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CLINICAL STUDY

The significance of miR-145 in the prediction of preeclampsia

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ABSTRACT

AIM: The aim of this study was to explain the effects of miRNA-145 in the pathogenesis of preeclampsia.

METHODS: Collecting the placental tissue of 40 severe preeclampsia patients and 20 normal pregnant women, and observation of the pathological findings by HE staining. Measuring the miR-145 by RT-PCR. EVCT were divided into NC group; MC group and miRNA group. The EVCT cells of MC and miRNA groups were simulated by hypoxia in vivo by $CoCl_2$. Measuring the proliferation rate of different groups by MTT testing. The cells apoptosis rates were measured by flow cytometry; evaluating PI3K, Akt, mTOR and P53 gene and protein expression of three groups by RT-PCR and WB.

RESULTS: Compared to the normal pregnant placental tissue. The miR-145 expression of preeclampsia pregnant placental tissue was significantly decreased ($p < 0.05$). In the cell experiments, the proliferation rate was significantly increased, and the cell apoptosis rate was significantly reduced in MC group compared to the MC group ($p<0.05$, respectively). Comparing with MC group, the PI3K, Akt and mTOR gene and protein expression of miRNA group were significantly up-regulated and the P53 expression was significantly down-regulated ($p<0.05$, respectively).

CONCLUSION: miR-145 might have effects to predict preeclampsia via PI3K/Akt/mTOR signalling pathways *(Fig. 5, Ref. 30)*. Text in PDF *www.elis.sk.*

KEY WORDS: preeclampsia, EVCT, MiR-145, PI3K/Akt/mTOR, P53.

Introduction

Preeclampsia is a disease in the late pregnancy, the disease originate in placenta and is a serious threat to the maternal and child health, placenta plays an important role in the pathogenesis of the disease (1). Nap outside nourish cells has played a key role in placenta formation and in a successful pregnancy to ensure the oxygen and nutrients exchange between foetus and mother, which has a vital significance (2). So far, the pathogenesis of preeclampsia was not clearly explained, but more recognized cause of hypothesis is increased apoptosis of Sertoli cell, leading to insufficient placental blood supply (3 – 6).

MicroRNAs are a class of about 21 ~ 26 bases, which widely exist in animals and plants of non-coding single small RNAs that are highly conservative, stable and evolution after the gene transcription level functions to regulate cell growth, and has an extremely important pathological and physiological significance (7). Related researches confirmed that there was a close correlation between miRNA and cell apoptosis and proliferation (8 – 11). In this study, we detected miRNA-145 expression in normal maternal and preeclampsia women, and explored miRNA-145's effects in the mechanism of the preeclampsia.

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Materials and methods

Study objects

Selecting 40 patients that were pregnant with severe preeclampsia from 2012.10 to 2015.9 treated in our hospital, the average age was (30.25 ± 5.16) years, the average gestational age was (37.15 ± 1.65) weeks. Selecting 20 pregnant women with normal late pregnancy as the control group in the same period, the average age was (29.74 ± 4.16) years, the average gestational age was (37.86 ± 1.32) weeks. There were no significant differences between the two pregnant women groups in age and gestational age ($p > 0.05$, respectively). Pregnant women included in this study had no history of smoking, blood transfusion, and immunotherapy, were singletons and other obstetric complications were ruled out. After caesarean section, the placenta tissue from the normal group, mild preeclampsia group and severe preeclampsia group was fully rinsed, fixed to 10 % Formaldehyde Solution, paraffin embedded, serial sections, HE stained to observe the histological characteristics of placenta and measuring the miRNA-145 expression in difference tissues.

Materials

Primitive culture EVCT; DMEM/F12 (U.S, Hyclone company); Trypsin, BSA, DAPI (U.S, Sigma company); SYBR Green qPCR Maser Mix (Japan, Toyobo company); rabbit anti human PI3K, Akt, mTOR and P53 (U.S, Abcam company); Primer and miRNA-145 (Shanghai biological engineering co.,LTD)

Cell culture

EVCT cells were divided into the three groups: NC group, MC group and miRNA group. NC group were cultured with DMEM/F12; MC group were using $CoCl_2$ chemical hypoxia induced cell hypoxia; miRNA group were based on MC group adding miRNA-145 transfection.

MTT Detection

We used MTT (Sigma, USA) manual kit following by instructions to determine the cell survival rate.

Cell apoptosis testing

Cells inoculated in six orifice by 5×10^4 density , culturing the cells in DMEM/F12 contained 10 % foetal bovine serum, until cell fusion achieved 50.0 % ~ 60.0 %. The cells were treated by different methods, collecting cell to the EP tube after pancreatic enzyme digestion, washing cells twice by PBS, centrifugal as 10000 r/min for 5min, measuring the cell apoptosis of difference groups.

RT-PCR

Exacting total RNA of the 3 groups by Trizol. We used spectrophotometer to measure the total RNA concentration and to evaluate purity. According to the Takara retroviruses kit prospectus method, reverse transcription to RNA synthesis for cDNA, reverse transcription system of 20 μl was used. 1μl product reverse transcription cDNA into the PCR cycle was taken. Reaction system: 95 °C 10 s, 60 °C 10 s, 72 °C 10 s. The whole reaction system for 45 cycle. GAPDH as reference in this study, using $2^{-\Delta\Delta Ct}$ methods to measure the gene expression. Primer sequences:

GAPDH: F: 5'-CGCTGAGTACGTCGTGGAGTC-3'
R: 5'-GCTGATGATCTTCAGGCTGTTGTC-3'
Akt: F: 5'-CAAGCCCAAGCACCGT-3'
R: 5'-GAATCACCTTCCCAAAGGTG-3'
PI3K: F: 5'-CATCACTTCCTCCTGCTCTAT-3'
R: 5'-CAGTTGTTGGCAATCTTCTTC-3'
mTOR: F: 5'-CGCTGTCATCCCTTTATCG-3'
R: 5'-ATGCTCAAACACCTCCACC-3'
P53: F: 5'-AGGCCTTGGAACTCAAGGAT-3'
R: 5'-CCCTTTTTGGACTTCAGGTG-3'

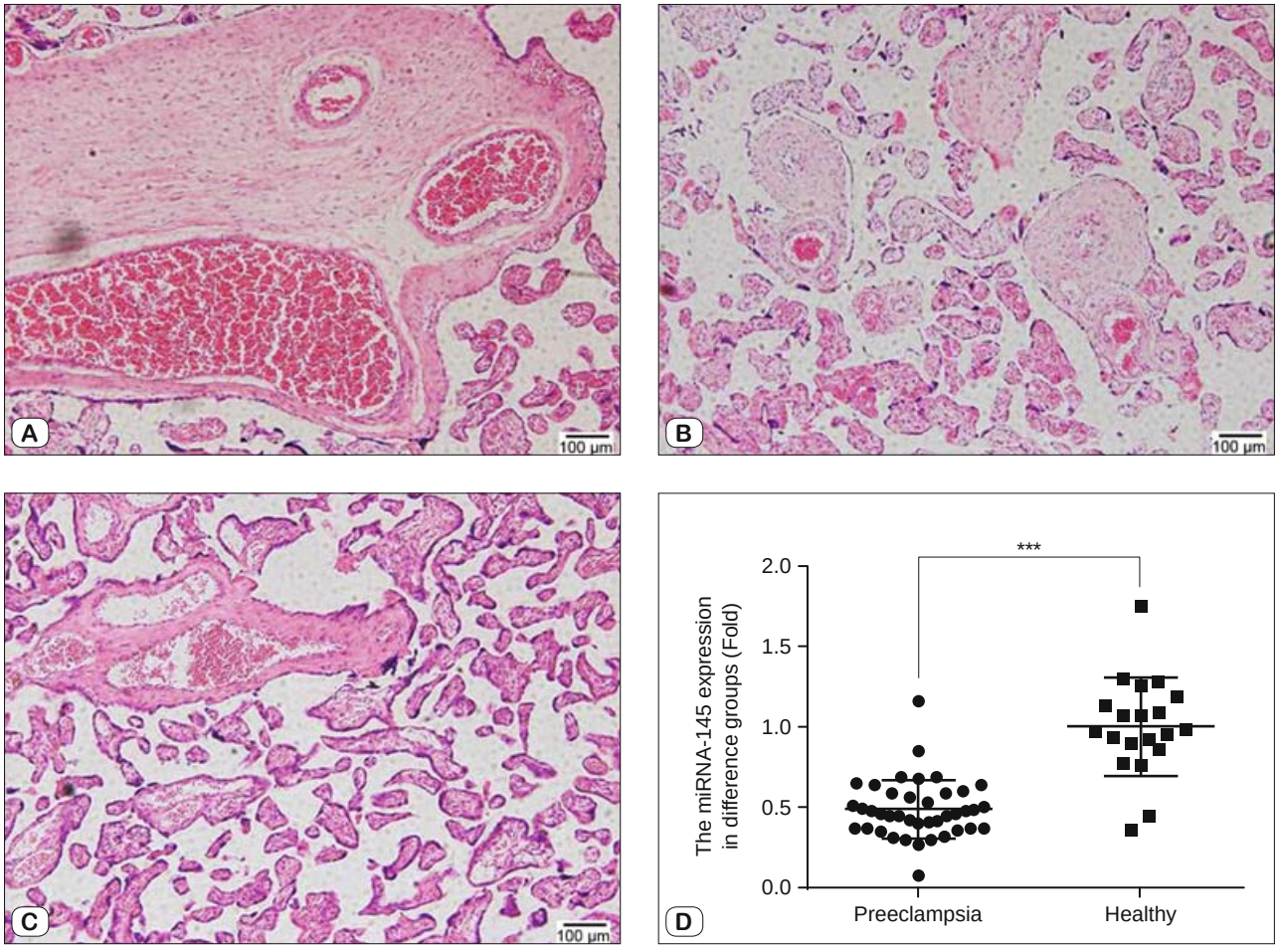

Fig. 1. The clinical data in difference tissues. A) The Placental tissue of Normal puerperal. B) The Placental tissue of Mild preeclampsia. C) The Placental tissue of severe preeclampsia. D) The miRNA-145 expression of difference tissues. ***: p < 0.05, Compared with Healthy puerperal.

WB assay

Total proteins were extracted from the cells of all groups. Protein concentrations were determinate by BCA. The sample was separated by 50 SDS-PAGE with a concentration of 12 % g, which was transferred to the membrane. The membrane was closed and added to the assay for the night. Washing the membrane, adding two anti, staining in room temperature incubation 1h, with TBST after washing the membrane.

Statistical analysis

All data were expressed by the mean±SD ($\bar{x}\pm s$), All data were used and included in SPSS19.0 (SPSS Inc., Chicago, USA) statistical package for statistical analysis. Using ANOVA and LSD analysis was used to analyse all the data, $p < 0.05$ showed that the difference was statically significant.

Results

Clinical data

The trophoblast cells in normal term placental villi and mild pre- eclampsia placental villi were mainly trophoblastic cells (Figs 1A, 1B). In severe preeclampsia placenta, trophoblast cell proliferation, basement membrane thickening of trophoblastic cells, villous interstitial fibrin deposition, syncytiotrophoblast nodules increased, syncytiotrophoblast budding, fibrinoid necrosis of villi increased, villous vessels increased, villi part of not mature decidual stromal cells showed a different size block, rhabditiform, decidual vascular endothelial cell fibrosis, decidual fibrinoid necrosis was also increased significantly (Fig. 1C). Compared to the healthy pregnant women, the miR-145 expression of preeclampsia women was significantly reduced ($p < 0.05$), the data are shown in Figure 1D.

MTT testing

The cell proliferation rate of miRNA group was significantly increased compared to that of MC group ($p < 0.05$), the data are shown in Figure 2.

Cell apoptosis

The cell apoptosis rate of miRNA group was significantly reduced compared to that of MC group ($P<0.05$), the data are shown in Figure 3.

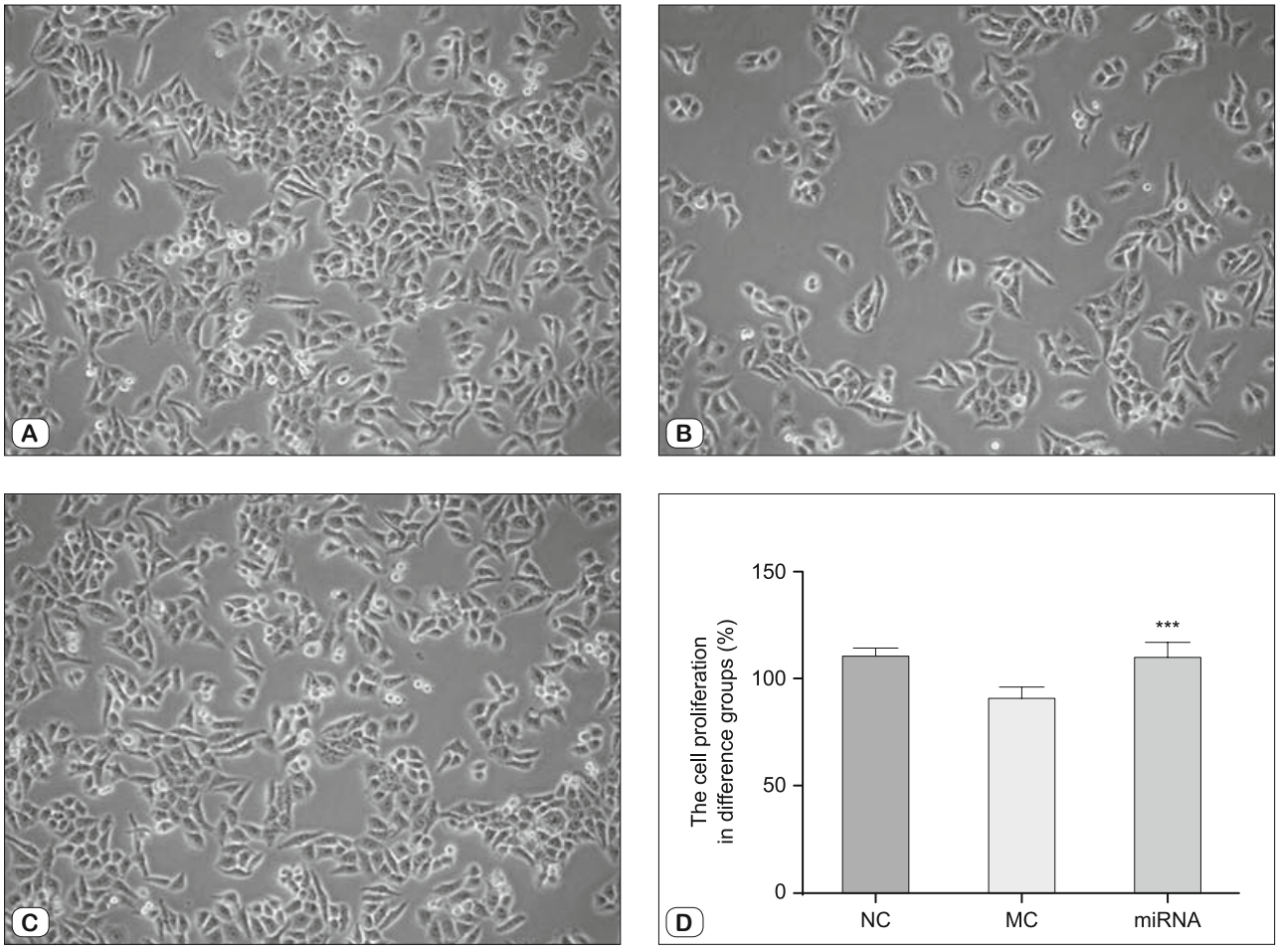

Fig. 2. The proliferation rate of difference groups. A) The proliferation cells of NC group. B) The proliferation cells of MC group. C) The proliferation cells of miRNA group. D) Comparing the proliferation rate of difference groups. ***: p < 0.05, Compared with MC group.

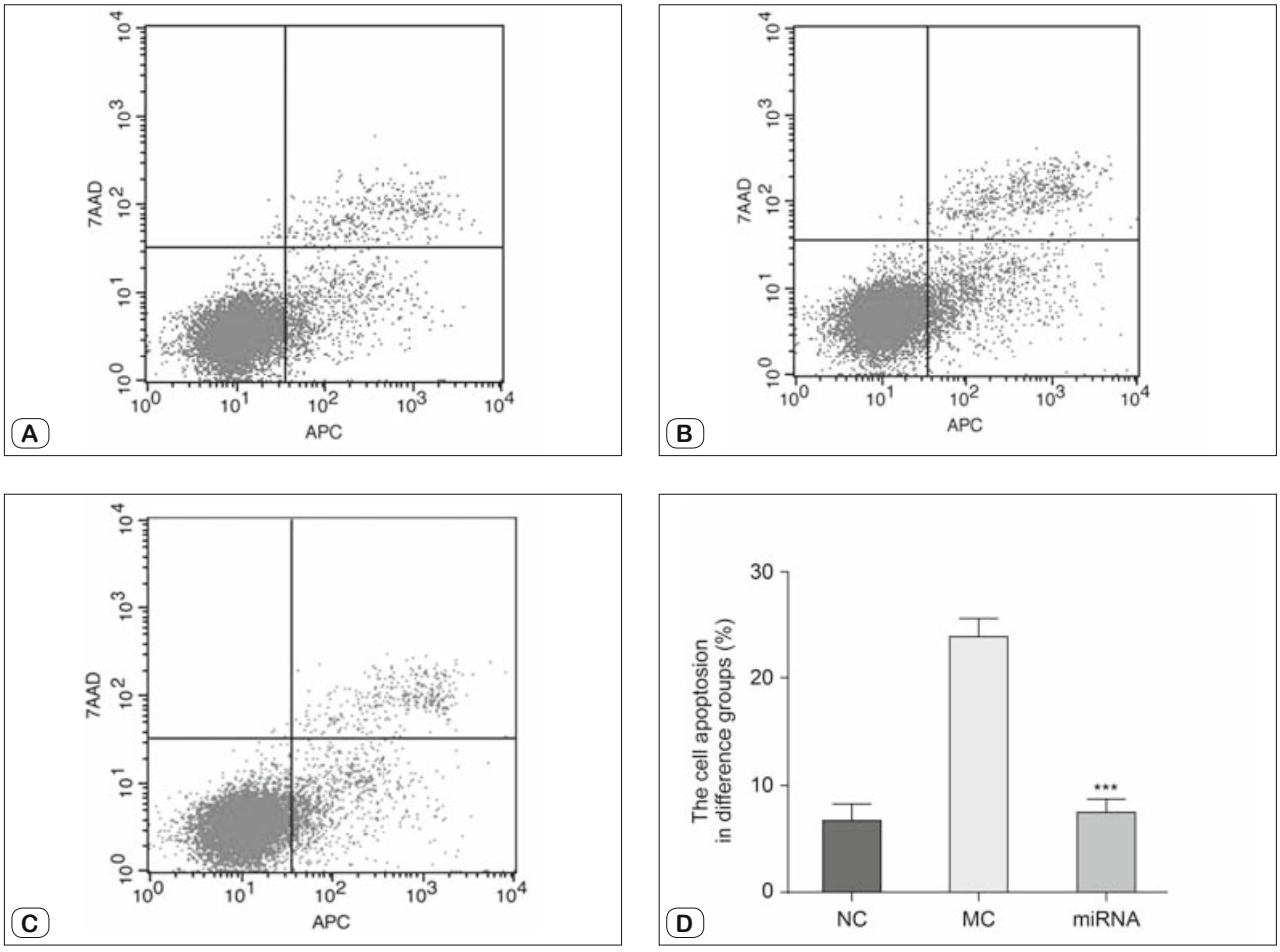

Fig. 3. The cell apoptosis rate of difference groups. A) The apoptosis rate of NC group. B) The apoptosis rate of MC group. C) The apoptosis rate of miRNA group. D) Comparing the apoptosis rate of difference groups. ***: p < 0.05, Compared with MC group.

RT-PCR

Comparing to the MC group, the PI3K, Akt and mTOR gene expression of miRNA group were significantly up-regulated, however, the P53 gene expression of miRNA group was significantly down-regulated (($p < 0.05$, respectively). The data are shown in Figure 4.

WB testing

Comparing to the MC group, the PI3K, Akt and mTOR protein expression of miRNA group were significantly up-regulated, however, the P53 protein expression of miRNA group was significantly down-regulated ($p < 0.05$, respectively). The data are shown in Figure 5.

Discussion

In normal pregnancy, Sertoli cell apoptosis with the growth of the placenta and the extension of gestational age. However, in preeclampsia, abnormal cellular renewal is present. Placental villi Sertoli cell apoptosis increases. Preeclampsia placenta lesion features include: fuzzy morphological changes, cell apoptosis and fit nodules fall off; these changes and the imbalance of oxygen and inflammatory factors have a close correlation (12–16). Apoptosis in preeclampsia and placental pathologic changes of the specific mechanism remains to be studied. Numerous studies confirmed that the miRNA-145 could effectively regulate the proliferation and apoptosis of tumour cells (17–20), however, the role of miRNA-145 in preeclampsia is still limited. This study was aimed to evaluate miRNA-145 in preeclampsia function and mechanism.

PI3K / Akt / mTOR signalling pathways is one of the important ways of the cell membrane receptor signal transduction, such as cell growth, proliferation, metabolism, apoptosis plays an important role in this process (21). PI3K can regulate AKT activation, phosphorylated AKT activation or inhibit further downstream target proteins, involved in cell proliferation, migration, survival and metabolism and other physiological activities (22). AKT as the central part of the pathway, in charge of by PI3K initiating biological information transmission, can be applied to the downstream of the mTOR targets (23, 24). Related studies confirmed the activation of mTOR and there was a close correlation between P53 gene expression (25–27). And studies have shown that excessive P53 expression could promote cell apoptosis (28–30).

In this study, we found that miRNA-145 gene expression of preeclampsia patient's placenta tissue was significantly lower than

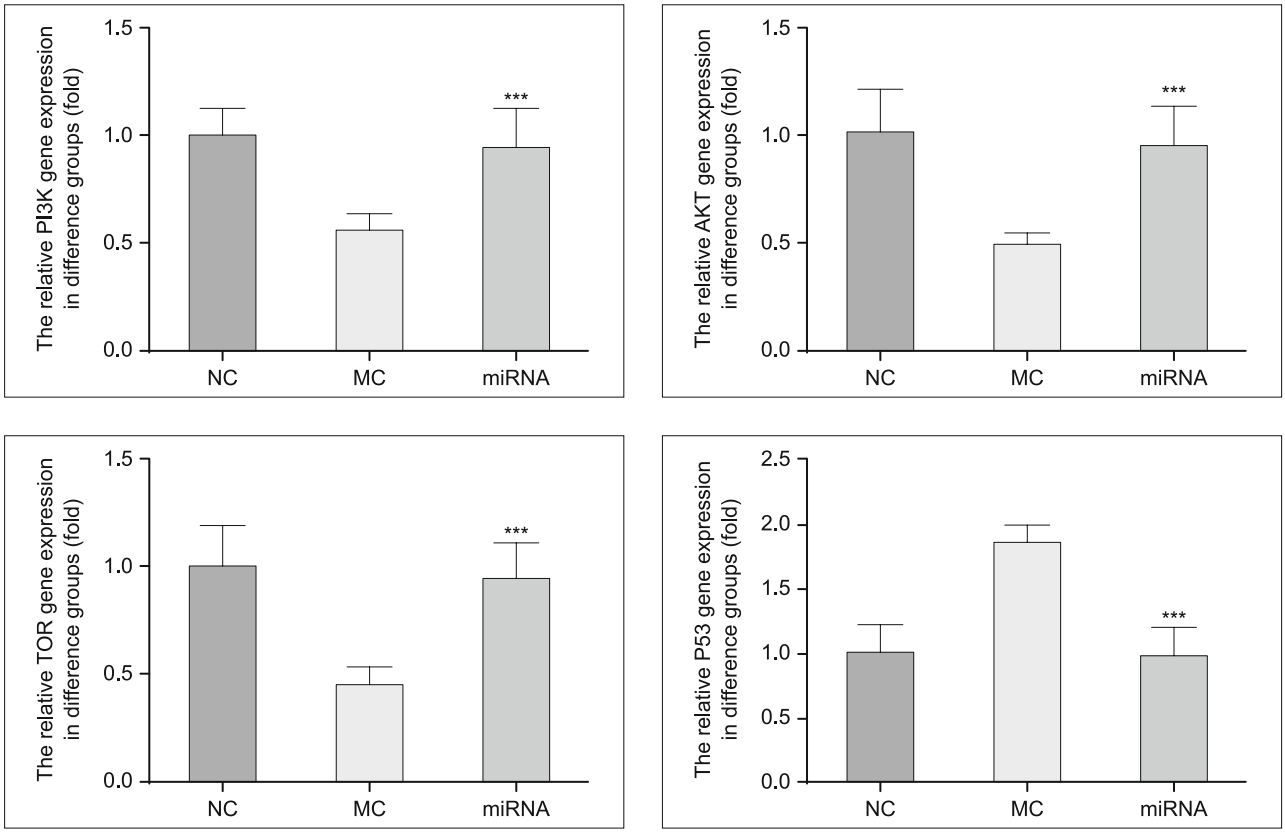

Fig. 4. The relative gene expression of difference groups. ***: p < 0.05, Compared with MC group.

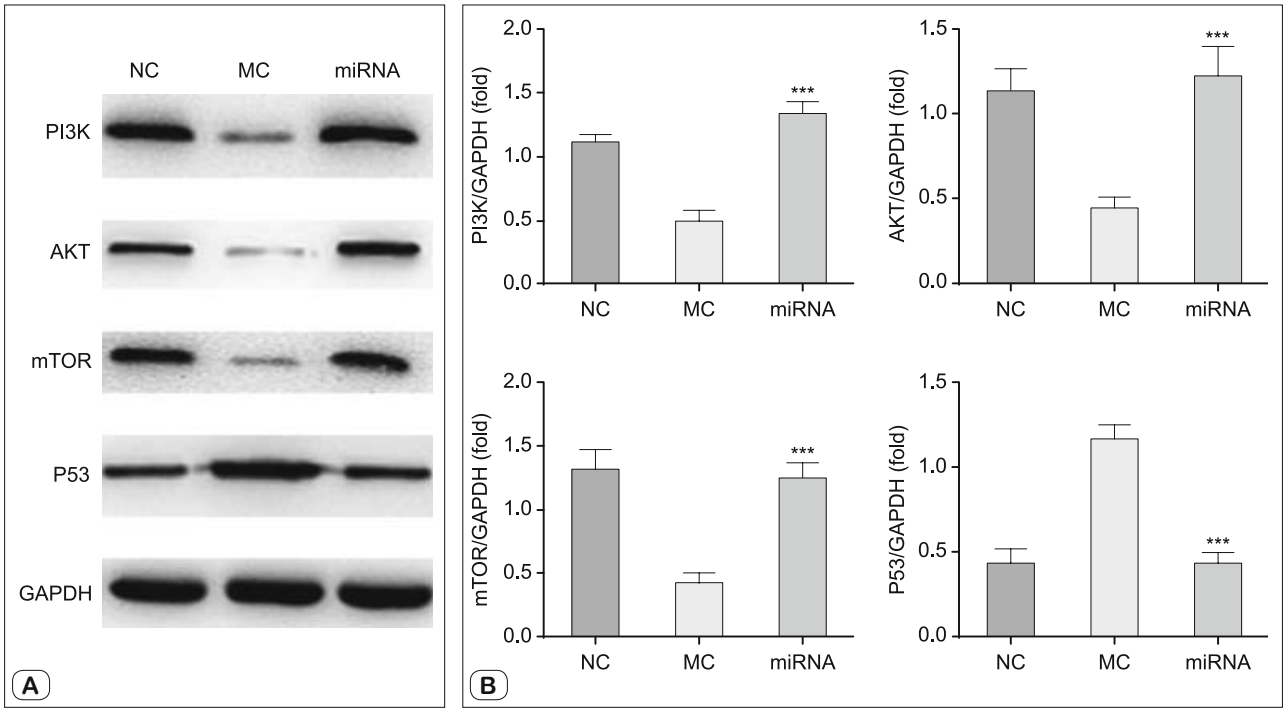

Fig. 5. The relative protein expressions of difference groups. A) The relative proteins expressions by WB assay. B) Comparing the relative protein expressions of difference groups. ***: p < 0.05, Compared with MC group.

that of normal pregnancy. Through the cell experiment, comparing to the MC group, miRNA-145 significantly enhanced cell proliferation rate and effectively reduced the EVCT cell apoptosis rate. In order to further explore its mechanism, we suggested PI3K/Akt/mTOR signalling pathways as the main factor for testing. The results showed that over-expression miRNA-145 could significantly promote the PI3K/AKT/mTOR signalling pathway and inhibit the P53 expression. We conclude that the low expression of miR-145 and inhibition of PI3K/Akt/mTOR signalling pathway and increased expression of P53 may are the important factors that result in preeclampsia.

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