophysiology of preeclampsia.

In summary, this study has provided new insights into the role of GATA1 in cellular signaling transduction and cell behavior, and has offered new ideas for the treatment

of related diseases. Future research will continue to explore the molecular mechanisms and clinical applications of GATA1, with the hope of bringing more benefits to human health. Our study will provide new targets and therapeutic ideas for the treatment of PE, and provide certain theoretical basis and data support for solving PE.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-024-02229-0.

Additional file 1.

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Not applicable.

Author contributions
WS L and BX C contributed to the study conception and design. WS L, H F, D C, FC G, J W, and BX C performed the experiments and interpreted the data. WS L was a major contributor in writing the manuscript. BX C was responsible for the manuscript revision, and all authors have read and approved the final manuscript for publication.

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

Declarations

Ethics approval and consent to participate
The present study was approved by the Ethics Committee of the School of Basic Medical Sciences, Shandong University (No. ECSBMSSDU2024-2-84). Consent to participate is not applicable.

Consent for publication
Not applicable.

Clinical trial registration
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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