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RESEARCH ARTICLE

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Mast cell-derived exosomal miR-181a-5p modulated trophoblast cell viability, migration, and invasion via YY1/MMP-9 axis

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Abstract

Background: Mast cells regulate the process of preeclampsia (PE). Since we previously identified mast cells specifically expressing miR-181a-5p in the placenta of PE patients, it is plausible to examine the effect and mechanism of mast cell-derived exosomal miR-181a-5p on trophoblast cells.

Methods: The miR-181a-5p and YY1 levels were determined by quantitative real-time reverse transcription-polymerase chain reaction. Exosomes were identified by transmission electron microscopy, Western blot, and PKH-26 labeling. Mast cells or trophoblast cell malignant phenotype were detected using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide, wound healing, and Transwell assays. Quantification of YY1 and metastasis-related proteins was performed using Western blot. TargetScan, JASPAR, dual-luciferase reporter genes, and chromatin immunoprecipitation were exploited to verify the relationship between miR-181a-5p, YY1, and MMP-9.

Results: MiR-181a-5p was overexpressed in mast cells of PE patients. Overexpressed miR-181a-5p restrained mast cell viability. Mast cell exosomes were successfully isolated, containing high expressions of CD63 and HSP70 and low expression of Calnexin and could be transported to the cytoplasm of trophoblast cells. Mast cell exosomes attenuated the viability, migration, and invasion of HTR-8/SVneo cells, inhibited YY1, N-cadherin, Vimentin, and MMP-9 protein expressions, and promoted E-cadherin protein expression. The effect of exosomes was enhanced by miR-181a-5p mimic but was reversed by miR-181a-5p inhibitor. MiR-181a-5p targeted YY1 which bound to the MMP-9 promoter. Overexpressed YY1 in HTR-8/SVneo cells accelerated the malignant phenotype of the cells and reversed the regulatory effects of exosomal miR-181a-5p.

Conclusion: Mast cell-derived exosomal miR-181a-5p modulates HTR-8/SVneo cell viability, migration, and invasion via YY1/MMP-9.

KEYWORDS
exosomes, mast cell, miR-181a-5p, preeclampsia, YY1

1 | INTRODUCTION

Preeclampsia (PE), a pregnancy-specific disease, seriously threatens maternal and fetal health.[1,2] The main pathological changes of the placenta in PE patients are diminished trophoblast invasion and abnormal spiral arterial remodeling.[3] Previous studies revealed that the above symptoms may be related to a decrease in uterine natural killer cell number, an increase in macrophage number around the spiral arteries, and a growing dendritic cell number in the placental bed.[4–6] Mast cells, one of the immune cells in the placental bed, appear to modulate trophoblast invasion, angiogenesis, and spiral artery remodeling as well.[5] In a PE model, mice with abnormal mast cells showed impairment of spiral artery remodeling and fetal developmental delay.[7] However, the existing study has not delved into the specific mechanism of mast cells. Therefore, further study of mast cells may add new theoretical evidence for the pathogenesis of PE.

Recently, the roles of miRNAs in PE have gradually attracted the attention of researchers.[8–11] For example, Zhang et al. proposed that miR-30b-5p alleviates PE symptoms in a rat model by mediating trophoblast ferroptosis.[12] Li et al. confirmed that miR-296-3p is highly expressed in PE and could directly target CEMP to block the activation of Wnt/β-catenin and PI3K/AKT pathways, thereby promoting the malignant phenotype of HTR-8/Svneo and JAR cells.[9] Hence, studying miRNAs in PE may enrich potential targets for the treatment of PE patients.

In our research, primary mast cells were isolated from the placenta of PE patients and normal pregnant women in the early stage, which were then applied to perform miRNA sequencing. According to bioinformatics analysis, we unveiled the abnormally overexpressed miR-181a-5p. Some research clarified that miR-181a is under-expressed during normal decidualization of endometrial stroma (hESCs),[13] while in several miRNA studies on PE, miR-181a is specifically overexpressed.[14,15] The latest research pointed out that the high expression of miR-181a-5p is connected with the adverse outcome of PE patients.[16] This diverts our attention to miR-181a. Nonetheless, some questions remain to be answered: what role does miR-181a play, which is abnormally highly expressed in mast cells in PE placenta and involved in the development of PE, and which cell secretes it specifically?

Furthermore, to enrich the mechanism of miR-181a-5p, we found that miR-181a-5p could target YY1 by bioinformatics analysis. It has been reported that YY1 silencing inhibits trophoblast cell migration and invasion.[17] Thus, based on the above analysis, we intend to verify whether mast cells secrete abnormally overexpressed miR-181a-5p and affect PE progression by regulating YY1.

2 | MATERIALS AND METHODS

2.1 | Ethics statement, tissues, and cells

The placental tissue was collected with the consent of all subjects and the collection was approved by the Ethics Committee of Ningbo Women & Children's Hospital Medical Ethics Committee (EC2022-025).

Placentas were harvested from pregnant women with PE (both mild and severe) in the third trimester (after 28 weeks) ($n = 15$) and those with normal pregnancies ($n = 20$). The placental tissue was digested into a single-cell suspension, and primary mast cells were isolated using the MagniSort™ Human CD117 (c-Kit) Positive Selection Kit (8802–6870-74; Invitrogen). Mast cells were cultured in IMDM supplemented with stem cell factor (SCF) (PCK019; Procell) and interleukin (IL)-6 (PCK085; Procell).

The immortalized human mast cell line Luva (EG1701-FP) was purchased from Kerafast (UK) and maintained in mast cell complete medium (CM-H110; Procell) at 37°C with 5% CO_2. Trophoblast HTR-8/SVneo cells (CRL-3271; ATCC) were cultured in RPMI-1640 medium (30-2001; ATCC) with fetal bovine serum (FBS) (30-2021; ATCC).

2.2 | Transfection

The miR-181a-5p mimic (M) (miR10000256-1-5), miR-181a-5p inhibitor (I) (miR20000256-1-5), and corresponding control (MC, IC) (miR1N0000002-1-5, miR2N0000002-1-5) were obtained from Ribobio. YY1 overexpression plasmid was constructed from pEX-3 vector (GenePharma), while pEX-3 empty vector was used as the negative control (NC). MiR-181a-5p mimic, miR-181a-5p inhibitor, miR-181a-5p mimic control and miR-181a-5p inhibitor control were separately transfected into Luva cells using Lipofectamine 2000 transfection reagent (11668027; Invitrogen), followed by extraction of exosomes as needed. YY1 overexpression plasmid and NC were also transfected into HTR-8/SVneo cells utilizing Lipofectamine 2000 and then co-cultured with exosomes.

2.3 | Exosome (exo) isolation and identification

ExoIsolator Exosome Isolation Kit (EX10) was bought from DOJINDO LABORATORISE. Briefly, the supernatant was collected by centrifugation of Luva cells, followed by filter sterilization via filter (0.22 μm, GVWP09050; Millipore). Next, the sample was filtered in vacuum using an Isolation Filter, and exosomes were recovered by repeatedly flowing phosphate-buffered saline (PBS) (806544; SAFC) over the surface of the Isolation Filter. Ultimately, the exosome was identified under the Tundra Cryo-Transmission Electron Microscope (Thermo Fisher) and through Western blot.

2.4 | PKH-26 labeling

For exosome uptake, Luva cells were incubated for 15 min with PKH26 (MINI26; Sigma-Aldrich) at 37°C and then applied to isolate exosomes. The PKH-26-labeled exosomes were co-incubated with HTR-8/SVneo cells for 6 h to allow internalization. After Hoechst 33258 staining (C1018; Beyotime) for nuclei, HTR-8/SVneo cell measurement was performed utilizing a fluorescent microscope (Zeiss Meditec AG) (magnification×200).

2.5 | Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

The miR-181a-5p or YY1 expression in mast cells, HTR-8/SVneo cells or exosomes was quantitated by qRT-PCR. RNA collections from cells were performed using TRIzol (15596026; Invitrogen). Total RNA from exosomes was harvested using Total Exosomal RNA & Protein Isolation Kit (4478545; Invitrogen). Subsequently, reverse transcription of total RNA was completed by RevertAid first strand cDNA synthesis kit (K1621; Thermo Scientific) or microRNA reverse transcription kit (4366597; Applied Biosystems). LightCycler 480II (Roche) and FastStart SYBR Green Premix (FSSGMMRO; Roche) were employed to run the real-time PCR reaction. GAPDH and U6 were served as internal control genes. The universal reverse miRNA primers were obtained from TaqMan™ MicroRNA Assay (4440888; Applied Biosystems). MiR-181a-5p forward (F) primer is AACATTCAACGCTGTCGGTGAGT, and U6 primer (5′-3′) is CTCGCTTCGGCAGCACA, AACGCTTCACGAATTTGCGT. Primer sequences of YY1 and GAPDH are as follows (5′-3′): YY1: ACGGCTTCGAGGATCAGATTC, TGACCAGCGTTTGTTCAATGT; GAPDH: ACAACTTTGGTATCGTGGAAGG, GCCATCACGCCAC AGTTTC.

2.6 | 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay

The MTT assay was carried out to evaluate the viability of Luva cells or HTR-8/SVneo cells and was performed by MTT kit (M1020; Solarbio). In the first part, Luva cells were transfected with miR-181a-5p mimic, miR-181a-5p inhibitor, miR-181a-5p mimic control, or miR-181a-5p inhibitor control for 24, 48, 72, or 96 h of culture. Then, cell viability was detected using the MTT kit. In the second and third parts, the exosomes were extracted from Luva cells transfected with miR-181a-5p mimic or miR-181a-5p mimic control, subsequent to which the exosomes were added to HTR-8/SVneo cells transfected with YY1 overexpression plasmid or NC for 48 h of culture. Afterward, cell viability was detected with MTT kit. Next, MTT solution was added to each group of cells and incubated for 4 h, followed by the addition of Formazan solution for 10-min incubation. Optical density (OD) value (490 nm) detection was conducted by a microplate reader (VL0L00D0; Thermo Scientific).

2.7 | Western blot

Proteins were collected by RIPA buffer (R0020; Solarbio) or Total Exosomal RNA & Protein Isolation Kit. The protein concentration was quantified with BCA kit (ab102536; Abcam). The protein separation was implemented using sodium dodecyl sulfate polyacrylamide gel electrophoresis gels. After that, proteins were loaded to nitrocellulose membranes (HATF04700; Millipore), followed by 1-h incubation using 5% skim milk. Next, membranes were cultivated first with primary antibodies overnight and then with secondary antibodies (ab205718, ab205719; Abcam) for 1 h. Thereafter, the enhanced chemiluminescence kit (10144; Proandybio) was used to detect intensities of band signals. Antibody information was as follows: CD63 (CY5253, 26 kDa, 1:1000; Abways); HSP70 (AB3138, 70 kDa, 1:1000; Abways); Calnexin (CY5839, 90 kDa, 1:1000; Abways); YY1 (CY5160, 68 kDa, 1:500; Abways); MMP-9 (CY1226, 78 kDa, 1:500; Abways); N-cadherin (ab76011, 100 kDa, 1:5000; Abcam); E-cadherin (ab40772, 97 kDa, 1:10000; Abcam); Vimentin (ab92547, 54 kDa, 1:1000; Abcam); GAPDH (ab8245, 36 kDa, 1:2000; Abcam).

2.8 | Wound healing assay

To determine the cell migratory capability, HTR-8/SVneo cells (1×10^5 cells/well) transfected with or without YY1 overexpression plasmid or NC were co-cultured with or without exosomes extracted from Luva cells to reach 100% confluency. Wound creation on the monolayers was accomplished using a sterile pipette tip. Post additional 48-h incubation in serum-free medium, cells were observed under a light microscope (×100, DP27; Olympus).

2.9 | Transwell assay

HTR-8/SVneo cells were firstly transfected with or without YY1 overexpression plasmid or NC and then co-cultured with or without exosomes extracted from Luva cells. Afterward, the treated HTR-8/SVneo cells in serum-free medium were added into the upper Transwell chamber (3422; Corning) pre-coated with Matrigel (356234; Solarbio). Additionally, the lower chamber was enriched with medium blended with 15% FBS and exploited as chemoattractant. Following the 48-h culture, invaded cells were fixed using 4% paraformaldehyde (P1110; Solarbio), reacted with crystal violet (G1062; Solarbio) for 20 min and finally detected by a microscope (magnification ×250).

2.10 | Bioinformatics assay

TargetScan (http://www.targetscan.org/vert_72/) analyzed the targeting relationship of miR-181a-5p to YY1. JASPAR (https://jaspar.genereg.net/) analyzed the targeting sequences of YY1 and MMP-9 promoter.

2.11 | Targeting relationship validation

Dual-luciferase reporter assay and chromatin immunoprecipitation (ChIP) were performed for targeting relationship verification. Two parts were included in dual-luciferase reporter assay. In the

first part, 293T cells (CL-0005; Procell) were co-transfected with YY1 wild-type (WT) or mutant (MUT) dual-luciferase plasmid and miR-181a-5p mimic or miR-181a-5p mimic control. YY1-WT or YY1-MUT dual-luciferase plasmid was constructed using pmirGLO vector (E1330; Promega Corporation). In the second part, 293T cells were co-transfected with YY1 overexpression plasmid or NC and MMP-9-WT, MMP-9-MUT1 or MMP-9-MUT2. MMP-9-WT, MMP-9-MUT1, and MMP-9-MUT2 were subcloned into pGL3-Basic vector (E1771; Promega). After 48-h transfection, dual-luciferase reporter assays were conducted by a dual-luciferase kit (HY-K1013; MedChemExpress).

For ChIP assay, the primer of MMP-9 is as follows: TATAGTTGGAAAATGGTAGAGTTGG, AATTACAAACATAAACCATAACACC. ChIP assays were carried out by ChIP Assay Kit (P2078; Beyotime). Cells were lysed and cross-linked with 1% formaldehyde (F8775; Sigma-Aldrich), followed by sonication and centrifugation to collect the supernatant. Then, an appropriate amount of primary antibody (anti-IgG (ab171870, 1 μg/m; Abcam) or anti-YY1 (703054, 2 μg/million cells; Invitrogen) was added and co-cultured with the sample at 4°C overnight. Protein A + G Agarose/Salmon Sperm DNA was added and mixed at 4°C for 60 min. DNAs were eluted and used for PCR to detect MMP-9 enrichment.

2.12 | Statistical analysis

Data were presented by mean ± standard deviation. Statistical analyses were analyzed by GraphPad 8.0 software (GraphPad Software). One-way analysis of variance and independent-samples *t* test were employed for difference comparison. A *p* value of <0.05 was deemed as statistical significance.

3 | RESULTS

3.1 | MiR-181a-5p was overexpressed in PE mast cells and affected cell viability

Abnormally high expression of miR-181a-5p was observed in mast cells of PE patients ($p < 0.001$, Figure 1A). To further verify the function of miR-181a-5p, Luva cells were transfected with miR-181a-5p mimic or inhibitor, and the result showed that miR-181a-5p mimic up-regulated yet miR-181a-5p inhibitor down-regulated the level of miR-181a-5p in Luva cells ($p < 0.001$, Figure 1B). Next, we observed that up-regulated miR-181a-5p suppressed Luva cell viability, whereas down-regulated miR-181a-5p played the converse role ($p < 0.05$, Figure 1C).

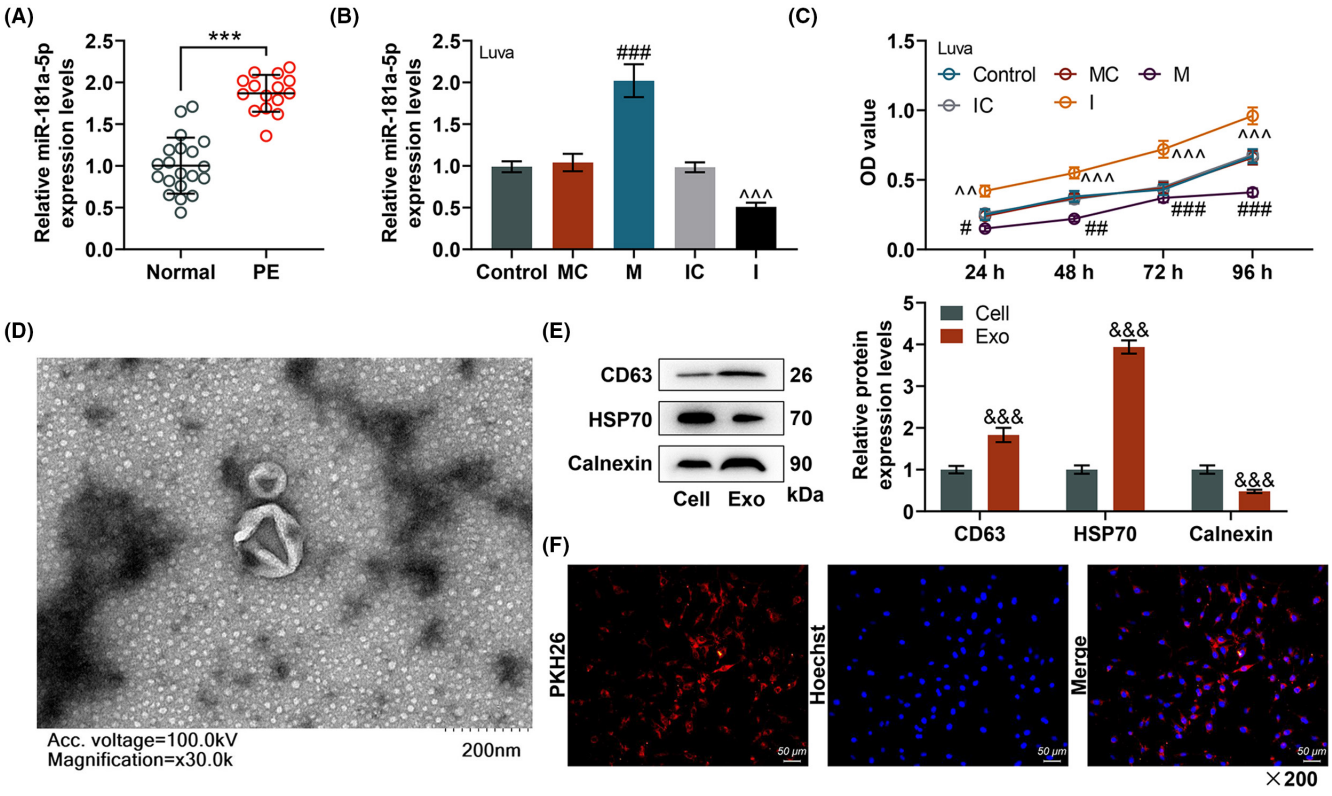

FIGURE 1 Expression and function of miR-181a-5p in mast cells and identification of mast cell-derived exosomes. (A) QRT-PCR was applied to detect the expression of miR-181a-5p in normal ($n = 20$) and PE ($n = 15$) mast cells. (B) QRT-PCR was exploited to gauge the transfection rates of miR-181a-5p mimic (M), inhibitor (I), mimic control (MC), and inhibitor control (IC). (C) MTT was used to detect the viability of mast cells in each group. (D) Transmission electron microscopy was used to observe the morphology of mast cell-derived exosomes. (E) Western blot was employed to quantify the expressions of exosome marker proteins CD63, HSP70, and Calnexin. (F) PKH26 labeling was utilized to identify internalization of exosomes (magnification ×200). qRT-PCR: quantitative real-time reverse transcription-polymerase chain reaction; PE: Preeclampsia; MTT: 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide. $^{***}p < 0.001$ versus normal; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus MC; $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$ versus IC; $^{\&\&\&}p < 0.001$ versus cell

3.2 | Mast cell exosomes secreted miR-181a-5p and transported into HTR-8/SVneo cells

To further examine the role of mast cell-derived miR-181a-5p in PE, we extracted exosomes from mast cells and identified their morphology (in the form of goblet structures with size ranging from 30 to 200 nm) by transmission electron microscopy (TEM) (Figure 1D). Western blot results showed that exosomes contained higher levels of CD63 and HSP70, and lower level of Calnexin ($p < 0.001$, Figure 1E). Furthermore, we noticed that PKH26-labeled exosomes were internalized into the cytoplasm of HTR-8/SVneo cells (Figure 1F). We transfected miR-181a-5p mimic into mast cells and then extracted exosomes. It can be noticed that miR-181a-5p mimic elevated the content of miR-181a-5p in exosomes, while miR-181a-5p inhibitor generated the inverse effect ($p < 0.001$, Figure 2A). Next, we co-cultured differently treated

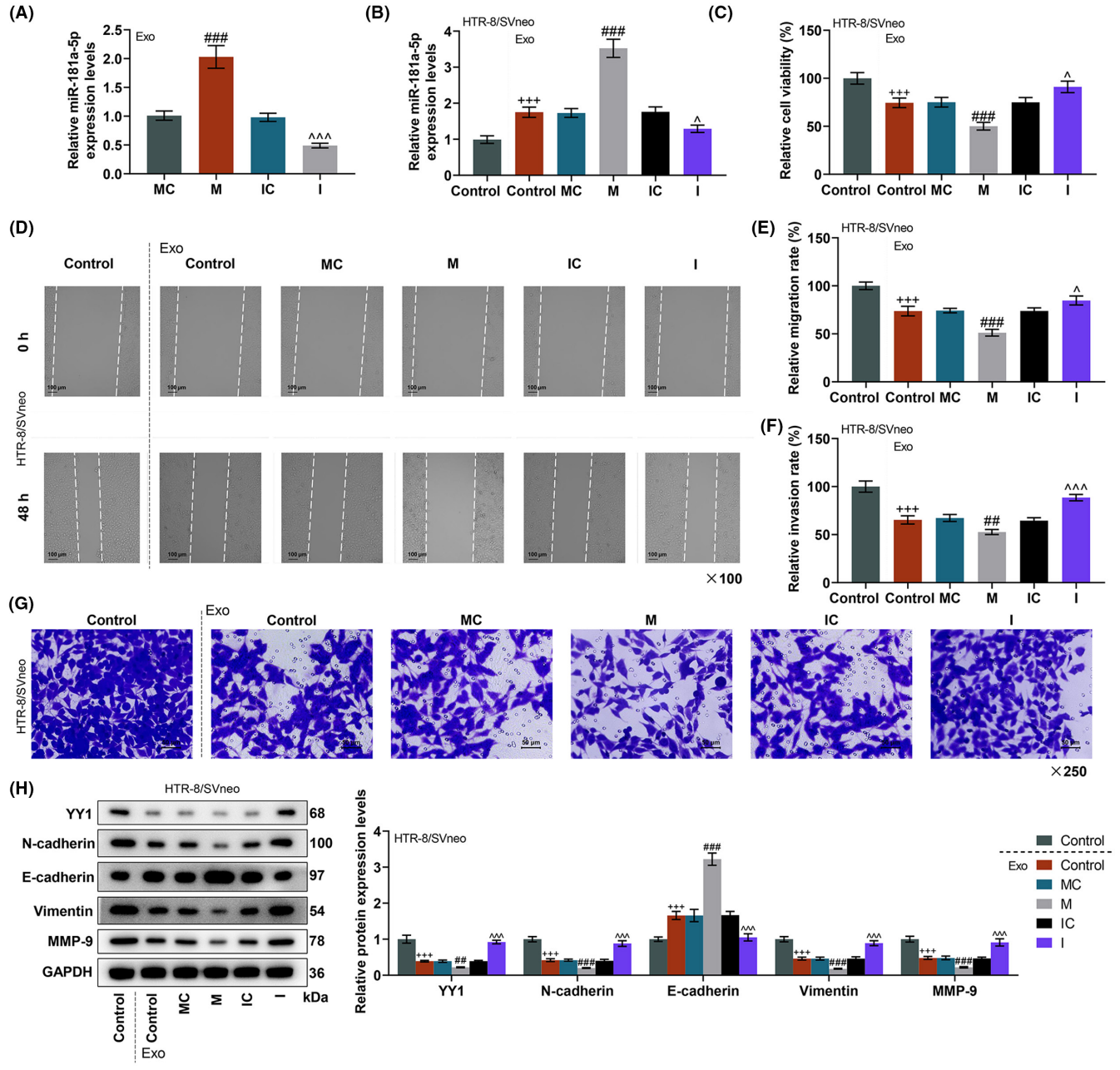

FIGURE 2 Mast cell-derived exosomal miR-181a-5p regulated trophoblast cell migration and invasion. (A, B) The effect of up- or down-regulation of miR-181a-5p on miR-181a-5p level in exosomes or co-culture systems was detected by qRT-PCR. (C) The effect of mast cell-derived miR-181a-5p on the viability of trophoblast cells was detected by MTT. (D, E) The effect of miR-181a-5p up- or down-regulation in exosomes or co-culture systems on trophoblast cell migration was determined using wound healing assay. (F, G) The cell invasion in each group was detected by Transwell. (H) Western blot was applied to measure the protein expressions of YY1, N-cadherin, E-cadherin, Vimentin, and MMP-9, with GAPDH as the control. $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus MC; $^{\wedge}p < 0.05$, $^{\wedge\wedge\wedge}p < 0.001$ versus IC; $^{+++}p < 0.001$ versus control (HTR-8/SVneo cells)

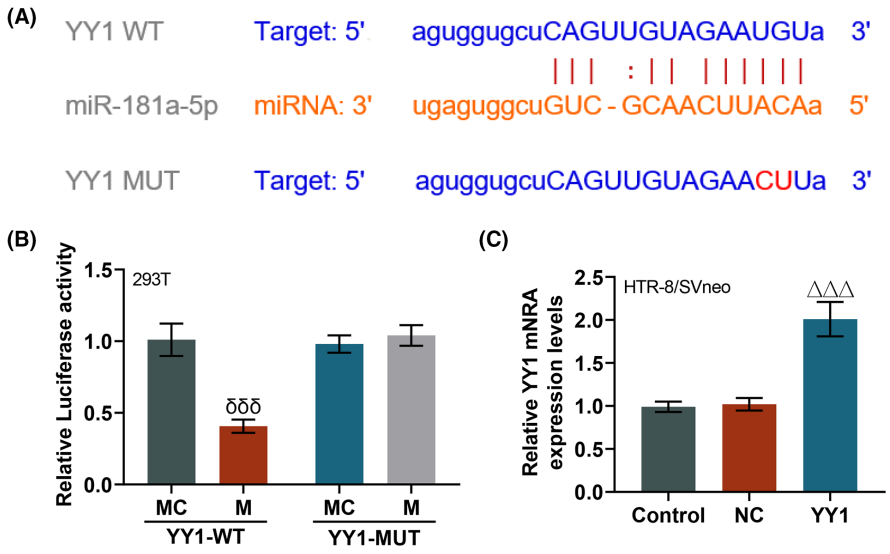

FIGURE 3 YY1 targeted by miR-181a-5p. (A) TargetScan (http://www.targetscan.org/vert_72/) was used to predict the targeting relationship of miR-181a-5p to YY1. (B) Dual-luciferase reporter assay was used to verify the binding of miR-181a-5p to YY1. (C) Transfection of YY1 overexpression plasmid increased YY1 level in HTR-8/SVneo cells as detected by qRT-PCR. $^{\delta\delta\delta}p<0.001$ versus YY1-WT + MC; $^{\Delta\Delta\Delta}p<0.001$ versus NC (negative control)

exosomes with HTR-8/SVneo cells and uncovered that exosomal miR-181a-5p mimic also increased whereas miR-181a-5p inhibitor decreased miR-181a-5p expression in HTR-8/SVneo cells ($p<0.05$, Figure 2B).

3.3 | Mast cell-derived exosomal miR-181a-5p modulated trophoblast cell viability, migration and invasion

Functional experiments revealed that exosomes from mast cells suppressed the viability, migration and invasion of HTR-8/SVneo cells. MiR-181a-5p mimic further enhanced the inhibitory effect of exosomes, while miR-181a-5p inhibitor attenuated the role of exosomes ($p<0.05$, Figure 2C–G). Mechanistically, mast cell exosomes repressed the expressions of YY1, N-cadherin, Vimentin, and MMP-9 proteins, and facilitated the expression of E-cadherin protein ($p<0.001$, Figure 2H). The effects of exosomes on the above proteins were reinforced by miR-181a-5p mimic but were neutralized by miR-181a-5p inhibitor ($p<0.01$, Figure 2H).

3.4 | Mast cell-derived exosomal miR-181a-5p may modulate trophoblast cell function through YY1/MMP-9

TargetScan analyzed the targeted binding sites of miR-181a-5p and YY1 as exhibited in Figure 3A. The YY1 wild-type sequence, instead of the mutant sequence, could reduce relative luciferase activity in combination with the miR-181a-5p mimic ($p<0.001$, Figure 3B). Next, we up-regulated YY1 expression level in HTR-8/SVneo cells by transfecting YY1 overexpression plasmid ($p<0.001$, Figure 3C).

The suppressive effect of overexpressed miR-181a-5p in mast cells on HTR-8/SVneo cell viability was offset by YY1 overexpression ($p<0.001$, Figure 4A). Likewise, YY1 overexpression boosted cell migration and invasion and reversed the suppressive effect of exosomal miR-181a-5p mimic on HTR-8/SVneo cells ($p<0.01$, Figure 4B–E). Mechanistically, overexpressed YY1 elevated N-cadherin, Vimentin, and MMP-9 protein expressions and lessened E-cadherin protein expression, which additionally, offset the effect of exosomal miR-181a-5p mimic on the above proteins ($p<0.001$, Figure 4F).

JASPAR predicted that YY1 targets MMP-9, which was validated by dual-luciferase reporter assay and ChIP ($p<0.001$, Figure 5A–C). Specifically, YY1 overexpression intensified the relative luciferase activity of MMP-9-WT but not MMP-9-MUT ($p<0.001$, Figure 5B). ChIP assay findings reflected that YY1 could efficiently enrich MMP-9 level ($p<0.001$, Figure 5C).

4 | DISCUSSION

The in-depth study regarding the pathogenesis of PE is of great significance for further developing disease diagnosis and treatment. Mast cells not only have immunomodulatory functions, but may also regulate trophoblast invasion, angiogenesis, and spiral artery remodeling.[5] Here, we indicated that miR-181a-5p was overexpressed in mast cells of PE patients, reflecting its possible role as a diagnostic marker for PE. Furthermore, mast cell-derived exosomal miR-181a-5p modulated trophoblast cell viability, migration and invasion via YY1/MMP-9.

Earlier, the role of placenta-derived exosomes in PE has been emphasized.[18] For example, one study indicated that extravillous trophoblast-derived exosomes modulate intrauterine vascular remodeling.[19] Exosomal miRNAs have also presented potential as diagnostic markers and in treating PE.[20] Huang et al. unraveled that human umbilical cord mesenchymal stem cell-derived exosomal miR-18b-3p inhibits the development of PE symptoms in rats by targeting LEP.[21] Notably, Wang et al. identified that placental exosomes derived from PE pregnant women hamper the proliferation and invasion of HTR-8/SVneo cells,[22] and such effect may be driven by exosomal miR-15a-5p which regulates PI3K/

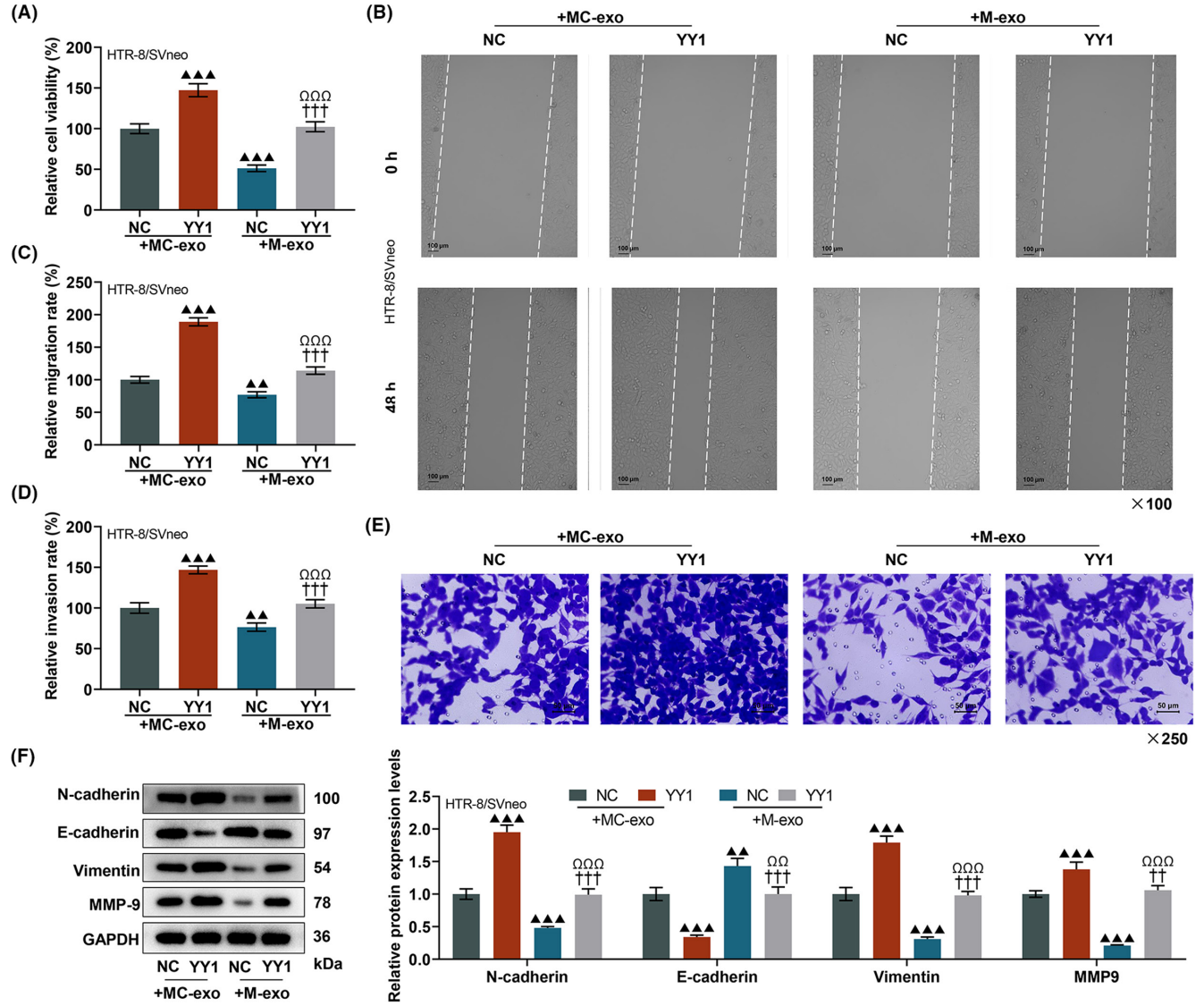

FIGURE 4 YY1 overexpression reversed the regulation of mast cell-derived exosomal miR-181a-5p on trophoblast cells. (A) After co-culture of exosomes containing miR-181a-5p mimic or mimic control with YY1-overexpressing HTR-8/SVneo cells, cell viability was detected by MTT assay. (B, C) Cell migration rates were measured in wound healing assays 48 h after cell treatment. (D, E) Transwell assay was employed to determine the effects of YY1 overexpression and exosomal miR-181a-5p on trophoblast cell invasion. (F) The protein expressions of N-cadherin, E-cadherin, Vimentin and MMP-9 were detected by Western blot. $^{▲▲}p<0.01$, $^{▲▲▲}p<0.001$ versus MC-exo + NC; $^{††}p<0.01$, $^{†††}p<0.001$ versus MC-exo + YY1; $^{ΩΩ}p<0.01$, $^{ΩΩΩ}p<0.001$ versus M-exo + NC

AKT signaling pathway through CDK1.[22] Here, we put forward a different opinion and discovered that mast cell-derived exosomes also can reduce the biological function of HTR-8/SVneo cells, but this effect includes a "credit" for the cargo miR-181a-5p. Mast cell-derived exosomes are often mentioned in the study of immune regulation.[23,24] Furthermore, in colitis-related studies, the function of miR-181a is proven to correlate with immunosuppression.[25] At the same time, miR-181a is also associated with epithelial-mesenchymal transition (EMT), myocardial remodeling, and hypertension.[25,26] In this study, a new piece of evidence was provided through forward and reverse validation showing that mast cell-derived exosomal miR-181a-5p inhibits trophoblast viability, migration, invasion, and EMT progression.

Another focus of our attention is the mechanism based on the role of mast cell-derived exosomal miR-181a-5p in HTR-8/SVneo cells. The most common regulatory mechanism of miRNA is to inhibit the transcription and translation of mRNA by binding to the 3′-UTR end of mRNA.[27] In PE, it has also been reported that miR-181a-5p accommodates the biological function of HTR-8/SVneo cells via targeting IGF2BP2.[15] On these bases, we predicted a different downstream target gene of miR-181a-5p, namely YY1 which was confirmed in our follow-up experiments.

As report goes, YY1 is aberrantly expressed in various diseases and has been documented as a possible cancer therapeutic target.[28–30] YY1 was demonstrated to be involved in regulating the pathological mechanism of PE and may be affected

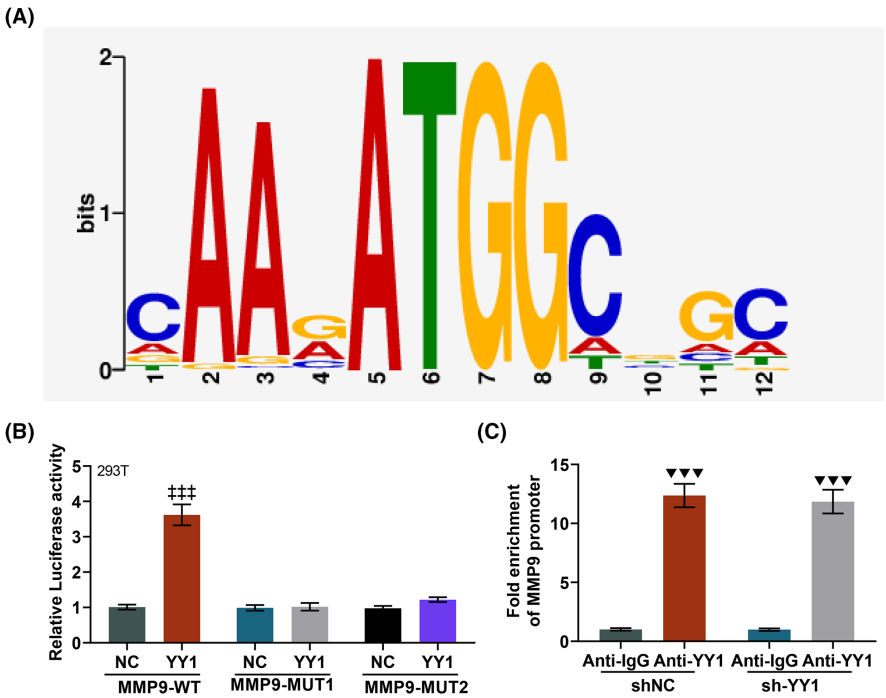

FIGURE 5 YY1 bound to MMP-9. (A) JASPAR (https://jaspar.genereg.net/) analysis of the targeting sequences of YY1 and MMP-9. (B, C) The binding relationship between YY1 and MMP-9 was verified using dual-luciferase reporter assay and chromatin immunoprecipitation. ‡‡‡$p < 0.001$ versus MMP-9-WT + NC; ▼▼▼$p < 0.001$ versus Anti-IgG

by lncRNA-ATB/miR-651-3p.[17] Currently, in the context of scarce studies on YY1 in PE, our data further demonstrated the role of YY1 in trophoblast cell invasion. Strikingly, the data supported another mechanism of YY1, namely inhibition by mast cell-derived exosomal miR-181a-5p.

What also drew our attention was the finding that MMP-9 is inversely connected with miR-181a-5p and is associated with poor prognosis in PE.[16] Previous literature has pointed out that MMP-9 is targeted by miR-183-5p and partially promotes the invasion and migration of HTR-8/SVneo trophoblast cells in PE.[31] Herein, we uncovered that YY1 can bind to the MMP-9 promoter and may be implicated in initiating the transcription-translation process of MMP-9. Combined with the previous results, we rationally speculated that miR-181a-5p secreted in mast cells could inhibit MMP-9 expression by targeting YY1 in trophoblast cells to affect cell migration and invasion.

Collectively, our study verified the specific expression of miR-181a-5p in mast cells from the placenta of PE patients and clarified that exosomal miR-181a-5p regulates the malignant biological phenomenon of trophoblast cells through the YY1/MMP-9 axis. Our study, from the immunological perspective, innovatively expounds the occurrence of PE, and provides a new approach and laboratory evidence for the clinical search of diagnostic markers.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Aner Chen. The first draft of the article was written by Yinfen Wang, and all authors commented on previous versions of the article. All authors read and approved the final article.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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