MicroRNA-144-3p may participate in the pathogenesis of preeclampsia by targeting Cox-2

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Abstract. Preeclampsia remains a major cause of maternal mortality and morbidity worldwide. It is generally accepted that the development of the placenta, including spiral artery remodelling, normal trophoblast cells function and maternal-fetal inflammation-immune interactions, is critical for the pathogenesis of preeclampsia. Several investigations have demonstrated that microRNAs (miRNAs/miRs) in the placenta may be potential molecular markers for diagnosis of preeclampsia. In the current study, the aim was to investigate the expression of miR-144-3p in the placenta of patients with preeclampsia and normal placentas, and to explore the potential target genes. miRNA microarray analysis was performed using three paired placentas (preeclampsia and normal) in order to find differential expression of miRNAs. Following this, miR-144-3p was selected as a differentially expressed miRNA and validated using *in situ* hybridization to determine the clinical significance in placentas with preeclampsia. A potential target gene of miR-144-3p, cyclooxygenase-2 (Cox-2), was identified by bioinformatics, luciferase reporter assay and western blotting. The expression of Cox-2 was also examined by immunohistochemical staining of samples of placenta from patients with preeclampsia and normal placenta. Western blot analysis was performed to investigate the effect of miR-144-3p on the expression of Cox-2 in HTR-8/SVneo cells *in vitro*. miR-144-3p was decreased in placentas from patients with preeclampsia. A luciferase reporter assay demonstrated that Cox-2 was a potential miR-144-3p target gene and the result was verified by western blotting. A negative correlation was observed between miR-144-3p and Cox-2 in preeclamptic placenta by immunohistochemical staining and *in situ* hybridization. Western blot analysis demonstrated that overexpression of miR-144-3p decreased Cox-2 expression by 38.2% in HTR-8/SVneo cells. Understanding the differential expression of miR-144-3p and its association with Cox-2 may aid the exploration of the pathogenesis of preeclampsia, and contribute to the development of miRNA-based therapies in the future.

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Introduction

Preeclampsia (PE) is a pregnancy-specific disorder that manifests as newly developed maternal hypertension and proteinuria during the second half of pregnancy, and remains a major cause of maternal mortality and morbidity worldwide (1,2). Newly defined features of preeclampsia also include maternal organ dysfunction, and long-term effects on cardiovascular disease in the mother later in the life (3,4). At present, the etiology and pathogenesis of preeclampsia remains elusive. Defective trophoblastic invasion, anomalous maternal-fetal inflammation-immune interactions and oxygen dysregulation have been reported to be associated with the pathogenesis of preeclampsia. A series of cascading reactions lead to preeclampsia syndrome, which eventually results in endothelial cell damage, followed by vasospasm, plasma leakage, ischemia and thrombosis. Delivery of the fetus and the placenta is the only effective method to eradicate the clinical manifestations of preeclampsia (5).

In the past decades, great efforts have been made to explore how microRNAs (miRNAs/miRs) regulate human placental physiology. miRNAs, a class of small, non-coding RNAs, modulate the expression of nearly a third of human genes (6). It has been demonstrated that miRNAs have essential roles in several biological processes, including proliferation, differentiation, cell growth and development (7). Equally, it has been demonstrated that there are numerous differentially expressed miRNAs in human placenta, which act via targeting of specific genes with diverse functions, and have an important role in normal and pathological placental physiology (8-10).

In the present study, the miRNA expression profile in the placenta of patients with preeclampsia was assessed using

microarray analysis. Based on the results of validation and literature retrieval, miR-144-3p was detected in the placentas of patients with preeclampsia and normal placentas using *in situ* hybridization (ISH) and immunohistochemical staining. A dual-luciferase reporter assay was used to investigate the potential miR-144-3p target gene cyclooxygenase-2 (Cox-2) and demonstrated that the expression of Cox-2 was negatively regulated by miR-144-3p. Taken together, it is speculated that miR-144-3p may be involved in the pathogenesis of preeclampsia by targeting Cox-2.

Materials and methods

Patients and tissue samples. The placental samples, from 25 women with severe preeclampsia and 25 with normal pregnancy, were obtained from the Department of Gynecology and Obstetrics, Yangzhou Women and Children Hospital (Yangzhou, China) between October 2014 and November 2016. The clinical data of the pregnant women are presented in Table I. Chorionic tissue blocks (~1 cm^3) were collected from the mother surface of the placenta and near the root of the umbilical cord. Tissues were washed with sterile PBS, then immediately frozen in liquid nitrogen at the time of surgery and stored at -70˚C until needed. This study was approved by the ethics board of Yangzhou Women and Children Hospital and informed consent was obtained from all 50 participants. The present study was approved by the ethics board of Yangzhou Women and Children Hospital and informed consent was obtained from all participants. All clinical investigations were conducted according to the principles of the Declaration of Helsinki.

miRNA microarray and reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and QIAGEN miRNeasy Mini kit (Qiagen GmbH, Hilden, Germany), quantitated on the NanoDrop 2000 (Thermo Fisher Scientific, Inc., Wilmington, DE, USA), labeled using the FlashTag Biotin HSR RNA Labeling kit (Affymetrix, cat no. 901911; Thermo Fisher Scientific, Inc.) and hybridized onto the GeneChip2 Hybridization (Affymetrix, cat no. 902413; Thermo Fisher Scientific, Inc.). Following the washing steps, the slides were scanned using the GeneChip2 Scanner 3000 7G (Affymetrix; Thermo Fisher Scientific, Inc.). All expressed data were normalized using the median normalization method, and significantly differentially expressed miRNAs were identified through volcano plot filtering. Data were analyzed by GCBI online software (GCBI, R3.3.1, http://www.gcbi.com.cn; Genminix Informatics Co., Ltd., Shanghai, China). RT-qPCR analysis for miR-337-3p, miR-187-3p, miR-122-5p, miR-26b-5p and miR-144-3p was performed with a miDETECT A TRACK™ miRNA qRT-PCR Starter kit (RiboBio Co., Ltd., Guangzhou, China). The miDETECT A TRACK™ Uni-RT primer was used for RT (Guangzhou RiboBio Co., Ltd., Guangzhou, China). The miDETECT A TRACK™ Forward Primer and Uni-Reverse Primer were used for qPCR (Guangzhou RiboBio Co., Ltd.). The reactions were incubated in a 96-well plate at 95˚C for 10 min, followed by 40 cycles at 95˚C for 2 sec, 60˚C for 20 sec and 70˚C for 10 sec. The miRNA Primers used in RT-qPCR were synthesized by RiboBio, Co., Ltd., and the primer sequences are commercially restricted. The expression of miRNA was normalized to the small nuclear RNA U6 expression as an endogenous control. The fold changes of mRNA expression were calculated by the $2^{-\Delta\Delta Cq}$ method.

Cell culture. The HTR-8/SVneo cells were maintained in the laboratory of Department of Gynecology and Obstetrics, the Affiliated Drum Tower Hospital of Nanjing University Medical School (Nanjing, China). The cells were cultured in RPMI-1640 (HyClone; GE Healthcare Life Sciences, Logan, UT, USA), supplemented with 10% heat-inactivated fetal bovine serum (Wisent, Inc., St. Bruno, Quebec, Canada), 100 U/ml penicillin and 100 mg/ml streptomycin, at 37˚C in a humidified 5% CO_2 incubator.

Luciferase reporter assays. The bioinformatics software TargetScan (version 7.0; http://www.targetscan.org/) (11) was used to predict the target genes of miR-144-3p. The Cox-2 3'untranslated region (3'UTR)-hRLuc reporters (PTGS2 WT) were created by ligation of the Cox-2 3'UTR PCR product into the *Xho*I and *Not*I site of the dual-luciferase reporter vector (pmiR-RB-REPORT™; RiboBio Co., Ltd.). The mutant reporters for the Cox-2 3'UTR (PTGS2 MT; CAUUUAAUG *G*GUACUGUAU) were generated by replacing the binding sites of miR-144-3p. Subsequently, HTR-8/SVneo cells were cultured in 96-well plates ($1.5x10^4$) and transfected with the wild or mutant luciferase reporters (100 ng), and miR-144-3p mimics (50 nM) using Lipofectamine® 2000 reagent (Invitrogen; Thermo Fisher Scientific, Inc.). All reactions were run in triplicate. After 48 h, luciferase activities were measured using the dual-luciferase reporter system (Promega, Madison, WI, USA). Normalized luciferase activity was reported as *Renilla* luciferase activity/luciferase activity.

miRNA ISH analysis. To detect the expression levels of human miR-144-3p in placenta tissue, ISH was performed using a miRCURY locked nucleic acid (LNA) detection probe for miR-144-3p (hsa-miR-144-3p/DigN/AGTACATCATCT ATACTGTA) (Exiqon; Qiagen GmbH; probe concentration 25 μM). The digoxigenin double-labeled LNA-modified probe, at a final concentration of 500 nmol/l, was added to the hybridization solution and hybridized according to the manufacturer's protocol. The sample images were captured using a light microscope at 100x magnification after the incubation of slides with 4-nitroblue-tetrazolium for 30 min at 25˚C and with nuclear fast red for 5 min at room temperature (Roche Applied Science, Indianapolis, IN, USA). The staining intensity and the proportion of miR-144-3p-positive cells were detected in 50 placenta samples (25 from preeclampsia women and 25 from women without preeclampsia). A total of 10 fields of view in each slide was randomly selected for analysis. The 4 intensity grades of staining cells were negative, weak, intermediate and strong, with corresponding scores of 0, 1, 2 and 3 respectively. Another score was given for the proportion of positive cells with the scoring rule as follows: No positive cells (0 point), 1-24% positive cells (1 point), 25-49% positive cells (2 points), 50-74% positive cells (3 points), 75-100% positive cells (4 points). The staining index (SI) was calculated

Table I. Clinical characteristics of normal and preeclamptic pregnancies.

Characteristics	Normal	Preeclampsia	P-value
Maternal age (years)	28.04±3.09	29.24±4.05	0.245
Systolic blood pressure (mmHg)	116.44±8.76	160.88±15.29	<0.001
Diastolic blood pressure (mmHg)	73.16±7.37	104.08±12.62	<0.001
Proteinuria	0	2.08±0.76	<0.001
Gestational age at delivery (weeks)	39.16±0.80	37.03±1.58	<0.001
Birthweight (g)	3490.80±344.27	2844.80±524.34	<0.001
miR-144-3p expression	235.48±105.49	163.91±92.33	0.014
Cox-2 expression	1.40±0.65	1.96±0.93	0.017

Cox-2, cyclooxygenase-2.

by multiplying the staining intensity and the proportion of positive cells. According to the definition of SI scores, low expression of miR-144-3p (1.0) was described as SI score ≤4, high expression of miR-144-3p (3.0) was described as SI score >8 and intermediate (2.0) >4 and ≤8.

Immunohistochemical staining. Immunohistochemical staining was performed using an immunohistochemistry detection kit (cat no. PV-6000; OriGene Technologies, Inc., Beijing, China) according to the manufacturer's instructions. A rabbit polyclonal antibody for Cox-2 (1:1,000; ab15191, Abcam, Cambridge, UK) was used as primary antibody. A goat anti-rabbit secondary antibody (1:1,000; A0208; Beyotime Institute of Biotechnology, Beijing, China) was used as the secondary antibody. The expression of Cox-2 was evaluated using a scoring system by the combining the staining intensity score and proportion of positive cells. Quantification of the staining intensity of Cox-2 was performed using image analysis as with ISH. A total of 10 fields of view in each slide was randomly selected for analysis.

Establishment of stably expressing miRNA-144-3p cell lines by lentivirus. HTR-8/SVneo cells with stable miRNA-144-3p expression and parental cell lines were established using a lentiviral expression system. The lentiviruses containing MiR-144-3p (MIMAT0000436: GAGCAGGGA GCAGGAAGCTGTGTGTGTCCAGCCCTGACCTGTCCT GTTCTGCCCCCAGCCCCTCACAGTGCTTTTCAAGCC ATGCTTCCTGTGCCCCCAGTGGGGCCCTGGCTGGG ATATCATCATATACTGTAAGTTTGCGATGAGACACTA CAGTATAGATGATGTACTAGTCCGGGCACCCCCAGC TCTGGAGCCTGACAAGGAGGACAGGAGAGATGCTG CAAGCCCAAGAAGCTCTCTGCTCAGCCTGTCACAAC CTACTGACTGCCAGGGCACTTGGGA) or negative control were constructed by GeneChem Co., Ltd., (Shanghai, China). All the lentiviral vectors expressed enhanced green fluorescent protein and puromycin resistance gene, which allowed the measurement of the infection efficiency and to select for infected cells. Cells ($5x10^6$/15 ml) were infected with Vector or MiR-144-3p lentivirus (15 μg), followed by culture in 5 μg/ml puromycin (Clontech Laboratories, Inc., Mountainview, CA, USA) to select stable lines. The resulting stable lines were used for further analysis.

Western blot analysis. Cells were collected and homogenized in lysis buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1% SDS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.02% sodium azide, 100 mg/l phenylmethylsulfonyl fluoride and 1 mg/l aprotinin. The supernatant was collected after centrifuging at 11,000 x g for 15 min at 4˚C. Protein concentration was determined using the bicinchoninic acid method (Pierce; Thermo Fisher Scientific, Inc.). SDS-PAGE using 10% gels was performed to separate protein (50 μg/lane) and separated proteins were then transferred to polyvinylidene difluoride membranes. PVDF membranes were blocked with 5% non-fat milk for 1 h at room temperature and incubated with primary antibodies against Cox-2 (1:1,000; ab15191; Abcam), GAPDH as a loading control (1:1,000; sc-32233; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and Cox-1 as a specific control (1:1,000; ab109025; Abcam) overnight at 4˚C. After washing, the membranes were incubated with goat anti-rabbit secondary antibody (1:5,000; cat no. sc-2004; Santa Cruz Biotechnology, Inc.) for 2 h at room temperature and visualized with enhanced chemiluminescence (Millipore, Billerica, MA, USA) by Quantity one software (version 62, Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Statistical analysis. Results were analyzed using the SPSS 22.0 statistical software (IBM Corp., Armonk, NY, USA). Experimental data are presented as the mean ± standard deviation. The differences for multiple groups were estimated using one-way analysis of variance followed by Dunnett's T3 post hoc test, and the association between miR-144-3p expression and the Cox-2 level was analyzed by Spearman correlation analysis. The independent two-tailed Student's t-test was performed for comparisons between two groups. P<0.05 was considered to indicate a statistically significant difference.

Results

miR-144-3p is downregulated in patients with preeclampsia compared with normal placentas. Firstly, miRNA microarray analysis of samples from six pregnant women (three from normal and three from preeclampsia cases) and the expression of miRNAs in paired placentas were compared to identify differential expressed miRNAs. The inclusion criteria of differential miRNAs was differential expression of ≥2.0 fold

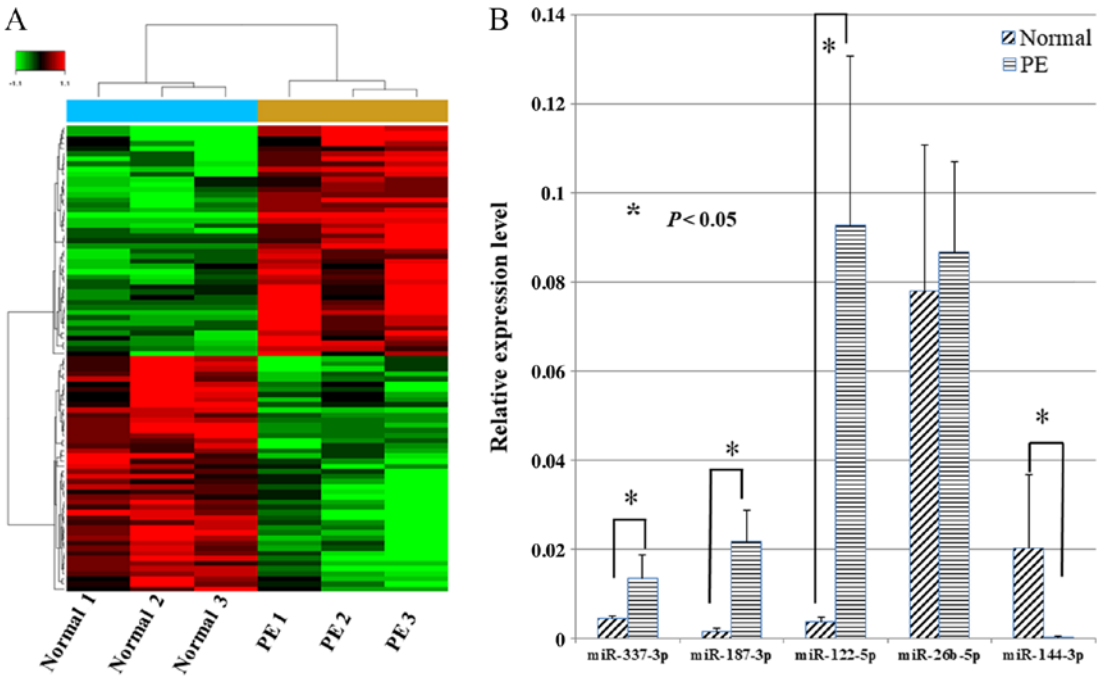

Figure 1. Differentially expressed miRs in preeclampsia placentas compared with normal placentas. (A) Microarray analysis of differentially expressed miRs from six pregnant women (three from normal and three from preeclampsia). Each row represents a miR and each column represents a sample pair of placentas from women with preeclampsia and normal pregnancy. (B) Bar graphs present reverse transcription-quantitative polymerase chain reaction expression of miR-337-3p, miR-187-3p, miR-122-5p, miR-26b-5p and miR-144-3p in placentas with preeclampsia and normal placentas. All RT-qPCR reactions were run in triplicate. The data are presented as relative expression following normalization. *P<0.05 vs. normal. PE, preeclampsia; miR, microRNA.

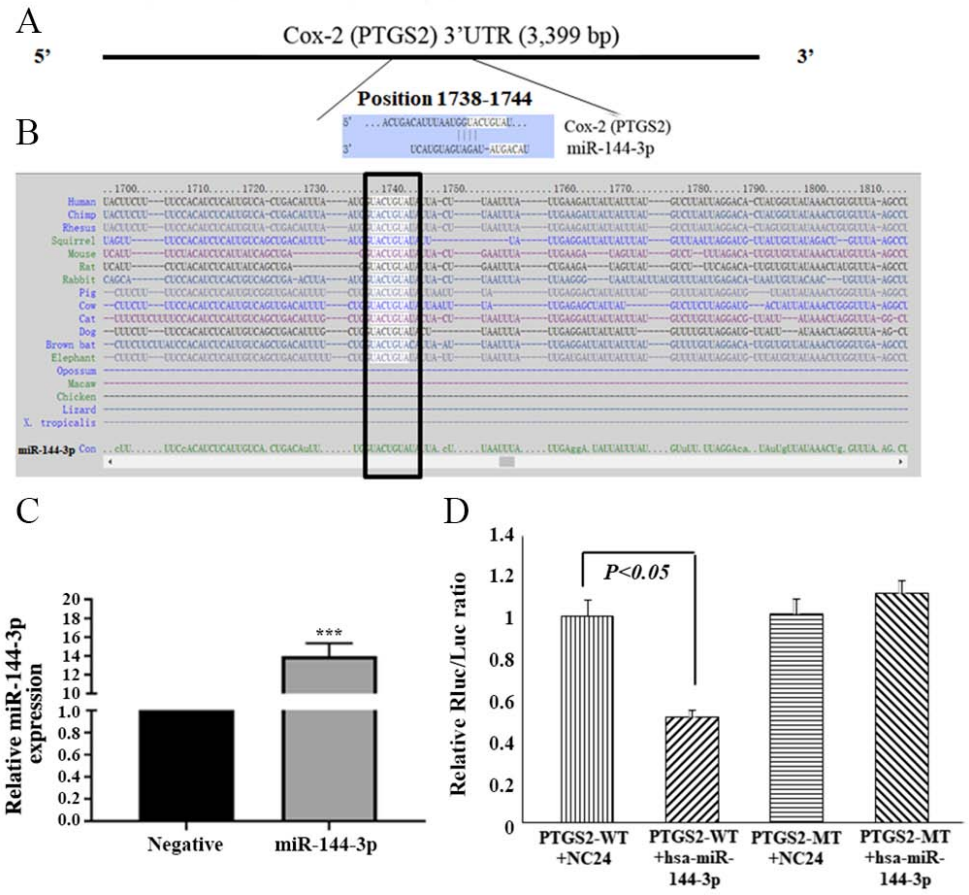

Figure 2. miR-144-3p targets the 3'UTR of Cox-2 directly. (A and B) The 3'UTR of Cox-2 harbors one complementary sites for the miR-144-3p sequence at its 3'UTR. (C) miR-144-3p overexpression. (D) Luciferase activity decreased by 46.5% when the 3'UTR of Cox-2 and miR-144-3p mimics were co-transfected in HTR-8/SVneo cells. ***P<0.05 vs. negative. Cox-2, cyclooxygenase-2; PTGS2, prostaglandin-endoperoxide synthase 2; UTR, untranslated region; miR, microRNA; WT, wild type; MT, mutant; NC, non-target Control.

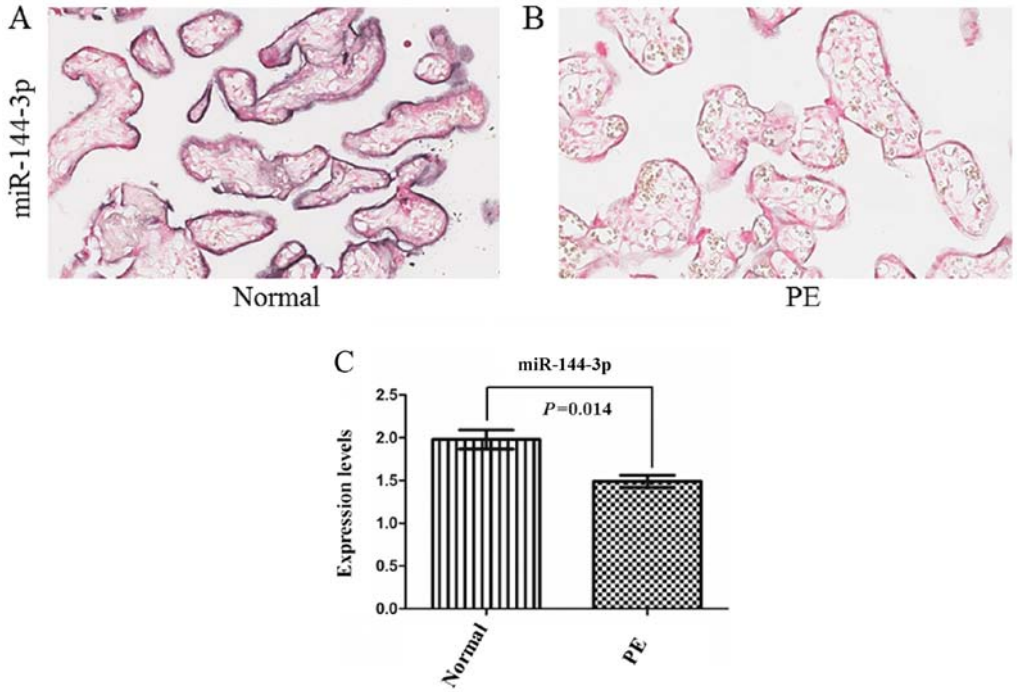

Figure 3. miR-144-3p was downregulated in placenta of patients with PE. (A) High miR-144-3p expression in normal placenta and (B) low miR-144-3p expression in PE placenta determined by *in situ* hybridization. (C) miR-144-3p was downregulated in placentas of patients with PE (P=0.014) compared with normal placenta. ISH Data are expressed as the mean ± standard error. miR, microRNA; PE, preeclampsia.

changes. In total, 46 miRNAs were downregulated and 45 were upregulated in the six samples (Fig. 1). Subsequently, the five miRNAs with the highest fold changes (differential expression of >4.0 fold changes), including miR-337-3p, miR-187-3p, miR-122-5p, miR-26b-5p and miR-144-3p, were selected to verify the results of the microarrays. Notably, RT-qPCR validation demonstrated that the fold changes in the five miRNAs expression were 2.99, 15.37, 24.91, 0.87 and 0.016, respectively, in preeclampsia placentas compared with the paired normal placentas (Fig. 1). Ultimately, miR-144-3p was selected as the focus of further research as it exhibited the greatest fold change.

miR-144-3p directly targets Cox-2 by interacting with the 3'UTR. Cox-2, also known as prostaglandin-endoperoxide synthase 2 (PTGS2), was selected as a candidate miR-144-3p target based on bioinformatics analysis (TargetScan) and functional knowledge. The 3'UTR of Cox-2 has a complementary site for the miR-144-3p seed sequence at its 3'UTR (Fig. 2A and B), and it is highly conserved among species. To further determine whether Cox-2 is a direct target of miR-144-3p, a luciferase reporter construct harboring a fragment of the Cox-2 3'UTR that contained the miR-144-3p binding site was prepared (PTGS2 WT). A construct with mutated miR-144-3p binding site in the Cox-2 3'UTR (PTGS2 MT) was also generated. Luciferase activity was decreased by 46.5% in HTR-8/SVneo cells co-transfected with Cox-2 3'UTR (PTGS2 WT) and miR-144-3p (Fig. 2C and D). By contrast, the luciferase activity was from the Cox-2 3'UTR-mut (PTGS2 MT) construct was not significantly affected by transfection with miR-144-3p levels in HTR-8/SVneo cells (Fig. 2D). These results indicate that miR-144-3p targets the Cox-2 3'UTR directly and downregulates its expression.

Clinicopathological features of clinical samples. The basic clinical characteristics of the patients in the preeclampsia and control group are presented in Table I. Patients in the preeclampsia group exhibited significant increase in the systolic pressure and diastolic pressure compared with the control group (P<0.0001). Notably, patients with preeclampsia exhibited proteinuria. Furthermore, the gestational age at delivery was significantly lower for patients with PE than for normal controls (P<0.0001). miRNA ISH analysis was used to compare the expression of miR-144-3p in placenta samples of the two groups (Fig. 3A and B). The results revealed that the expression of miR-144-3p was significantly decreased in placentas from the PE group compared with the normal group (P<0.05; Fig. 3C).

Negative correlation between miR-144-3p and Cox-2 expression was observed in placentas. Tissue assays comprising 25 pairs of placentas with preeclampsia and normal placentas were examined for miR-144-3p expression using ISH. ISH revealed differential expression of miR-144-3p in preeclampsia and normal placentas (Fig. 3). Immunohistochemical staining for Cox-2 was performed using the same placenta tissues used for miR-144-3p ISH analysis (Fig. 4A and B). The expression of Cox-2 was negatively correlated with miR-144-3p, as demonstrated by Spearman correlation analysis (r=-0.509, P=0.001; Fig. 4C).

miR-144-3p regulates the expression of Cox-2 in HTR-8/SVneo cells in vitro. To further investigate the association between miR-144-3p and Cox-2, HTR-8/SVneo cells were transfected with miR-144-3p vector or Vector control. The lentiviral transduction efficiency was assessed (Fig. 5A-C). Western blot analysis was performed to investigate the effect of miR-144-3p

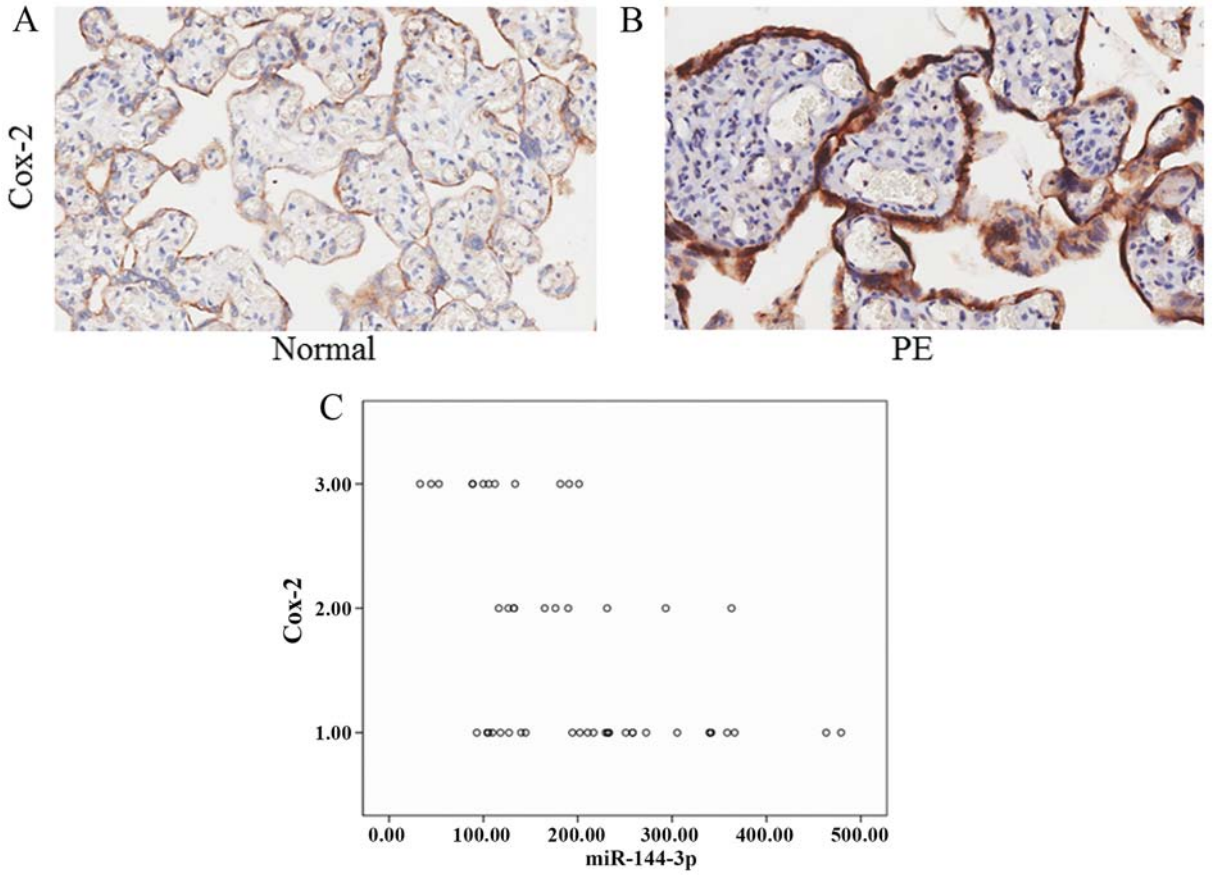

Figure 4. Cox-2 expression in placenta of normal controls and patients with PE. (A) Low Cox-2 expression in normal placenta and (B) high Cox-2 expression in PE placenta. (C) The expression of Cox-2 was negatively correlated with miR-144-3p, Spearman correlation analysis (r=-0.509, P=0.001). PE, preeclampsia; Cox-2, cyclooxygenase-2; miR, microRNA.

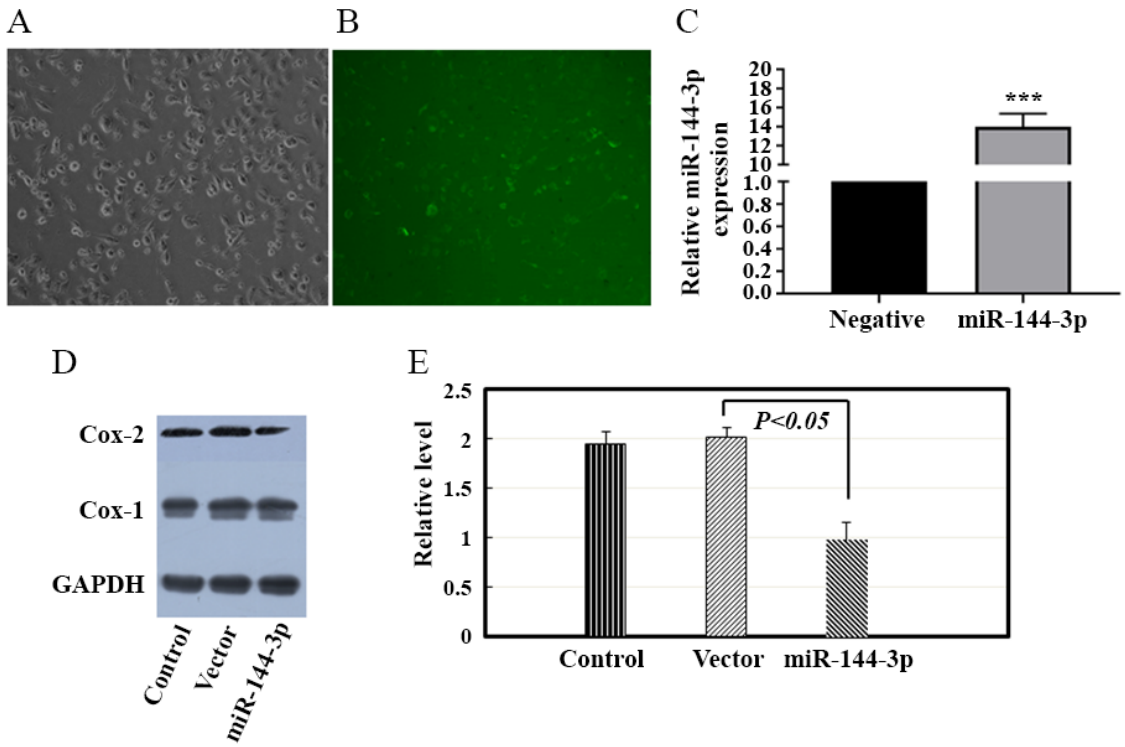

Figure 5. Overexpression of miR-144-3p decreased Cox-2 expression in HTR-8/SVneo cells. (A) Optical and (B) corresponding fluorescence images of HTR-8/SVneo cell infected with stable miRNA-144-3p-expression lentivirus vectors. (C) Effect of miR-144-3p overexpression. (D) Western blot of Cox-2 expression in HTR-8/SVneo cells with and without miR-144-3p overexpression. (E) Changes in expression levels of Cox-2 in HTR-8/SVneo cells induced by miR-144-3p. Overexpression of miR-144-3p decreased Cox-2 expression by 38.2% in HTR-8/SVneo cells.***P<0.05 vs. negative. miR, microRNA; Cox, cyclooxygenase.

on the expression of Cox-2 in HTR-8/SVneo cells *in vitro*. Overexpression of miR-144-3p decreased Cox-2 protein expression by 38.2% in HTR-8/SVneo cells (Fig. 5D and E).

Discussion

Because of the severity of preeclampsia, its pathogenesis has been the focus of continuous research. Increasing evidence indicates that miRNAs may be involved in the pathogenesis of preeclampsia (8-10). In the current study, miR-144-3p was selected as an RNA of interest based on microarray and RT-qPCR results. The expression of miR-144-3p and Cox-2 were compared in placenta samples from patients with preeclampsia and paired normal placentas, using ISH and immunohistochemical staining. The expression of miR-144-3p was downregulated in placentas of patients with preeclampsia compared with paired normal placentas, which was consistent with previous reports (12,13). In addition, the current study also demonstrated that the expression of miR-144-3p was negatively associated with the expression of Cox-2. A dual-luciferase reporter assay suggested that Cox-2 was a direct target of miR-144-3p and its expression was negatively correlated with expression of miR-144-3p.

miRNAs are short, non-coding RNA molecules that post-transcriptionally regulate gene expression by perfect or imperfect binding to the 3'UTR of target mRNAs, thus causing mRNA degradation or modification of mRNA translation. miRNAs control diverse biological possesses, including cell proliferation, apoptosis, migration and invasion. Pineles *et al* (8) first reported that miRNAs were expressed differentially in preeclampsia placentas. Certain miRNAs have been reported to be involved in the pathogenesis of preeclampsia. For instance, miR-181a-5p, miR-299, miR-30a-3p and miR-155 have been reported to be involved in the pathogenesis of preeclampsia by targeting different genes (14-17). Xiao *et al* (12) discovered that miR-144 contributed to preeclampsia by targeting phosphatase and tensin homolog in trophoblastic cells. Consistently, the expression of miR-144-3p was downregulated in placentas of patients with preeclampsia compared with paired normal placentas in the current study; however Cox-2 was identified as the miR-144-3p target in the current study. Several studies have identified the targets of miR-144-3p in different disease; for instance, miR-144-3p inhibits cell proliferation and induces apoptosis in multiple myeloma by targeting c-MET proto-oncogene, receptor tyrosine kinase (18); miR-144-3p suppresses proliferation and migration of colorectal cancer cells via the G1 to S phase transition 1 gene (19); other reported targets of miR-144-3p also include serum/glucocorticoid regulated kinase 1 (20), TNF superfamily member 11 (21) and runt related transcription factor 1 (22), which have predominantly been described in cancer research. There is no report on the interaction between these targets and miRNAs in the pathogenesis of preeclampsia. Yao *et al* (23) validated that Cox-2 was a direct target of miR-144 and that miR-144 negatively regulated the expression of Cox-2 in gastric cancer. Together, these studies indicate that each miRNA can have multiple target genes, and several miRNAs can regulate the same gene. Therefore, miRNAs and the target genes may form a complex regulatory network during different pathological and biological possesses. However, the relationship between miR-144-3p and Cox-2 on preeclampsia has not been reported previously.

Cox-2, as a rate-limiting enzyme of prostaglandin synthesis, can regulate the levels of prostaglandins (24). The roles of Cox-2 in preeclampsia have been discussed in previous studies (25-27). Goksu *et al* (26) reported that the expression of Cox-2 was increased in the placenta of patients with preeclampsia, presumably due to the decreased levels of prostaglandin E and prostaglandin F in the placenta. Inflammation and vascular endothelial damage can lead to vasospasm, which is one of important physiological manifestations of preeclampsia (28). Endothelial cell injury can also activate platelets and clotting factors, which will cause an aggravation of current hypercoagulable state in pregnant women. A large number of inflammatory substances can stimulate and adhere to the white blood cell molecules expressed on the surface of endothelial cells in a short time. Excessive prostaglandin production can stimulate the production of pro-inflammatory cytokines and increase vascular permeability, promote the adhesion of monocytes and macrophage migration, and induce macrophage chemotaxis (29-31). All of these ultimately lead to systemic inflammation and endothelial cell injury in preeclampsia (32).

In summary, the present investigation demonstrated that the expression of miR-144-3p was decreased that Cox-2 was increased in preeclamptic placentas, and the expression of the two markers had a negative correlation, and the association between miR-144-3p and Cox-2 was also verified *in vitro*. However, the research has some limitations. One limitation is that the sample size was relatively small, and more samples should be included to confirm the conclusions, which could be solved through multi-center cooperation in the future. Another limitation of this study is that the association between miR-144-3p and Cox-2, and the mechanisms in preeclampsia should be further explored *in vivo* using animal models; for example, in a mouse with a miR-144-3p conditional deletion or overexpression in the placenta. Additionally, the microarray analysis also identified other miRNAs, including miR-337-3p, miR-187-3p, miR-122-5p and miR-26b-5p, which may be involved in the pathogenesis of preeclampsia. Further work is required to study the miRNA network associated with the pathogenesis of preeclampsia. The utility of changing the expression of miRNAs may provide novel strategies for preeclampsia therapy.

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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.

Authors' contributions

SH, JL and LM designed the experiments; SH, MT, QL and YC performed the experiments; HL and YW analyzed the data; SH and LM wrote the manuscript.

Ethics approval and consent to participate

The present study was approved by the ethics board of Yangzhou Women and Children Hospital (Yangzhou, China) and informed consent was obtained from all participants.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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