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RAR-Related Orphan Receptor: An Accelerated Preeclampsia Progression by Activating the JAK/STAT3 Pathway

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Purpose: To investigate the effect and underlying mechanism of RAR related orphan receptor A (RORA) on preeclampsia (PE).
Materials and Methods: Differentially expressed genes (DEGs) in four datasets were obtained by using the Venn diagram method. RORA mRNA and protein expressions were detected by qRT-PCR, western blot, and immunohistochemistry. HTR-8/SVneo cell viability, proliferation, invasion, migration, and angiogenesis were detected by CCK-8 assay, EdU assay, Transwell, wound healing assay, and tube formation assay, respectively. The concentration of Ang-1 in cells was assessed using available ELISA kit. Epithelial-mesenchymal transition, proliferation, and angiogenesis-related proteins were detected by western blot. GSEA analysis were performed for common DEGs, and the expression of enriched pathway-related proteins was also detected.
Results: The expression of RORA was increased in PE tissue and HTR-8/SVneo cells. Silencing RORA could promote the migration, invasion, epithelial-mesenchymal transition, proliferation, and angiogenesis of hypoxia-treated HTR-8/SVneo cells. Mechanistically, RORA contributed to the deterioration of PE by activating the JAK2/STAT3 signaling pathway to promote cell proliferation, migration, invasion, and angiogenesis.
Conclusion: RORA was up-regulated in PE and affected HTR-8/SVneo cell proliferation, invasion, migration, apoptosis, and angiogenesis via the JAK2/STAT3 signaling pathway. This provided a novel strategy for the prevention and treatment of PE.

Key Words: Preeclampsia, RAR related orphan receptor A, JAK2/STAT3 signaling pathway, HTR-8/SVneo cells

INTRODUCTION

Preeclampsia (PE) is a pregnancy-specific disease characterized by emerging hypertension and proteinuria.[1] The incidence of PE in pregnant women ranges from 2% to 8%, and approximately over 50000 PE-related maternal deaths occur worldwide each year.[2] So far, the etiology of PE has not been clear, but the study of hypoxia plays an vital role in the pathophysiological process of PE.[3,4] Currently, there is no effective treatment for PE, and delivery of the fetus and placenta is needed to limit maternal morbidity and prevent fetal death.[5] However, frequent premature delivery increases the risk of fetal complications, and the risk to the mother does not end with pregnancy.[6] Therefore, early and accurate diagnoses are of key significance to the occurrence and development of PE. Besides, screening potential therapeutic targets and strengthening the study of PE can also help to improve perinatal outcomes.

Improving the understanding of the pathophysiology of PE and screening potential therapeutic targets may also be conducive to improving perinatal outcomes.

RAR related orphan receiver A (RORA), the first member of the ROR subfamily located at 15Q22.2, acts as a transcription factor in the regulatory region of the target gene by binding to an ROR response element.[7] Emerging evidence indicated that RORA mediates key cellular adaptions to hypoxia and con-

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tributes to the pathophysiology of many disease states. For example, Li, et al.[8] confirmed that hypoxia induced RORA expression and late differentiation in keratinocytes. Chauvet, et al.[9] revealed that RORA was up-regulated by hypoxia in HepG2 human hepatoma cells. In addition, RORA was also involved in migraine,[10] acute kidney injury,[11] endothelial cell function,[12] and post-traumatic stress disorder.[13] However, whether RORA affected PE and how it affected PE still remained a mystery.

Here, according to the GSE147776, GSE66273, GSE102897, and GSE65271 database, RORA was found to be up-regulated in PE tissues, indicating the potential association of RORA with PE pathogenesis. Therefore, in this study, we focused on RORA to explore its role in the pathogenesis of PE and its potential molecular mechanism.

MATERIALS AND METHODS

Tissue samples

Normal placental tissue and PE placenta tissue were taken from normal parturient and PE parturient who visited our hospital for treatment. The placental tissues of PE used in this experiment were taken from pregnant women who underwent Cesarean section, with no clinical evidence of intrauterine infection, intact fetal membrane, and singleton pregnancy. The severity of PE was divided into mild (13) and severe (7).[14] Each author signed an informed consent form, and the study was approved by the Ethics Committee of 960th Hospital of the Joint Logistics Support Force of the Chinese People's Liberation Army (2019 scientific research ethics review No. 002). All patients have signed written informed consent. The characteristics of PE and normal pregnant women are shown in Table 1.

Transfection

Cells [HTR-8/SVneo and 3A (tPA 30-1)] purchased from ATCC (Manassas, VA, USA) were incubated with the Roswell Park Memorial Institute (RPMI) medium (5% CO_2 at 37°C). The siRNA specifically targeting RORA was synthesized and purified by RiboBio (Guangzhou, China). HTR-8/SVneo cells were transfected with the recombinant lentiviral vector and recombinant plasmid using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA).

Table 1. Clinical Characteristics of Normal and Preeclamptic Pregnancies

Parameters	Normal group (n=20)	PE group (n=20)	*p* value
BMI (kg/m^2)	27.01±2.44	28.45±3.01	0.105
Age at delivery (yr)	32.66±3.11	33.01±3.45	0.738
Pregnancy duration at delivery (days)	273.21±8.25	251.39±9.45	<0.001
Systolic blood pressure (mm Hg)	113.55±8.14	171.24±7.54	<0.001
Diastolic blood pressure (mm Hg)	71.44±6.54	103.75±8.15	<0.001
Proteinuria (g/day)	-	3.55±0.88	-
Birth weight (g)	3501.57±45.11	2335.24±53.22	<0.001
Placental weight (g)	658.14±36.44	541.19±47.64	<0.001

BMI, body mass index; PE, preeclampsia.
Data are presented as mean±standard deviation.

Hypoxic treatment

To simulate trophoblast hypoxia, HTR-8/SVneo cells were cultured under hypoxic (1% oxygen) condition in a hypoxia workstation incubator (Catalog #27310, STEMCELL Technologies, Victoria, Australia) or under normoxic condition (8% oxygen) for 72 h prior to analysis.

qRT-PCR

Total RNA was isolated by homogenization with Trizol (Invitrogen). Then, cDNA synthesis was performed using a cDNA Cycle Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). After the sample was prepared, the expression level was detected with SYBR green (Shanghai Zheyan Biotech Co., Ltd., Shanghai, China). The primers sequences used for qPCR amplification were as follows: RORA, F: AGGAGCCAGAAGCTCT TCAA; R: TCTCAATGCAGGGAGCAGAA; GAPDH, F: AAT GAATGGGCAGCCGTTAG; and R: CCCAATACGACCAAAT CAGAGAAT. The expression level was used to normalize the mRNA expression, and the following formula was used to calculate the fold change: fold change=$2^{-\Delta\Delta CT}$.

Western blot

Total proteins were isolated and quantified by a protein concentration detection kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). The protein samples were separated by 10% SDS-PAGE and then transferred to PVDF membranes (Millipore Corp., Billerica, MA, USA). Non-specific sites were blocked with 5% milk powder diluted in TBS with 0.05% Tween 20. The membranes were incubated with primary antibodies overnight at 4°C: RORA (1:1000, abs133463), E-cadherin (1:1000, abs130068), N-cadherin (1:1000, abs145401), vimentin (1:1000, abs131996), cyclin A (1:1000, abs130079), proliferating cell nuclear antigen (PCNA, 1:1000, abs106186), cyclin-dependent kinase 1 (CDK1, 1:1000, abs111146), vascular endothelial growth factor A (VEGFA, 1:1000, abs101354), vascular endothelial growth factor receptor-2 (VEGFR2, 1:1000, abs148891), Janus kinase 2 (JAK2, 1:5000, abs148946), p-JAK2 (1:2000, abs130650), signal transducer and activator of transcription 3 (STAT3, 1:2000, abs145357), p-STAT3 (1:2000, abs130919), and GAPDH (1:1000, abs132004). After rinsing, the membranes were incubated with secondary goat anti-mouse (1:2000, abs20039) or goat anti-rabbit antibodies (1:2000, abs20040, all Absin, Shanghai, China), and the blots were assessed by enhanced chemiluminescence using an ECL reagent (Thermo Fisher Scientific Inc.). The protein expression was semi-quantified using the ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Immunohistochemistry
In brief, after conventional dehydration and dewaxing, the tissue sections were incubated with the RORA primary antibody (ab70061, Abcam, Waltham, MA, USA). Then, the HRP-labeled secondary antibody (ab6759, Abcam) was added. After that, the samples were stained with DAB (Wuhan wingroup Pharmaceutical Co., Ltd, Wuhan, China) and visualized by microscopy (magnification, ×200; Leica, Wetzlar, Germany).

Transwell
The Transwell chambers without/with Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) were used to detect the migration ability and invasion ability of HTR-8/SVneo cells. For simplicity, transfected cells (5×10^5 cells/mL) were cultured in serum-free RPMI 1640 on the upper Transwell chamber, while RPMI 1640 (Beijing Baierdi Biotechnology Co. LTD, Beijing, China) were added to the lower chamber. After 24 h, cells migrating to or invading the lower surface were stained with 0.1% crystal violet (Beyotime Biotechnology, Shanghai, China). Finally, the cells were counted in five random selected fields using a stereomicroscope (magnification, ×100, Thermo Fisher Scientific Inc.).

Wound healing
HTR-8/SVneo cells were digested with trypsin. Then, the cell suspension (50 μL; 4.5×10^5 cells/mL) were inoculated on 6-well plates. When the confluency of cells reached 80%, the cells were scratched with a 200-μL pipette tip after 24 h incubation. After that, the cells were incubated at 37°C with 5% CO_2 with serum-free medium, and recorded as 0 h, 24 h scratch growth photos. The distance of wound areas was measured using a microscope (magnification, ×100; Leica).

CCK-8
In short, HTR-8/SVneo cells adjusted to the appropriate concentration (3×10^3 cells/well) were inoculated on 96-well plates and treated in DMEM (10 mL) containing 5% FBS. Then, each well was added with 10 μL of CCK-8 solution (Sangon Biotech Co., Ltd., Shanghai, China) and incubated for 2 h in the dark. Finally, the optical density (OD) at 450 nm was measured using a microscope (magnification, ×100; Leica).

EdU
First, log phase (3×10^3 cells/well) HTR-8/SVneo cells were seeded on a 48-well plate. After 24 h of culture, 150 μL of EdU kit medium (C10339, Thermo Fisher Scientific Inc.) was added to each well (37°C for 2 h). Then, the samples were fixed with paraformaldehyde and washed with PBS, and 200 μL of Apollo staining solution and DAPI were added into the darkroom for staining for 30 min at room temperature. Finally, the staining was observed by fluorescence inverted microscope (magnification, ×100; Leica) and photographed.

Tube formation assay
Firstly, melted Matrigel was coated on a pre-cooled 24-well plate on ice, and then placed in a 37°C incubator for 30 min. Secondly, the HTR-8/SVneo cell suspension was added to the Matrigel. Finally, after being fixed with 4% paraformaldehyde for 15 min, the cultures were analyzed by light and fluorescence microscopy, and the images were then analyzed for the number of tubule branches using the Image J software (magnification, ×100).

Measurement of Ang-1
The supernatant of transfected cells was collected. After that, the concentration of Ang-1 was detected by ELISA kit (USCN Business Co., Ltd, Wuhan, China).

The specific concentration of Ang-1 was obtained according to the OD value at 450 nm.

Bioinformatics analysis
We searched for "preeclampsia" and "hypoxia" in the Gene Expression Omnibus (GEO) datasets support center (https://www.ncbi.nlm.nih.gov/geo) and downloaded the gene chip data. The Venn diagram method was applied to obtain the common differentially expressed genes (DEGs) of each dataset. For GSEA analysis, we used RORA as the phenotype, and selected the "c2.cp.kegg.v6.1.symbols.gmt" sub-collection as the reference gene sets using the GSEA software (GSEA v4.1.0, Broad Institute, Inc. Cambridge, MA, USA).

Statistical analysis
All statistical analyses were performed using the SPSS 22.0 software (IBM Corp., Armonk, NY, USA). Comparisons were performed by one-way analysis of variance to analyze the differences between groups. For a pairwise comparison between groups, least-significant difference test was performed. $P<0.05$ were considered statistically significant.

RESULTS

RORA was overexpressed in PE tissues and HTR-8/SVneo cells
A total of four datasets were retrieved through GEO (GSE147776, GSE66273, GSE102897, and GSE65271), of which GSE65271 was the hypoxic group and the normoxia group, and the other chips were the PE group and the conventional group. The Venn diagram method showed that RORA was a DEG in PE (Fig. 1A). Moreover, qRT-PCR data revealed that RORA transcription level was significantly up-regulated in the PE group compared to the normal group (Fig. 1B). Consistently, RORA protein level also increased remarkably in the PE group (Fig. 1C and D). To further study the role of RORA in PE, we constructed si-RORA. Western blot showed that the expression of RORA protein in the si-RORA group was significantly lower than that in si-NC

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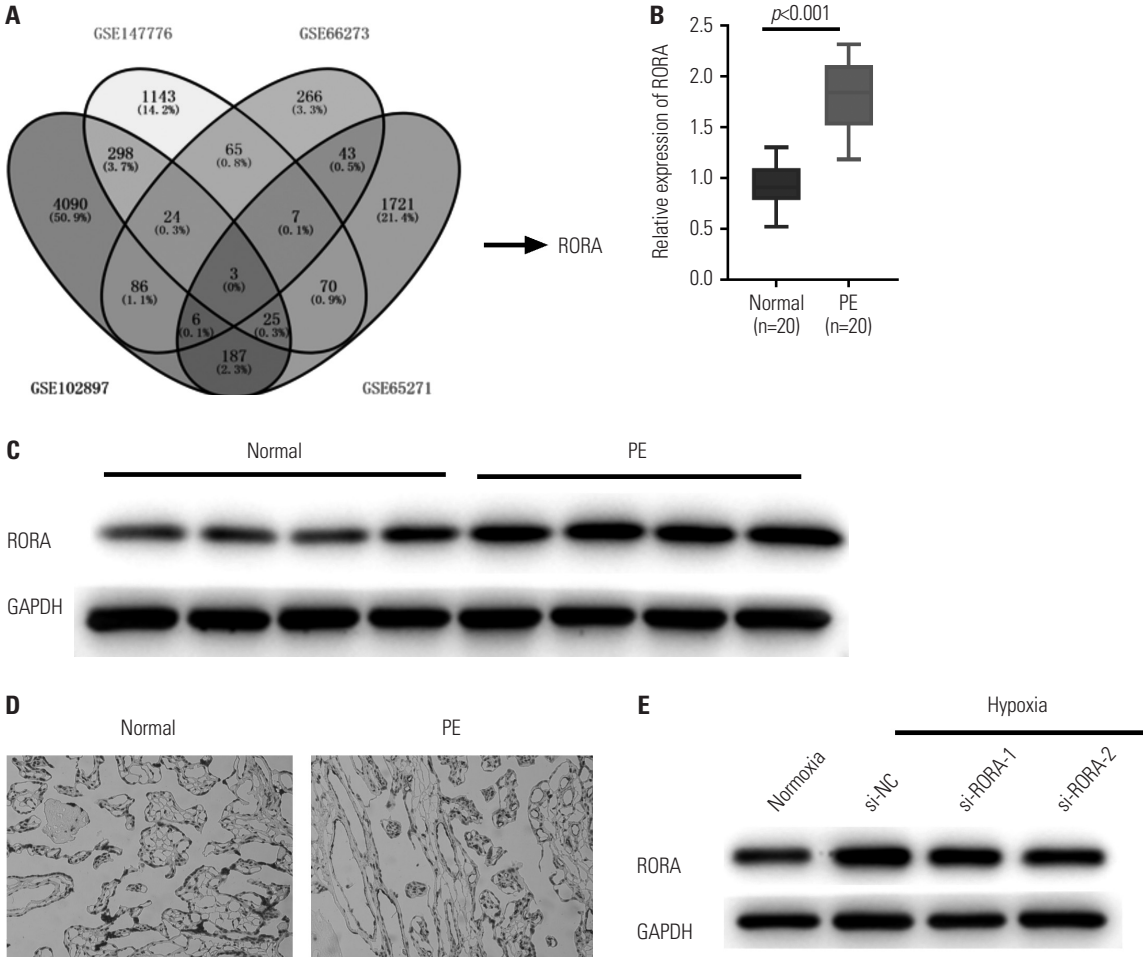

Fig. 1. RORA was highly expressed in PE tissues. (A) The common differentially expressed genes in four datasets were obtained using the Venn diagram method. (B) mRNA expression of RORA was determined by qRT-PCR. (C) RORA protein expression was detected by western blot. (D) RORA protein expression was determined by immunohistochemistry. (E) Transfection efficient was determined by western blot. RORA, RAR related orphan receptor A; PE, preeclampsia.

under hypoxia condition (Fig. 1E), indicating that the transfection was successful.

RORA inhibited HTR-8/SVneo cells migration, invasion, and epithelial-mesenchymal transition

Transwell results showed that compared to the normoxia group, hypoxia inhibited the proliferation and migration of HTR-8/SVneo cells. However, after knockdown of RORA expression, the migration and proliferation activities of si-RORA group were significantly increased (Fig. 2A). The results of wound healing assay showed that wound closure rate in the si-NC group under hypoxia was significantly lower compared to the normoxia group, while wound closure rate was markedly higher in the si-RORA group than that in the si-NC group (Fig. 2B). Furthermore, we detected the expression of epithelial-mesenchymal transition (EMT)-related proteins (E-cadherin, N-cadherin, and vimentin). As presented in Fig. 2C, silencing RORA inhibited the expression of E-cadherin in hypoxia-treated HTR-8/SVneo cells and conversely developed the expression of N-cadherin and vimentin, compared to the si-NC group.

RORA inhibited activity and proliferation of HTR-8/SVneo cells

As presented in Fig. 3A, The results of CCK-8 demonstrated that hypoxia weakened the activity of HTR-8/SVneo cells. Notably, compared to the si-NC group, the knockdown of RORA generated a heavy increase in cell activity. Moreover EdU data showed that EdU-positive cells in the si-NC group under hypoxia were significantly lower compared to the normoxia group, while EdU-positive cells in the si-RORA group were markedly higher than those in the si-NC group (Fig. 3B). Additionally, proliferation-related proteins (cyclin A, PCNA, and CDK1) were also detected. As expected, compared to the si-NC group, silencing RORA significantly promoted the expression of proliferation-related proteins (cyclin A, PCNA, and CDK1) in the hypoxia-induced HTR-8/SVneo cells (Fig. 3C).

RORA inhibited angiogenesis in trophoblasts

As shown in Fig. 4A, compared to the normoxia group, hypoxia evidently inhibited the angiogenesis of HTR-8/SVneo cells. Also, after knockdown of the expression of RORA, neovascu-

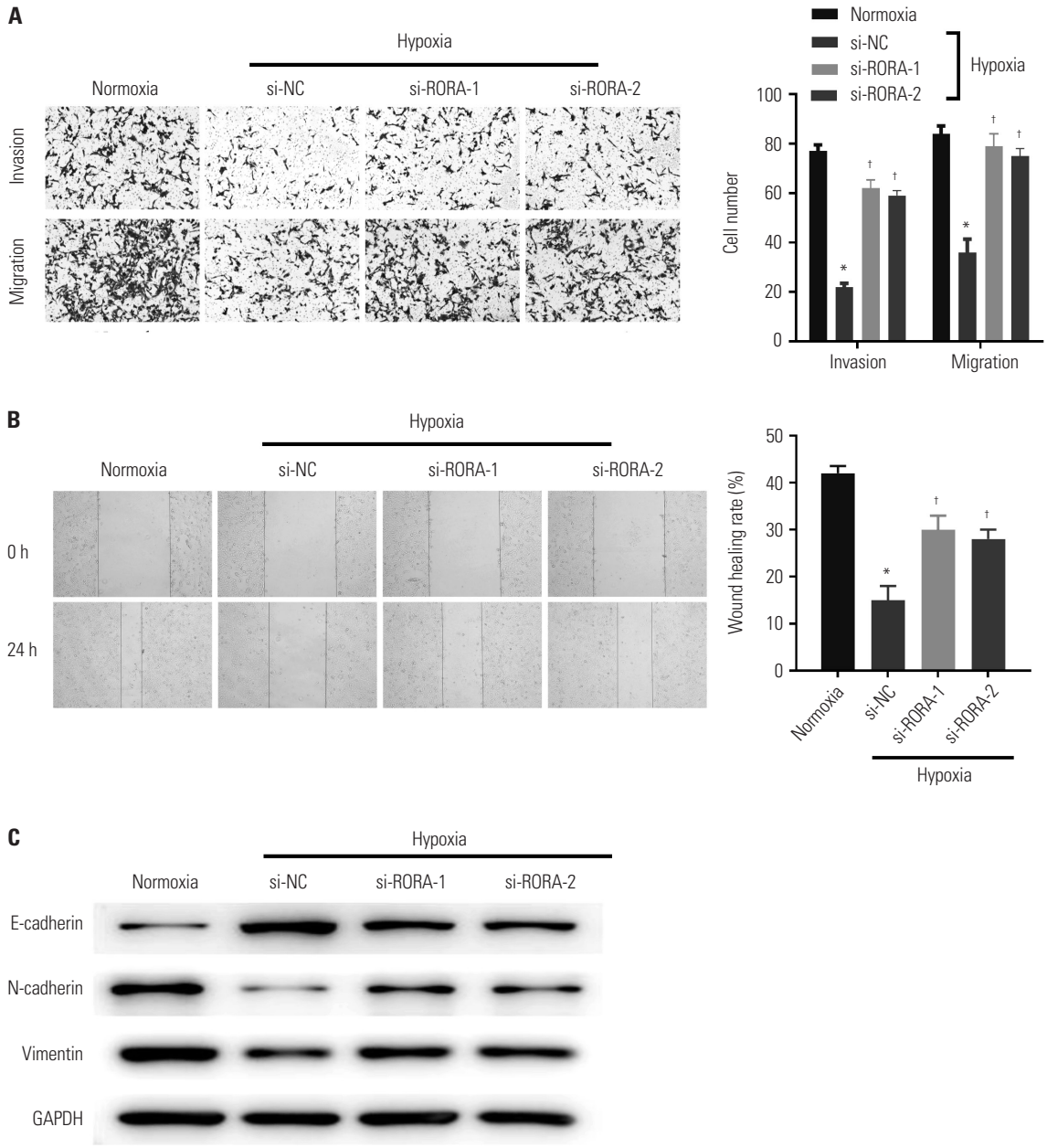

Fig. 2. RORA inhibited HTR-8/SVneo cells migration, invasion, and epithelial-mesenchymal transition. (A) Cells invasion and migration were detected by Transwell assay. (B) Cells migration was determined by wound healing assay. (C) E-cadherin, N-cadherin, and vimentin protein were determined by western blot. *p<0.01 signifies statistically significant difference compared to normoxia group; †p<0.01 compared to si-NC group. RORA, RAR related orphan receptor A.

larization was increased in the cells compared to the si-NC group. Similarly, we observed that si-RORA significantly increased the content of Ang-1 in the hypoxia-treated HTR-8/SVneo cells compared to the si-NC group (Fig. 4B). In addition, we also tested the expression of angiogenesis-related proteins (VEGFA and VEGFR2) and RORA, and found that hypoxia reduced the protein expressions of RORA, VEGFA, and VEGFR2 in the si-NC group. However, after silencing RORA, the protein levels of VEGFA and VEGFR2 in the si-RORA group increased significantly compared to the si-NC group (Fig. 4C).

RORA accelerated PE progression through the JAK2/STAT3 signaling

The GSEA analysis revealed that the JAK2/STAT3 signaling pathway was significantly enriched in PE (Fig. 5A and B), and western blot data confirmed that RORA did affect the expression of pathway-related proteins. Specifically, compared to the normoxia group, p-JAK2 and p-STAT3 were significantly increased in the si-NC group under hypoxia condition. However, si-RORA inhibited p-JAK2 and p-STAT3 protein expressions in HTR-8/SVneo cells by the opposite effect (Fig. 5C). To further verify the molecular mechanism of RORA on PE, we added the path-

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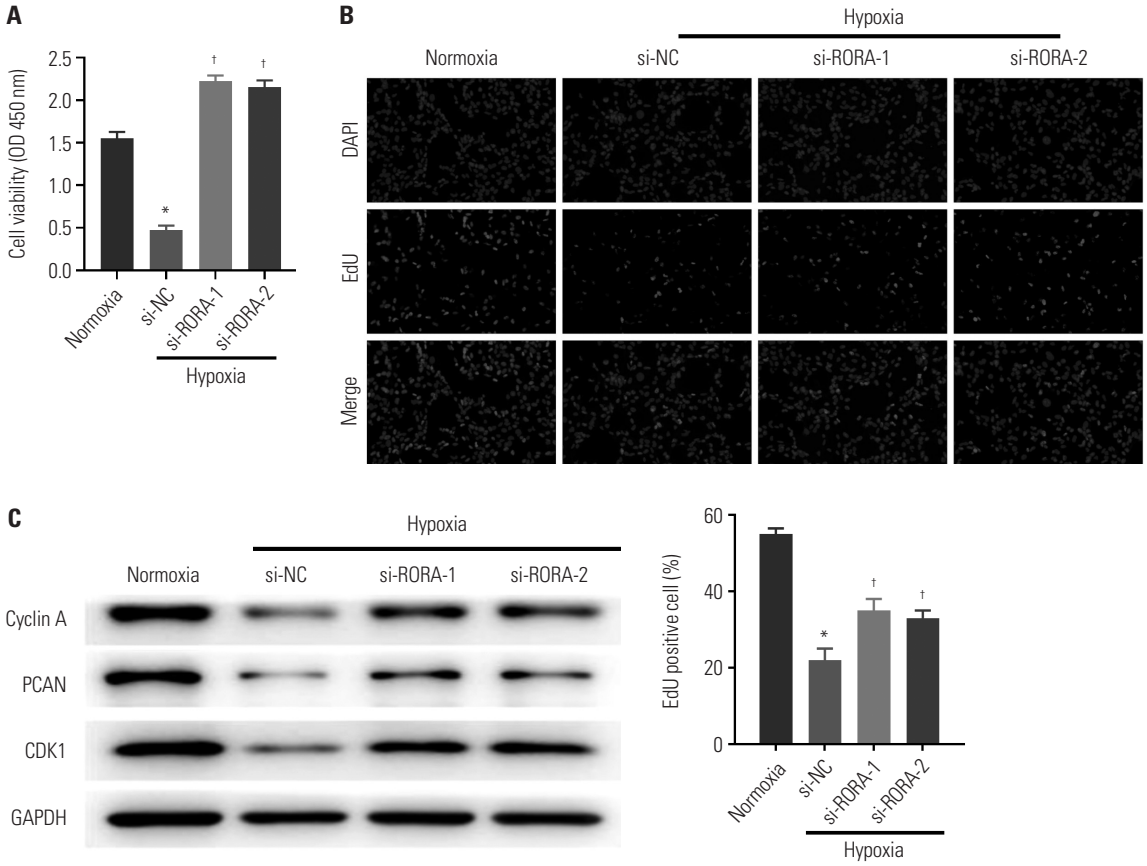

Fig. 3. RORA inhibited HTR-8/SVneo cell activity and proliferation. (A) Cell viability was determined by CCK-8. (B) Cell proliferation was determined by EdU assay. (C) Cyclin A, PCNA, and CDK1 protein levels were determined by western blot. *p<0.01 signifies statistically significant difference compared to normoxia group; †p<0.01 compared to si-NC group. RORA, RAR related orphan receptor A; OD, optical density.

way activator, RO8191. Western data showed that RO8191 reversed the inhibitory effect of si-RORA on p-JAK2 and p-STAT3 proteins (Fig. 5D). In the invasion and migration experiments, the invasion and migration cell number increased significantly in the si-RORA group compared to the si-NC group, while the number was reduced sharply in the si-RORAZ+RO8191group compared to the si-RORA (Fig. 5E). In addition, we examined the expression of proteins associated with proliferation and angiogenesis in cells and found that silencing RORA significantly promoted the expression of cyclin A, PCAN, CDK1, VEGFA, and VEGFR2 proteins in hypoxic-treated HTR-8/SVneo cells. In contrast, when RO8191 was added, the levels of these proteins in the cells were significantly reduced (Fig. 5F).

DISCUSSION

PE is a major and direct cause of maternal and neonatal morbidity and mortality.[15] Due to its wide range of clinical features and symptoms, the diagnosis of PE is challenging. Therefore, the discovery of new diagnostic markers and therapeutic targets is beneficial to the treatment of PE.[16] A previous study showed that in human melanomas, RORA, as a target of hypoxia-inducible factor 1 alpha, was up-regulated in hypoxia.[17] Furthermore, Qiu, et al.[18,19] reported that the SNPs of RORA in the placental genome were associated with placental abruption risk. Behnia, et al.[20] reported that DNA methylation in fetal leukocyte DNA in RORA was associated with preterm birth. Here, we reported that RORA was highly expressed in PE tissues and hypoxic-treated cells, indicating that RORA may play a significant role in the treatment of PE. Furthermore, low expression of RORA was achieved in the HTR-8/SVneo cells by transfection, and an in-depth study regarding the effect of RORA on cell function was conducted. Trophoblast cells are closely related to the development of the placenta, and are migratory and invasive.[21] Trophoblast cells anchor the fetus by moderately invading the maternal endometrium and musculus, while trophoblast cells can replace maternal endothelial cells to complete placental vascular reconstruction and maintain placental blood supply.[22] Accumulated evidence suggested that abnormal trophoblast cell migration and invasion can cause PE.[23,24] Here, it was observed that, under hypoxia, high expression of RORA inhibited the migration and invasion of HTR-8/SVneo cells, while the knockdown of RORA promoted the migration and invasion. In order to verify the effect of RORA on the behavior of other trophoblast cells, we

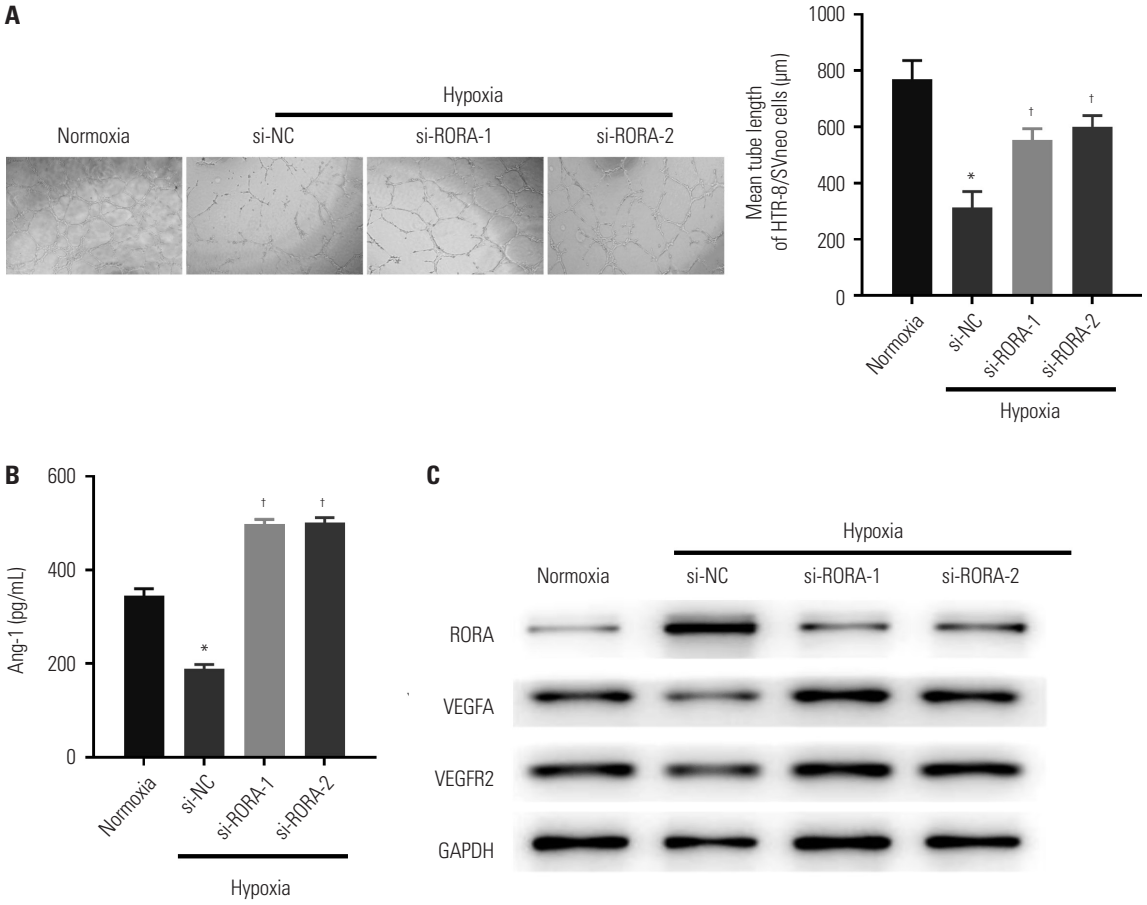

Fig. 4. RORA inhibited angiogenesis in trophoblasts. (A) Tube formation was determined by tube formation assay. (B) Concentrations of Ang-1 in the HTR-8/SVneo cells were assessed using the available ELISA kit. (C) RORA, VEGFA, and VEGFR2 proteins were determined by western blot. *p<0.01 compared to normoxia group; †p<0.01 compared to si-NC group. RORA, RAR related orphan receptor A.

selected the 3A (TPA 30-1) cells lines for Transwell assay and EdU assay. The effects of RORA gene knockout on pathway proteins and angiogenesis-related proteins were detected by western blot. The results were consistent with the previous experimental results (Supplementary Fig. 1, only online). This was also consistent with the results that high mobility group A1 inhibited trophoblast migration under hypoxia.[23] Recently, EMT has been known to play an important role in the regulation of cell migration and invasion. During pregnancy, extravasated trophoblast cells migrate and invade through the EMT, successfully inducing them to invade the muscular spiral artery.[25] As the three recognized markers of EMT, vimentin, E-cadherin, and N-cadherin are required for cell migration and invasion.[26,27] Consistently, our data have suggested that RORA knockout inhibited E-cadherin expression, and induced N-cadherin and vimentin in the placenta of PE. Taken together, si-RORA inhibited HTR-8/SVneo cell migration, invasion, and EMT.

It has been reported that the decreased proliferation capacity of trophoblast cell is another important cause of spontaneous abortion, fetal growth restriction, and PE.[28] During normal pregnancy, trophoblast cells infiltrate into the decidua to complete the spiral artery remodeling and placenta formation, while PE placenta uterine spiral artery recasting is impaired, and trophoblast cell proliferation is reduced.[29,30] Our study found that, in the hypoxic-treated cells, high expression of RORA inhibited cell proliferation and the expression of related proteins, while the knockdown of RORA had the opposite effect. Angiogenesis is a key factor in placenta formation and vascular remodeling, involving a variety of growth factors.[31] Abnormal angiogenesis balance is a key factor in the onset of PE. Ang-1 is a unique growth factor, which can induce diverse angiogenic activities.[32] VEGFA is a key regulator of angiogenesis, which can enhance the migration and invasion function of trophoblast cells.[4] VEGFR2 is the primary signal transduction responsible for VEGFA, and plays a necessary role in regulating angiogenesis and angiogenesis during embryonic development.[33] In a previous study, Zeng, et al.[34] demonstrated that hypoxia induced miR-150-5p expression, and the inhibition of miR-150-5p enhanced the invasion, migration, and tubulogenesis of trophoblast cells. Therefore, we investigated the effect of RORA on angiogenesis in both normoxic and similar hypoxic conditions, and found that up-regulation of RORA inhibited cell angiogenesis while down-regulation of RORA was

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beneficial to angiogenesis, which was consistent with the findings from previous research. In summary, RORA is a very important gene in the occurrence and progress of PE, and the underlying mechanism of these functions should be further investigated. The JAK/STAT3 is an important signaling pathway that is involved in many biological processes, including

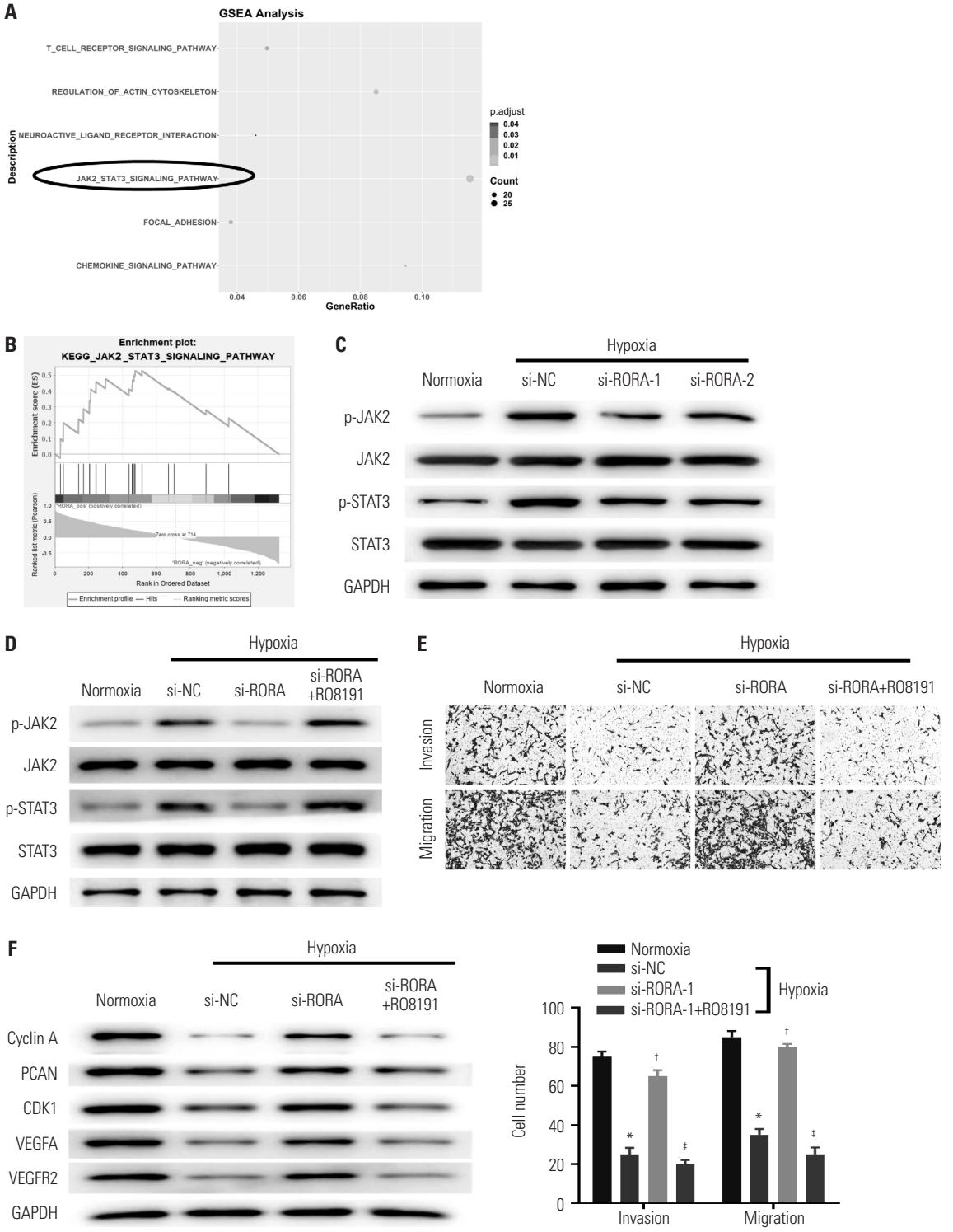

Fig. 5. RORA accelerated PE progression by activating the JAK2/STAT3 signaling pathway. (A) Dot plot of dysregulated pathways in PE tissue. The JAK2/STAT signaling pathway was activated. (B) GSEA pathways enriched by DEGs. (C) Protein levels of p-JAK2, JAK2, p-STAT3, and STAT3 were determined by western blot. (D) After RO8191 was added, the protein levels of p-JAK2, JAK2, p-STAT3, and STAT3 in indicated groups were detected. (E) Cells invasion and migration in indicated groups were measured by Transwell assay. (F) Protein levels of cyclin A, PCAN, CDK1, VEGFA, and VEGFR2 were determined by western bot. *p<0.01 compared to normoxia group; †p<0.01 compared to si-NC group; ‡p<0.01 compared to si-RORA-1 group. RORA, RAR related orphan receptor A; PE, preeclampsia; DEG, differentially expressed gene.

cell proliferation, inflammatory response, immune response, and cancer.[35,36] JAK protein is activated by intracellular receptors through autophosphorylation, which in turn phosphorylates and promotes the STAT3 protein.[37] The JAK2/STAT3 signaling pathway has been found to play a role in maintaining embryonic stem cell pluripotency.[38] In addition, it has also been demonstrated that the JAK/STAT signal pathway is significantly activated in trophoblast cells, and promotes the progress of PE. For example, Qu, et al.[36] reported that HIF-3α can inhibit the proliferation of JAK/STAT signal pathway and promote the development of PE in extravillous cytotrophoblasts. Xu, et al.[39] revealed that hypoxia can significantly activate the JAK/STAT3 signaling pathway and promote trophoblast activity and angiogenesis. Results of the present study found that up-regulation of RORA promoted the JAK/STAT3 pathway while down-regulation of RORA inhibited the JAK/STAT3 pathway, suggesting that RORA regulated PE via the JAK/STAT3 signaling pathway. In this regard, we have verified the regulatory effect of RORA on JAK2/STAT3 by using the JAK2/STAT3 activator to act on the cells. The results of our study showed that silencing RORA promoted the proliferation, migration, invasion, and angiogenesis of trophoblast cells by inactivating the JAK2/STAT3 pathway in PE.

The present study provided a novel insight that RORA activated the JAK2/STAT3 signaling pathway, which led to the decline of trophoblast cell proliferation, migration, invasion, and angiogenesis, and ultimately accelerated the progression of PE.

The limitation of this study was that an in vitro hypoxia model (trophoblast) was used in the study design. Further construction of a mouse PE model and study on the effect of hypoxic preconditioning on the JAK2/STAT3 pathway in placental tissue will become the next step in our research.

AVAILABILITY OF DATA AND MATERIAL

The datasets used and analyzed in the current study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

Conceptualization: Ying Yu. Data curation: Ying Yu. Formal analysis: Ying Yu. Funding acquisition: Tongyu Zhu. Investigation: Ying Yu. Methodology: Tongyu Zhu. Project administration: Tongyu Zhu. Resources: Tongyu Zhu. Software: Tongyu Zhu. Supervision: Tongyu Zhu. Validation: Tongyu Zhu and Ying Yu. Visualization: Tongyu Zhu and Ying Yu. Writing—original draft: Tongyu Zhu and Ying Yu. Writing—review & editing: Tongyu Zhu and Ying Yu. Approval of final manuscript: Tongyu Zhu and Ying Yu.

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