

Materials and methods

Subjects 20 women with normal pregnancies and 20 women with PE from Jiaying Municipal Maternal and Child Health Care Hospital were enrolled in the study. All specimens collected from patients were confirmed by pathology prior to storage at -80°C . All enrolled patients signed informed consent. The research study design was approved by the Ethics Committee of Jiaying Municipal Maternal and Child Health Care Hospital affiliated with Jiaying University.

Cell lines and transfection JAR and JEG-3 cell lines were all obtained from the Chinese Academy of Sciences (Shanghai, China). The JAR cell line was cultured in RPMI-1640 medium (Sigma-Aldrich, St. Louis, MO, USA) and the JEG-3 cell line was cultured in minimum Eagle's medium (MEM; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% (v/v) fetal bovine serum (FBS; Thermo Fisher Scientific, Inc.), 100 IU/ml penicillin and 100 IU/mL streptomycin (both from Thermo Fisher Scientific, Inc.) at 37°C in a humidified atmosphere containing 5% (v/v) CO_2 . The appropriate number of cells were seeded into 6-well plates, and cells were grown to approximately 80% confluency. Transfection was performed using lipofectamine 3000 (Invitrogen, L3000015) transfection reagent according to the manufacturer's instructions. Mfn2 small interfering (si)RNA, forward 5'-CCAUGAGGCCUUUCUCCTT-3' and reverse 5'-GGAGAAAGGCCUCAUGGTT-3' were synthesized at Sangon Biotech Inc. (Shanghai, China).

Western blot As described in our previous publications (Wan et al., 2019), total protein was extracted according to the protein extraction kit instructions, and subjected to 12% SDS-PAGE electrophoresis. Then the SDS-PAGE was transferred to a PVDF membrane and blocked with 5% (v/v) skim milk at room temperature for 2 hours and the membrane was incubated with either anti-Mfn2 (Abcam, ab124773), anti-CHOP (Abcam, ab124773), anti-GRP78 (Abcam, ab108613), anti-PERK (Abcam, ab229912), anti-Flag (Huabio, 0912-1), or anti- β -actin (Abcam, ab8226) primary antibodies at 4°C overnight. After washing, the membranes were subsequently incubated with secondary antibody for 1 hour at 37°C . An ECL chemiluminescence kit was used to visualize

the protein bands. β -actin was used as the loading control.

Cell proliferation assay. We used a CCK-8 kit (Dojindo, Kumamoto, Japan) to examine cell proliferation according to the manufacturer's instructions. JAR and JEG-3 cells were seeded into 96-well culture plates (1×10^4 cells/well). 24, 48, or 72 hours after transfection, the cells were inoculated with 100 μ L of WST-8 and incubated at 37 °C for 4 hours. The absorbance of the cells at OD₄₅₀ was then measured.

Wound healing assay JAR and JEG-3 cells were seeded in 6-well plates. After 24 hours in culture, the cells were transfected with lipofectamine 3000. After 12 hours, wounds (1 mm wide) were created and then imaged over a 72-hour period using a microscope (CX31; Olympus Corporation, Tokyo, Japan), as described in our previous publications (Wan et al., 2019).

Transwell assay After transfection with Mfn2 siRNA, according to the manufacturer's protocol, transwell experiments were performed to evaluate cell migration and invasion. In the invasion experiment, the upper chambers were pre-coated with Matrigel (BD Biosciences, NJ), while in the migration experiment, the matrigel coating was omitted. Then a total of 500 μ L of medium supplemented with 10% (v/v) FBS was added to the lower chamber respectively. Following a 72-hour incubation, the cells were fixed, stained and counted under a microscope (CX31; Olympus Corporation, Tokyo, Japan).

Establishment of stable cell lines The Mfn2 lentiviral overexpression vector was constructed by integrating the coding sequence (CDS) of Mfn2 into pLVX-3 \times FLAG-Puro. 293T cells were transfected with pLVX-Mfn2-3 \times FLAG-Puro or empty pLVX-3 \times FLAG-Puro lentiviral vector for 8 hours using lipofectamine 3000. Recombined lentiviral vectors were collected 48 hours post transfection and used to infect JEG-3 cells. The empty pLVX-3 \times FLAG-Puro (Vector) was used as a negative control.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) Total RNA was extracted from cells using TRIzol reagent (Invitrogen) according to the manufacturer's protocol.

For the detection of Mfn2 expression, a PrimeScript II 1st Strand cDNA Synthesis Kit was used to synthesize cDNA, and qPCR was performed using the SYBR® Premix Ex Taq™ kit on an Applied Biosystems 7500 thermocycler. Primers were purchased from Sangon Biotech Co., Ltd. The sequence-specific primers for Mfn2 were as follows: F: 5'-ATCTGTGCCAGCAAGTTGACA-3' and R: 5'-AAGTGAATCCAGAGCCTCGAC-3'. The sequence-specific primers for β -actin were as follows: forward, 5'-ATTGCCGACAGGATGCAGAA-3' and reverse, 5'-GCTGATCCACATCTGCTGGAA-3'. The β -actin gene was used as an internal reference. Relative expression levels were computed using the $2^{-\Delta\Delta CT}$ method.

ROS detection by flow cytometry To confirm the ROS levels in JEG-3 cells following treatment, the cells were digested with 0.25% (v/v) trypsin and 500 μ L of 1 μ mol/L CM-H2DCFDA (C6827, Invitrogen) was added to each tube, which were then incubated at room temperature in the dark for 30 min. Next, cells were washed twice with phosphate-buffered solution (PBS) and gently resuspended in DMEM medium containing 10% (v/v) FBS before being analyzed using flow cytometry (ACEA NovoCyte, China).

Detection of SOD levels Protein supernatants were used to evaluate cellular SOD levels using the SOD detection kit protocol provided by the manufacturer (A001-1-1, Jiancheng, China).

Statistical analysis GraphPad Prism 8 software (La Jolla, CA, USA) was used for statistical analysis. Data are expressed as the mean \pm standard deviation (SD). A Student's *t*-test was used to compare groups, and a *P* value of <0.05 was considered statistically significant.

Reference

Wan LX, Sun DD, Xie JM, et al., 2019. Declined placental PLAC1 expression is involved in preeclampsia. *Medicine*, 98:44. <http://dx.doi.org/10.1097/MD.00000000000017676>