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A study of protective effects of Mus81 in preeclampsia

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ABSTRACT

This study was to investigate the effect and mechanism of Mus81 in severe PE. 20 cases of pregnant women with severe PE and 20 cases of healthy pregnant women were enrolled. Placental tissues were collected after delivery, and the expression of Mus81 in placental tissues was detected by qRT-PCR and Western blot (WB). The si-Mus81 adenovirus was used to construct a pregnant mouse model of Mus81 down-expression in vivo, to clarify the effect of Mus81 on pregnant mice and blood pressure, urinary protein, serum sFLT1 and fetal weight in PE. After overexpression of Mus81 in HTRB-S/Vneo cells, the proliferation, migration and apoptosis of the cells were measured by EdU staining, flowcytometry, qRT-PCR and cell scratch test. Protein expression of the Wnt/β-catenin signaling pathway was detected by WB. To further explore the mechanism, Wnt/β-catenin inhibitor DKK1 inhibitor was added to HTRB-S/Vneo cells and then Ad-Mus81 was added for co-incubation for 48 h. Protein expressions p-β-catenin and activated-β-catenin were detected by WB. Bax and Bcl-2 were detected by qRT-PCR, and the proliferation of HTRB-S/Vneo cells was measured by EdU staining. Cell migration was detected by scratch test. The expression of Mus81 in the placental tissues of pregnant women with severe PE was lower than that in normal placental tissues. The blood pressure, urine protein and serum sFLT1 protein levels of Mus81 knockdown mice were all upregulated and the fetal weight was decreased after the injection of si-Mus81, which successfully simulated the characteristics of PE. After overexpression of Mus81, the proliferation and migration of HTRB-S/Vneo cells were enhanced, while the apoptosis was decreased. After overexpression of Mus81, the expression levels of p-β-catenin decreased while active-β-catenin increased obviously. Then, DKK1 inhibitor and Ad-Mus81 were added to the HTRB-S/Vneo cells and co-incubated for 48 h. Compared with the Ad-Mus81+DMSO group, the expression of p-β-catenin increased while activated-β-catenin decreased in the Ad-Mus81+DKK1 inhibitor group. The proliferation and migration decreased, but apoptosis of HTRB-S/Vneo cells was increased. Mus81 can regulate the proliferation, migration and apoptosis of trophoblast cells through the Wnt/β-catenin pathway, which plays an important role in maintaining the normal physiological function of trophoblast cells and is also involved in the occurrence and development of severe PE.

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Introduction

Preeclampsia (PE) is one of the common complications during pregnancy, and it is also a common cause of the clinical death of mothers and babies. PE causes more than 60,000 maternal deaths each year around the world and is the third leading cause of maternal death after postpartum hemorrhage and amniotic fluid embolism (1, 2). Complications of PE or eclampsia include liver rupture, pulmonary edema, and acute kidney injury (3, 4). Therefore, it is particularly important to be able to diagnose and effectively treat PE as soon as possible.

At present, the pathogenesis of PE has not been fully elucidated. The generally accepted theory is that the occurrence of PE is divided into two stages. The first stage occurs in early pregnancy, and is characterized by abnormal invasion of trophoblasts and remodeling obstacles of spiral arteries, which leads to hypoperfusion of the placenta. In the second stage, hypoperfusion causes ischemia, hypoxia and metabolic dysfunction in the placenta, which leads to a large number of placenta-derived adverse factors being released into the mother's blood, causing excessive systemic inflammatory response and vascular endothelial injury in the mother, and ultimately leading to a series of clinical symptoms of PE (5). The placenta is an important accessory organ of the fetus during pregnancy. Abnormal function of the placenta and the change of gene expression in the placenta are closely related to the occurrence of various pregnancy complications (6). Trophoblast cells are the most powerful functional cells in the placenta. Trophoblast cell proliferation barriers and invasion defects are important symptoms in the course of PE (7). Exploring the regulatory mechanism of trophoblast cell proliferation and invasion will help to understand the pathogenesis of PE.

Mus81 is a DNA repair gene discovered in recent years. It plays an important biological function in maintaining the stability of the cell genome (8), so it was once considered a tumor suppressor gene. However, recent studies have found that Mus81 has diverse biological functions and can also play a role in promoting tumor development. YIN and other studies have found that Mus81 can promote the migration of gastric cancer cell lines by regulating epi-

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thelial-mesenchymal transition (EMT) (9). Other previous studies also found that Mus81 silencing can reverse the chemotherapy resistance of human liver cancer and colon cancer cell lines and inhibit the proliferation of human colon cancer cell line HCT116 and promote its apoptosis (10). But the role of Mus81 in the proliferation and apoptosis of human trophoblast cells is not yet clear. To this end, this study specifically analyzed the changes of Mus81 in the PE placenta and its regulation of trophoblast proliferation, migration and apoptosis.

Materials and Methods

Collection and preservation of clinical tissue specimens

After passing the review of the ethics committee of Taizhou People's Hospital Affiliated to Nanjing Medical University, collect the inpatients who terminated their pregnancy due to cesarean section in the obstetric department of our hospital: (1) Normal pregnancy group: 20 cases of normal pregnant women who underwent cesarean section due to relative cephalopelvic disproportion or social factors were selected. (2) Severe preeclampsia group: another 20 patients with severe preeclampsia who met the diagnostic criteria and had no other complications were delivered by cesarean section. The diagnostic criteria are in accordance with the latest guidelines, and other pregnancy complications and other underlying diseases are excluded. Within 10 minutes after the delivery of the placenta during the cesarean section, randomly and completely took 3 pieces of tissue on the fetal surface of the placenta and about 2 cm around the root of the umbilical cord, the size of which was about 1 cm^3, and rinsed with pre-cooled and sterilized PBS. Then stored it in the refrigerator to -80°C.

Experimental animal

Female and male CD1 mice (SPF grade, 42-62 days old) were purchased from Beijing Weitong Lihua Laboratory Animal Technology Co., Ltd. (Beijing, China). The operation of this experiment was approved by the Experimental Animal Ethics Committee of Taizhou People's Hospital Affiliated to Nanjing Medical University.

Establishment of si-Mus81 pregnant mouse model

The male and female mice were mated in a cage at a ratio of 1:2. The day the female mice saw the thrombus was recorded as the first day of pregnancy (GD1), and they were randomly divided into PBS control group, si-Mus81 control group, and si-NC experimental group. The pregnant mice were injected through the tail vein at GD6 (200 μL each of sterile PBS, si-Mus81 and si-NC respectively). All pregnant mice were sacrificed at GD18 by cervical dislocation method, and the weight of the GD18 fetus was tested.

Non-invasive blood pressure test in pregnant mice

The BP-2000 non-invasive blood pressure meter was used to detect the tail vein systolic blood pressure of GD2, 4, 6, 8, 10, 12, 14, 16, and 18 pregnant mice.

24-hour urine protein test in pregnant mice

The 24-hour urine of GD2, 6, 10, 14, and 18 pregnant mice was collected in a metabolic cage, and the urine protein level was detected by the IDEXX automatic biochemical analyzer.

Detection of sFLT1 in serum

Eyeball blood was taken from pregnant mice of GD9, 15, 18. The collected blood was allowed to stand at room temperature for 1 hour to separate the serum, and the sFLT1 level in the serum was detected according to the instructions of the enzyme-linked immunosorbent assay (ELISA) test kit (Becton Dickinson, Heidelberg, Germany).

Cell culture

The HTR8-S/Vneo cell line used in this experiment was purchased from the Cell Culture Center (Beijing, China). We used Dulbecco's Modified Eagle's Medium (DMEM; Life Technology, Wuhan, China) complete medium containing 10% fetal bovine serum (FBS; Life Technology, Wuhan, China), penicillin (100 U/mL) and streptomycin (100 mg/mL) to culture cells in a cell incubator at 37°C and 5% CO_2.

Cell transfection

The overexpression adenovirus of Mus81 was synthesized by Beijing Olinger Biotechnology Co., Ltd. (Beijing, China). A certain amount of cells were cultivated in a 6-well cell culture dish, and when they grow to an appropriate density, cell transfection was performed. The adenovirus stock solution with 250μL of serum-free Opti was diluted, and incubated for 6 min at room temperature. The diluted adenovirus was inhaled into the diluted lipo2000 (Olinger, Beijing, China) and then mixed, and incubated at room temperature for 20 min. Then, the medium was replaced in the 6-well cell culture dish, 1.5 mL of complete DMEM medium was added, and then the mixed solution prepared in the third step was added to the 6-well cell culture dish. The RNA was extracted 48 hours after the transfection was completed, and the transfection efficiency was tested.

EdU staining

Cells were cultured in a 24-well plate. When they grow to a suitable density, cell transfection was conducted. After stimulation for 48 hours, EdU (30 uM) was added to the culture medium of the 24-well plate and then incubated for 2 hours. The cells were washed with PBS, cell fixation solution was added and incubated at room temperature for 25 min. After washing the cells with PBS, 2 mg/mL glycine was added and incubated on a shaker for 5 min. After washing the cells with PBS, 0.5% Triton X-100 (Ye Sen, Shanghai, China) was added and incubated for 10 min. Apollo staining solution was added and incubated for 30 min in the dark. After washing the cells with PBS, DAPI (Ye Sen, Shanghai, China) was added and incubated for 5 min in the dark. The staining results were observed under a fluorescent inverted microscope, and the results were analyzed with Image J software.

Scratch test

About 2×10^6 cells were inoculated in each 6-well culture dish for 24 hours until the cell density was about 70-80%. These cells were transfected with Ad-Mus81 or Ad-NC for 6 h. A 200 μL pipette tip was used for vertical scribing. Then it was washed twice with 1 mL of medium, and then cultured in a cell incubator. At 0 h and 24 h after the scratch, the image of the scratch was acquired with a digital camera at the same position. Standard calipers were used to quantitatively evaluate wound width (um).

Western Blot (WB)

RIPA lysis buffer (Construction, Nanjing, China) was used to lyse cells or tissues on ice to extract protein samples. Then, the protein samples were separated by gel electrophoresis. and the protein on the gel was electrotransferred to the polyvinylidene difluoride (PVDF, Thermo Fisher Scientific, Waltham, MA, USA) membrane. The PVDF membrane was sealed with 5% skimmed milk powder for 1.5 h. A blocking solution was used to dilute the primary antibody (Mus81, Mouse, 1:2000, Abcam, Cambridge, MA, USA; activated-β-catenin, Rabbit, 1:1000, Abcam, Cambridge, MA, USA; p-β-catenin, Rabbit, 1:1000, Abcam, Cambridge, MA, USA; β-actin, 1:5000, Abcam, Cambridge, MA, USA) and incubated it with PVDF membrane at 4°C overnight. After washing the PVDF membrane with TBST 3 times, incubated with horseradish peroxidase (HRP; Abcam, Cambridge, MA, USA) coupled secondary antibody for 1 h. After the PVDF membrane was washed, it was developed by chemiluminescence color-developing solution and detected by the ChemiDocTM XRS$^+$ system.

RNA Isolation and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

For tissue samples, 1 mL TRIzol (Thermo Fisher Scientific, Waltham, MA, USA) was added to 50 mg of tissue and ground thoroughly with a homogenizer on ice. For cell samples, after washing the cells with PBS, 1 mL TRIzol was added to fully lyse on ice. Then the lysate was transferred into a 1.5 mL enzyme-free EP tube and centrifuged at 12000 rpm for 10 min at 4°C. The supernatant was taken and transferred to a 1.5 mL enzyme-free EP tube, 200 μL of chloroform was added and shanked vigorously for 30 seconds, and placed on ice for 15 minutes, centrifuged at 4°C, 12000 rpm for 10 minutes. The upper colorless and transparent solution was slowly transferred into a new 1.5 mL enzyme-free EP tube, 2 times the volume of isopropanol was added, vigorously shanked for 30 s, and centrifuged at 12000 rpm at 4°C for 10 min. After centrifugation, a white RNA precipitate can be seen. Carefully discarded the supernatant, added 75% ethanol, and washed with shaking. It was centrifuged at 12000 rpm at 4°C for 10 min, discarded the supernatant, and dyed at room temperature for 5 min. 30 μL DEPC water (Thermo Fisher Scientific, Waltham, MA, USA) was added to dissolve the white RNA precipitate, and the RNA concentration was measured. Quantitative primers were designed using Primer 6.0, synthesized and purified by Gene (China). The primer sequences are shown in Table 1. After RNA extraction, it was reverse transcribed into cDNA by PrimeScript™ RT Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), reaction program: 37°C, 15 min, 85°C, 5 s, 4°C storage. Then carried out the qRT-PCR reaction: 95°C, 4 min, 95°C, 5 s, 60°C, 34 s, 40 cycles. The amplification signal was detected by using CFX Connect, each experiment was repeated three times, GAPDH was used as an internal reference marker for gene expression, and the relative gene expression level was calculated by the $2^{-\Delta\Delta Ct}$ method.

Cell cycle

The cells were digested into single cells with trypsin digestion solution, and centrifuged at 1000 rpm for 5 min. Then, the cell pellet was washed and resuspended in PBS, centrifuged at 1000 rpm for 5 min, and the supernatant was discarded. 100 μL PBS was added to resuspend the cells, and 500 μL pre-cooled 75% ethanol was slowly added. And centrifuged at 1000 rpm for 5 min, then washed twice with PBS. 1 mL of DNA staining solution (KeyGen, Nanjing, China) was added and the cell cycle was checked by flow cytometer after staining for 15 minutes.

Statistical analysis

GraphPad Prism 6.0 (La Jolla, CA, USA) was used for statistical analysis. The t-test was used for comparisons between the two groups. Comparison between multiple groups was done using a One-way analysis of variance (ANOVA) test followed by Post-Hoc Test (Least Significant Difference). The least Significant Difference (LSD) test or Student-Newman-Keuls (SNK) test was used for pairwise comparison under the condition of homogeneity of variance, and $P<0.05$ was statistically different.

Results

Mus81 expression decreases in the placental tissues of patients with severe PE

We collected clinical data of patients, and there was no significant difference in age, BMI, and gestational age of pregnant women between the severe PE group and the normal group (Table 2). qRT-PCR detected the expression of Mus81 in the placenta of pregnant women in the normal group and the pregnant women in the PE group. It was found that compared with the normal placental tissue, the expression of Mus81 in the placenta tissue of patients with severe PE was obviously reduced (Figure 1A). At the same time, the result of WB was similar to qRT-PCR, and the

Table 1. Real-time PCR primers.

Gene name	Forward (5'>3')	Reverse (5'>3')
Bax	CAGTTGAAGTTGCCATCAGC	CAGTTGAAGTTACCATCAGC
Bcl-2	GACTGAGTACCTGAACCGGCATC	CTGAGCAGCGTCTTCAGAGACA
Caspase3	TGGAACAAATGGACCTGTTGACC	AGGACTCAAATTCTGTTGCCACC
Mus81(Human)	CAGCGGGAAGGAAGCTAAGAT	GCAGGAACTGGCATGGAAG
Mus81(Mouse)	TCGTGTTTCAAAAGGCATTGC	TCACCGCCTGATGCTAGGT
Fas	TATCAAGGAGGCCCATTTTGC	TGTTTCCACTTCTAAACCATGCT
FasL	TCCGTGAGTTCACCAACCAAA	GGGGGTTCCCTGTTAAATGGG
Cyclin B1	AAGGTGCCTGTGTGTGAACC	GTCAGCCCCATCATCTGCG
CDK2	CCTGCTTATCAATGCAGAGGG	TGCGGGTCACCATTTCAGC
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

Table 2. Clinical characteristics of patients with severe preeclampsia and normal pregnancy.

Variable	NT group (n=20)	PE group (n=20)
Age	28.85 (25.88, 31.88)	27.88 (22.21, 34.89)
BMI(kg/m^2)	26.55 (21.71, 36.98)	29.44 (22.74, 36.12)
gestational age (week)	38.11 (37.04, 39.84)	36.95 (34.17, 38.57)

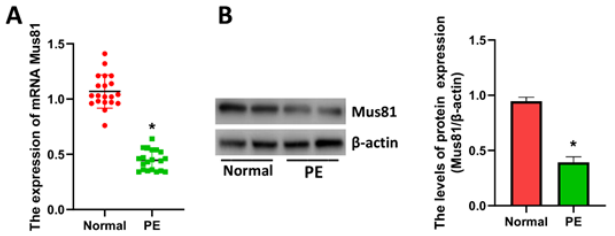

Figure 1. Mus81 expression decreases in the placental tissues of patients with severe PE. (A). qRT-PCR was used to detect the mRNA level of the Mus81 gene in tissues. (B). WB was used to detect the protein level of the Mus81 gene in tissues. (Normal and PE groups). * vs. Normal group P<0.05.

expression of Mus81 protein decreased in the PE group (Figure 1B). The result showed that Mus81 expression decreases in placental tissues of patients with severe PE.

Pregnant mice showing signs of PE after knocking down Mus81

In order to further explore the role of Mus81 in PE, in this study, GD6 pregnant mice were injected with si-Mus81 adenovirus through the tail vein to obtain Mus81 knockdown mice. The results showed that the mRNA and protein levels of Mus81 in the placenta of pregnant mice in the si-Mus81 group were down-regulated (Figure 2A & 2B). Then, we continuously monitored the changes in blood pressure, urine protein, sFLT1, and fetal weight in the mice. The results showed that the blood pressure, urine protein, and sFLT1 levels of the si-Mus81 group increased, and the fetal weight decreased (Figure 2C-F). The above results indicated that the knockdown of Mus81 successfully demonstrated human PE-like symptoms in pregnant mice.

The effect of Ad-Mus81 on trophoblast cells proliferation, apoptosis and migration

Treatment of HTRB-S/Vneo with Ad-Mus81 for 48 h, qRT-PCR and WB results showed that Ad-Mus81 can promote Mus81 gene expression (Figure 3A & 3B). EdU staining showed that overexpression of Mus81 can obviously promote the proliferation of trophoblasts (Figure 3C). The results of cell cycle analysis by flow cytometry showed that the number of HTRB-S/Vneo cells in the G0/G1 phase of the Ad-Mus81 group decreased, and the number of cells in the S phases increased (Figure 3D). The above results indicated that Mus81 can promote the proliferation of trophoblast cells. Compared with the Ad-NC group, the mRNA expression levels of Cyclin B1 and CDK2 in the Ad-Mus81 group were increased, and the difference was statistically significant (Figure 3E). In addition, compared with the Ad-NC group, the expression of Bcl-2 mRNA in the Ad-Mus81 group was obviously increased, and the mRNA expression of Bax, Fas, and FasL was decreased (Figure 3F). All the above results showed that Mus81 can promote the proliferation and migration, and reduce apoptosis of trophoblast cells.

Mus81 regulates the proliferation, apoptosis and migration of trophoblasts through the Wnt/β-catenin pathway

We treated HTRB-S/Vneo cells with Ad-Mus81 for 48 h, and the WB results showed that overexpression of Mus81 could obviously inhibit p-β-catenin and promote the expression of activated-β-catenin (Figure 4A). After the Wnt/β-catenin pathway inhibitor DKK1 inhibitor was added to the HTRB-S/Vneo cell line, WB results showed that the protein expression of p-β-catenin increased obviously, and the expression of activated-β-catenin decreased in the Ad-Mus81+DKK1 inhibitor than Ad-Mus81+DMSO group (Figure 4B). At the same time, qRT-PCR results showed that Bcl-2 expression decreased, and Bax increased obviously in the Ad-Mus81+DKK1 inhibitor (Figure 4C). Edu staining results showed that the addition of DKK1 inhibitors inhibited cell proliferation and migration (Figure 4D). The above results indicated that the overexpression of Mus81 can promote the proliferation and migration of trophoblast cells through the Wnt/β-catenin signaling pathway, and inhibit the apoptosis of trophoblast cells.

Discussion

PE is caused by multiple factors. The currently accepted theory is that in the early stage of placenta formation, the infiltration capacity of trophoblast cells is reduced and the invasion of the uterine wall is restricted, resulting in in-

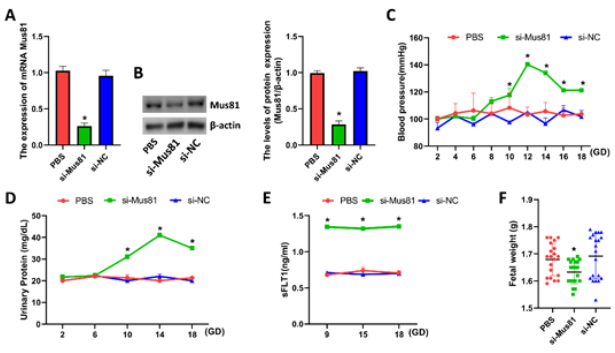

Figure 2. Pregnant mice showing signs of PE after knocking down Mus81. (A). qRT-PCR was used to detect the mRNA level of the Mus81 gene after adenovirus transfection in normal placental tissues. (B). WB was used to detect the protein level of the Mus81 gene after adenovirus transfection in normal placental tissues. (C). Blood pressure changes of pregnant rats were monitored for 2-18 days. (D). Urine protein levels of pregnant mice were monitored for 2-18 days. (E). Serum levels of sFLT in pregnant mice were monitored from 0 to 18 days. (F). Fetal weight on the GD18 was detected. (PBS, si-Mus81, si-NC groups). * vs. si-NC group P<0.05.

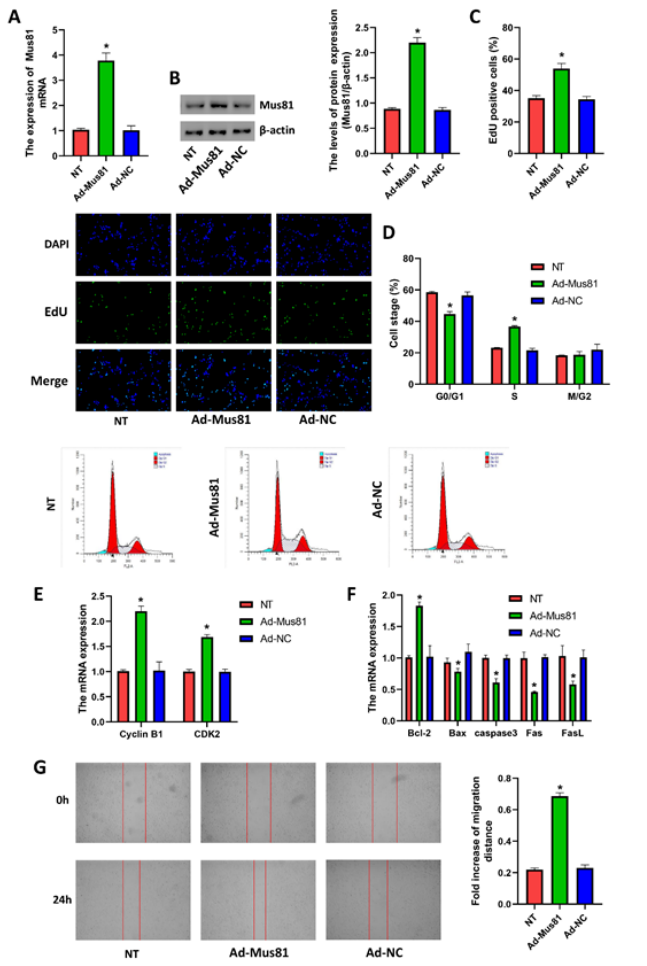

Figure 3. The effect of Ad-Mus81 on trophoblast cell proliferation, apoptosis and migration. (A). qRT-PCR was used to detect the mRNA level of the Mus81 gene after adenovirus transfection in HTRB-S/Vneo cells. (B). WB was used to detect the protein level of the Mus81 gene after adenovirus transfection in HTRB-S/Vneo cells. (C). EdU staining was used to detect the proliferation in 3 groups. (D). flow cytometry was used to detect the cell cycle in 3 groups. (E). qRT-PCR was used to detect the mRNA level of Cyclin B1 and CDK2 gene in 3 groups. (F). qRT-PCR was used to detect the mRNA level of Bcl-2, Bax, caspase3, Fas and Fasl gene in 3 groups. (G). Scratch test was used to detect HTRB-S/Vneo cell migration. (NT, Ad-Mus81, Ad-NC groups). * vs. Ad-NC group P<0.05.

sufficient recasting of the uterine spiral arterioles and shallow implantation of the placenta. Low blood flow leads to the occurrence of PE (11, 12). Therefore, this experiment started with the proliferation, migration and apoptosis of trophoblast cells, and looked for corresponding pathways for research.

The wnt/β-catenin signaling pathway is considered as an important signaling pathway that regulates cell proliferation and differentiation (13, 14). Wnt ligands can exert important influences on the development and homeostasis maintenance of multiple organs through step-β-catenin-dependent and step-β-catenin-independent signaling pathways. It has been reported in the literature that β-catenin entering the nucleus is an important indicator of Wnt/β-catenin signal activation (15). This study showed that overexpression of Mus81 could activate β-catenin, promote its expression, and inhibit the expression of β-catenin phosphorylated at Ser33/37/ Thr41, thus inhibiting the degradation of β-catenin, indicating that overexpression of Mus81 in trophoblast cells could activate Wnt/β-catenin signal. After the Wnt/β-catenin signaling pathway is activated, β-catenin enters the nucleus and combines with TCF/LEF to realize the regulation of downstream target genes, thereby affecting various life activities (16). This study showed that the expression of p-β-catenin decreased obviously after overexpression of Mus81, suggesting that Mus81 may regulate the proliferation, migration and apoptosis of HTRB-S/Vneo cells through the Wnt/β-catenin signaling pathway. To further explore the mechanism, we added the Wnt/β-catenin inhibitor DKK1 inhibitor before overexpression of Mus81 (17). The results showed that compared with the Ad-Mus81 group, the DKK1 inhibitor obviously increased the expression of p-β-catenin protein. Thereby inhibiting the proliferation and migration of trophoblast cells and promoting apoptosis. In summary, this experiment believes that Mus81 can affect the proliferation, migration and apoptosis of trophoblast cells in patients with severe PE by regulating the Wnt/β-catenin signaling pathway.

There have been many reports on the method of simulating human PE-like characterization by injecting adenovirus through the tail vein of pregnant mice. Li Shen et al injected CD81 overexpressed adenovirus through the tail vein of pregnant mice, resulting in abnormal placental development of pregnant mice and the appearance of human PE-like symptoms (18). In addition, Peng W et al. injection of ACTN4 to interfere with adenovirus also caused pregnant mice to exhibit human PE-like symptoms (19). Therefore, in this study, Mus81 knockdown adenovirus was injected into pregnant mice to construct a Mus81 knockdown pregnant mouse model, which resulted in obviously increased blood pressure, urine protein, serum

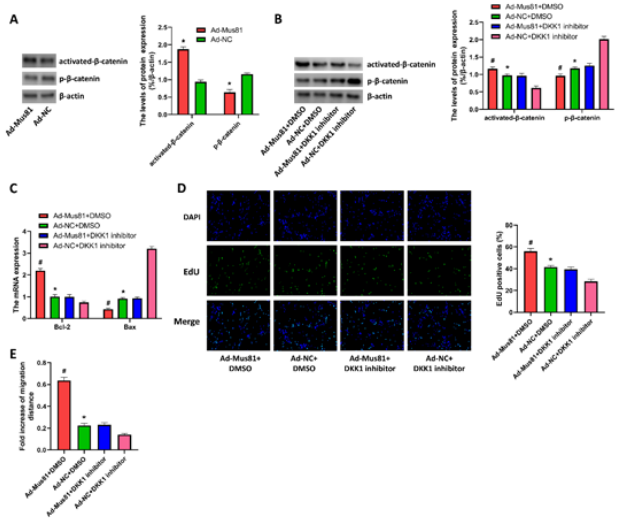

Figure 4. Mus81 regulates the proliferation, apoptosis and migration of trophoblasts through the Wnt/β-catenin pathway. (A). WB was used to detect the protein level of activated-β-catenin and p-β-catenin gene in 2 groups. (Ad-Mus81 and Ad-NC groups). * vs Ad-NC group P<0.05. (B). WB was used to detect the protein level of activated-β-catenin and p-β-catenin gene in 4 groups. (C). qRT-PCR was used to detect the mRNA level of Bcl-2 and Bax gene in 4 groups. (D). EdU staining was used to detect the proliferation in 4 groups. (E). HTRB-S/Vneo Cell migration analysis. (Ad-Mus81+DMSO, Ad-NC+DMSO, Ad-Mus81+DKK1 inhibitor, Ad-NC+DKK1 inhibitor groups). * vs. Ad-NC+DMSO group P<0.05, # vs. Ad-Mus81+DMSO group P<0.05.

sFLT1 level in pregnant mice, and obviously reduced fetal weight and other human PE-like symptoms (20). It is worth noting that the knockdown of adenovirus by Mus81 may result in low expression in multiple organs of pregnant mice. Therefore, it is unclear whether the aforementioned PE is only caused by the low expression of Mus81 in placental trophoblasts. However, using placental-specific Mus81 knockout mice can help us to further study the role of Mus81 in the differentiation of placental trophoblasts.

Conclusion

In summary, our research results showed that Mus81 plays an important role in trophoblasts. Mus81 can regulate the proliferation, migration and apoptosis of trophoblasts through the Wnt/β-catenin pathway, which is of great significance for maintaining the normal physiological functions of trophoblasts. and participated in the occurrence and development of severe PE disease.

Disclosure of conflict of interest

None.

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