Original Article

miR-335 targets CRIM1 to promote the proliferation and inhibit the apoptosis of placental trophoblast cells in preeclamptic rats

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Abstract: Objective: To investigate the role of miRNA-335 on the proliferation and apoptosis of placental trophoblast cells in preeclamptic rats and its potential mechanism. Methods: Placental trophoblast cells were isolated from preeclamptic model rats and normal ones. Trophoblast cells from the model group were divided into six groups for transfection: the blank (empty vector) group, the NC (negative control) group, the miRNA-335 mimic group, the miRNA-335 inhibitor group, the CRIM1 (overexpressed recombinant plasmid) group, and the miRNA-335 inhibitor + CRIM1 group. The miRNA-335 expressions after the transfection were determined using qRT-PCR. The mRNA and protein expressions of CRIM1, the transforming growth factor (TGF-β1), and the apoptosis-related factors (Bax and Bcl-2) in each group were determined using qRT-PCR and Western blotting. The cell proliferation and apoptosis were determined using MTT assays and flow cytometry, respectively. Results: Compared with normal rats, the systolic blood pressure, diastolic blood pressure, and urinary protein levels were increased in the model rats, which had increased miRNA-335 expressions, but a decreased CRIM1 expressions (all P<0.05). The inhibition of miRNA-335 promoted the expressions of CRIM1, TGF-β1, and Bcl-2 and inhibited the expression of Bax in trophoblast cells (all P<0.05). miRNA-335 inhibition or CRIM1 over-expression promoted the proliferation and reduced the apoptosis of trophoblast cells. The combined effect of miRNA-335 inhibition or CRIM1 over-expression had an even more significant effect on the changes in the above indicators (all P<0.05). Conclusion: miRNA-335 inhibition can enhance the expression of CRIM1 to promote the proliferation and reduce the apoptosis of trophoblast cells in preeclamptic rats.

Keywords: miR-335, CRIM1, preeclampsia, cell proliferation

Introduction

Preeclampsia, a placenta-derived disease, is a serious complication of pregnant and lying-in women [1]. Trophoblast cells are considered to be the most important cell type in the placenta, and their dysfunction, such as insufficient proliferation and differentiation and abnormal syncytialization, can cause uterine spiral artery remodeling disorder, which is a critical process in the pathogenesis of preeclampsia [2]. Therefore, the proliferation and apoptosis of placental trophoblast cells are of great significance for the normal development of the placenta and vascular bed [3]. It was found that a large number of apoptotic trophoblast cells in the placentas of preeclamptic patients were positively correlated with the onset of preeclampsia [4]. Therefore, exploring the biological behavior of trophoblast cells in preeclampsia is of great significance in clarifying the pathogenesis of the disease.

The expression of miRNAs in the placenta of preeclamptic patients is significantly different from the expressions of miRNAs in the normal placenta, suggesting that miRNAs may play an important role in the pathogenesis of preeclampsia [5]. Lu et al. found that the expression of miRNA-137 is enhanced in the placenta in preeclampsia and can inhibit the proliferation and migration of trophoblast cells by targeting ERRα [6]. Furthermore, miR-41 is up-regulated in placental tissue and regulates tro-
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phoblast cell infiltration, and miR-128 plays an important role in promoting the apoptosis of trophoblast cells in preeclampsia [7, 8]. In addition to the above disorders in miRNA expression, miRNA-335 has also been found to play a role in promoting the pathogenesis of preeclampsia. The overexpression of miR-335 can inhibit the migration of trophoblast cells and advance severe preeclampsia [9].

Cysteine-rich motor neuron protein 1 (CRIM1) is a transmembrane protein and an important member of the BMP protein family [10]. CRIM1 was found to play a vital role in the adhesion and invasion of cancer cells as well as in the development of embryos and organs [11]. Pennisi et al. found that CRIM1 is not only necessary for placental development, but also key for maintaining the correct differentiation of trophoblast cells [12]. Previous studies have found that a decrease in CRIM1 expression can promote the migration and invasion of renal cancer cells as well as affect the migration and adhesion of lung cancer cells [13, 14]. However, the role CRIM1 plays in the regulation of placental trophoblasts and the pathogenesis of preeclampsia has not been fully clarified.

Therefore, in this study we investigated the effect of miR-335 on the placental trophoblast cells of preeclamptic rats and explored the relationship between miR-335 and CRIM1.

Materials and methods

Ethical statement

The study was approved by the Animal Ethics Committee of The Affiliated Hospital of Youjiang Medical University for Nationalities and complied with the requirements for the use of experimental animals in Guidelines for the Care and Use of Laboratory Animals published by the National Institutes of Health, and corresponding measures were taken to reduce animal suffering [15].

Animal model construction

SPF-grade SD rats (30 females and 10 males) aged 2-3 months and weighing 200-220 g were purchased from the Shanghai Laboratory Animal Research Center (Shanghai, China). All the rats were fed in a constant ventilation environment with a constant temperature of 25-26°C and a controlled humidity of 70%. Every day both darkness and illuminations lasted 12 h, and the rats were disinfected regularly. After 7 days of adaptive feeding, the rats were fed in cages according to the female to male ratio of 2:1. On the second day, vaginal smears were taken on the rats and a microscopic observation of sperm was defined as day 0 of pregnancy. The pregnant rats (n=20) were randomly divided into a PE model group (n=15) and a normal group (n=5) on the 7th-8th days of pregnancy. After the 13th day of pregnancy, the rats in the PE model group were subcutaneously injected with nitroso L-arginine methyl ester (Shanghai Beinuo Biotechnology Co., Ltd., China) at a dosage of 75 mg/(kg·d) once a day until delivery to establish the PE model [16]. The normal group was given the same amount of a saline injection. The blood pressure and proteinuria of the model group rats were evaluated, and the model was considered successfully established if the blood pressure and proteinuria levels were significantly higher than the corresponding levels in the normal group [17].

Blood pressure measurement

A rat tail blood pressure meter (MRBP, Shanghai Yuyan Scientific Instrument Co., Ltd., China) was used to measure the blood pressure of rats on the 6th and 18th days of pregnancy. The rats' blood pressure was measured at around 8:00 in the morning after 2 h of fasting. The thermostat was controlled at approximately 25°C, and the rats were placed for 10 min and monitored with a MouseOx Plus mini-animal non-invasive monitor (012000, Hedebio Technology Co., Ltd., China). After their heart rates stabilized, the meter was placed at the tail root of the rats to measure their blood pressure.

Automatic biochemical analysis

On the 6th and 18th days of pregnancy, rat urine was collected with a rat metabolic cage (HB, Huaibei Biowill Co., Ltd., China) for 24 h. The urine protein content of the rats was determined with an automatic biochemical analyzer (AU5800, Beckman coulter, USA). The rat urine was first centrifuged at a speed of 658×g for 15 min at 4°C. After sediment removal, the rat urine protein content was determined with biuret reagent (Shanghai Jingke Chemistry Technology Co., Ltd., China).
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Prediction and verification of the target genes

TargetScan (http://www.targetscan.org/vert_72/) was used to explore the possible targeting relationship between miR-335 and CRIM1, and then the relationship was validated using a double-luciferase reporter system assay. The wild-type CRIM1-3'-UTR-wt and site-directed mutagenesis of the binding sites (CRIM1-3'-UTR-mut) were designed and built. Luciferase reporter vectors were constructed and transfected into CHO cells (ThermoFisher, USA) and cultured on 24-well plates. The samples' luciferase activity was measured using a dual luciferase reporter assay kit (Shanghai Beyotime Biotechnology Co., Ltd., China).

Isolation and culture of the placental trophoblast cells

At 21 days of gestation, rat placental tissue was taken and cut into 2 mm × 2 mm tissue pieces, which were rinsed with D-Hank's solution (Wuhan Yipu Biotechnology Co., Ltd., China). Afterwards, DMEM low-sugar medium containing 0.2% collagenase (Sigma, USA) was added to the tissue pieces. The tissue pieces were then transferred to a centrifugal tube, treated with an electromagnetic stirrer (DF-101XP, Zenith Lab (Jiangsu) Co., Ltd., China) at 37°C for 20 min, and then the sample was centrifuged for 3 min at 860×g. The precipitation was re-suspended by culture medium. The cell suspension was blown and mixed and transferred to a disposable petri dish. After they were washed with D-Hank's solution, the cells were supplemented with trypsinase containing 0.02% EDTA (Beijing Reagene Biotechnology Co., Ltd., China), and placed in an incubator (5% CO₂, 37°C) for 5 min. The digestion was terminated by adding a medium containing 20% fetal bovine serum, and the mixture was centrifuged at 860×g for 3 min, and the supernatant was discarded. The cells were re-suspended with a MEM medium containing 20% fetal bovine serum and then inoculated into a new dish. The culture dish was transferred to an incubator (5% CO₂, 37°C), and the culture medium was replaced every three days.

Identification of the placental trophoblast cells

The rat placental trophoblast cells were inoculated on a sterile blood cap (YM-HC-YH253-268, Shanghai Yuanmu Biology, China) that was placed in a 12-well plate in advance. After 48 h of culturing, the cells were washed with PBS twice and then fixed with polyformaldehyde (Boster Biology, Wuhan, China) for 15 min and washed with PBS. The cells were incubated with 10% rabbit serum (Beijing Solarbio Science & Technology Co., Ltd., China) for 30 min and then incubated with rabbit anti-rat vimentin (1:1,000, Abcam, UK) at 4°C overnight. The cells were washed with PBS 3 times and incubated with Alexa Fluor®488-labeled IgG (1:4,000, Abcam, UK). Then, the cells were incubated with DAPI (1:1,000, Beijing Solarbio Science & Technology Co., Ltd., China) for 30 min at room temperature, followed by washing with PBS 3 times. Then the cells were sealed with anti-fluorescence quenching sealing tablets (Shanghai Beyotime Biotechnology Research Institute, China). Inverted microscopy (CX41, Olympus, Japan) was used for the cell observation and imaging. Image-Pro Plus 7.0 software was used to determine the proportion of positive cells.

Grouping and transfection of placental trophoblast cells

Placental trophoblast cells isolated from normal rats were used as the normal control group (no sequence was transfected). Placental trophoblast cells isolated from the model rats were divided into 6 groups according to the transfection methods: the blank group (empty vector), the NC group (negative control plasmid), the miRNA-335 mimic group (miRNA-335 mimic), the miRNA-335 inhibitor group (miRNA-335 inhibitor), the CRIM1 group (CRIM1 overexpressed recombinant plasmid), and the miRNA-335 inhibitor + CRIM1 group (miR-335 inhibitor and CRIM1 overexpressed recombinant plasmid). The cells were transfected according to the Lipofectamine 2000 (Invitrogen, USA) instructions. All the plasmids used in this study were purchased from Nanjing Shuangling Biotechnology Co., Ltd. (China).

qRT-PCR

After 48 h of transfection, the cells in each group were lysed using a pre-cooled cell lysis buffer (Nanjing SenBeiJia Biological Technology Co., Ltd., China). Then the total RNA was extracted using the one-step method and using Trizol kits (Invitrogen Company, USA). The RNA
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reverse transcription was performed according to the reverse transcription kit instructions (TaKaRa, China). The ABI7500 fluorescence quantitative PCR instrument (Prism® 7500, ABI Company, USA) was used for the RNA quantification. The reaction system was operated according to the instructions of the PCR kit (Invitrogen, USA). The reaction conditions were as follows: 50°C for 2 min, 95°C for 2 min, denatured at 95°C for 3 sec, annealed at 60°C for 30 sec, and extended at 70°C for 10 sec, for a total of 40 cycles. The reaction system included: Premix Ex Taq or SYBR Green Mix (12.5 μL), forward primer (1 μL), reverse primer (1 μL), cDNA (1-4 μL), and ddH2O up to 25 μL. The internal reference for miRNA-335 was U6, and for other mRNAs it was GAPDH. The primers were synthesized by the BGI company and their sequences are shown in Table 1. A dissolution curve was used to evaluate the reliability of the PCR results. The quantification adopted the 2^{-ΔΔCT} method. Each experiment was repeated 3 times and the means were calculated.

Western blot

The rat trophoblast cells were processed using a cell lysis buffer (AR0107, Boster Biology, Wuhan, China), and then crushed using ultrasound on ice to an exacted total protein and its concentration was determined using the BCA method. 20 μL of protein sample was loaded and polyacrylamide gel electrophoresis was carried out. The proteins were transferred onto a membrane using the wet transfer method and then sealed at room temperature for 1 h with skimmed milk. The blocking buffer was discarded and then the proteins on the membrane were incubated with following primary antibodies at 4°C overnight: rabbit anti-CRIM1 rat polyclonal antibody (1 μg/mL, Abcam, UK; prepared with 5% BSA), rabbit anti-TGF-β1 anti-rat polyclonal antibody (4 μg/mL, Abcam, UK), rabbit anti-Bax anti-rat monoclonal antibody (1:2,000, Abcam, UK), and rabbit anti-Bcl-2 anti-rat monoclonal antibody (1:1,000, Abcam, UK). GAPDH (1:10,000, Abcam, UK) was used as the internal reference. The next day the membranes were rinsed with TBST 3 times, and the secondary antibody goat anti-rabbit IgG (1:10,000, Abcam, UK) was added onto the membrane. After incubation for 4-6 h at 4°C, the membrane was washed with TBST again. The chemiluminescent solution (Boster Biology, Wuhan, China) and the ECL developer were used for the color development. The relative optical density of the immunoblot bands was analyzed. The experiment was repeated three times and the mean values were calculated.

MTT assay

The cells in a logarithmic growth phase were inoculated into a 96-well plate with a cell density of 1 * 10^4/mL, and divided into seven groups. After 24 h, 48 h, and 72 h of routine culture, 10 μL of MTT solution (5 mg/mL; Beijing Solarbio Science & Technology Co., Ltd., China) was added to the wells. After 4 h of culturing, the absorbance of each well was measured at 570 nm using a microplate reader (Varioskan Flash 3001, ThermoFisher Science, USA). The experiment was repeated three times and the means were calculated.

Measuring the apoptosis with flow cytometry

The apoptosis was measured with the Annexin-V-FITC/PI apoptotic detection kit (Beyotime Biotechnology, Shanghai, China). The cells in each group were inoculated into 96-well plates with 1 * 10^6 cells/well. After 48 h of culturing, the cells were digested using trypsinase without EDTA (Beijing Leagene Biotechnology Co., Ltd., China) and collected. After washing with PBS twice, the cells were centrifuged at 650-g

<table>
<thead>
<tr>
<th>Table 1. Real time-PCR primer sequences</th>
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<tr>
<td>Gene</td>
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<tr>
<td>miR-335 Forward: GTCGTATCCAGTGCAGGGTCCG</td>
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<tr>
<td>Reverse: AGGTATCGCACTGGATACGACA</td>
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<tr>
<td>CRIM1 Forward: TTAGGTGTGGCAGTGGCAGCATC</td>
</tr>
<tr>
<td>Reverse: CCGAGGACATGGTGGTCCAC</td>
</tr>
<tr>
<td>TGF-β1 Forward: CGACCATCGGAGGTGCGCATGAC</td>
</tr>
<tr>
<td>Reverse: CTGGGCGACCTCTCTCTTG</td>
</tr>
<tr>
<td>Bax Forward: AGCCACATGGACCCCTTTGCCAGAG</td>
</tr>
<tr>
<td>Reverse: GTAGTGATGTGACGACGACGACGACGAC</td>
</tr>
<tr>
<td>Bcl-2 Forward: TGAACAGTCCATACCGGGCC</td>
</tr>
<tr>
<td>Reverse: CTCAGTCTGAGGAAGCCCAAGG</td>
</tr>
<tr>
<td>GAPDH Forward: GCAGGCAATCAAGGGGCTATG</td>
</tr>
<tr>
<td>Reverse: ATCCAGGGTAAAGGACGGGT</td>
</tr>
<tr>
<td>U6 Forward: ACATGCACGAGATACTGACATTC</td>
</tr>
<tr>
<td>Reverse: GTGGAACGGAATTTGCCAGTGA</td>
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Note: TGF-β1, transforming growth factor.
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for 5 min, and the supernatant was discarded. The apoptosis was measured using an Annexin-V-FITC/PI apoptotic detection kit (Beyotime Biotechnology, Shanghai, China). The FITC and PI fluorescences were quantified by activating the band pass filters of 525 nm and 620 nm with a wavelength of 488 nm. The experiment was repeated three times.

Statistical methods

SPSS 21.0 statistical software (SPSS, Inc., Chicago, IL, USA) was used to process the data in this study. The results are expressed as the mean ± standard deviation. For the comparisons of the normal distribution data among the groups, one-way ANOVA followed by Tukey post-hoc tests were carried out. For the comparisons of the skewed distribution data among the groups, Kruskal-Wallis tests and Dunn's multiple comparisons were carried out. P<0.05 was considered statistically significant.

Results

Blood pressure and urinary protein levels of the established animal models

Before modeling (the 6th day of pregnancy), there were no significant differences in the systolic blood pressure, diastolic blood pressure, or urinary protein levels between the normal and model groups (all P>0.05). After the modeling (the 18th day of pregnancy), these indexes in the model group all increased significantly compared with the normal group (all P<0.05), indicating that the model was successfully established (Figure 1).

miR-335 targeting downregulates CRIM1

The TargetScan website (http://www.targetscan.org/vert_72/) found that there were binding sites between miRNA-335 and CRIM1 (Figure 2A). The results of the dual-luciferase reporter system assay demonstrated that,
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compared with the cells that underwent co-transfection with the NC and CRIM1-3’UTR-WT plasmids, the luciferase activity of those that underwent the co-transfection with the miR-335 mimic and the CRIM1-3’UTR-WT plasmids decreased significantly (P<0.05, Figure 2B), which indicates that miRNA-335 targets CRIM1 mRNA and negatively regulates the CRIM1 expression.

Isolation and identification of the placental trophoblast cells

A low vimentin expression is considered a basic marker of trophoblast cells. We used immunofluorescence to identify the vimentin expressions in isolated trophoblastic cells. Positive cells exhibited green fluorescence (vimentin) in the cytoplasms and blue fluorescence (DAPI) in the nuclei. The positive expression rate of vimentin in the isolated cells was only 4.25±0.53%, indicating that the trophoblast cells were successfully extracted (Figure 3).

miR-335 inhibition enhances the expression of CRIM1, TGF-β1, and Bcl-2, and decreases the Bax expression

qRT-PCR and Western blot were used to quantify the miRNA-335, CRIM1, TGF-β1, Bcl-2, and Bax expressions in the rat placental trophoblast cells. The results showed that compared with the normal group, all the other groups exhibited increased miRNA-335 expression, and decreased CRIM1, TGF-β1, and Bcl-2 expressions, and increased Bax expression (all P<0.05). Compared with the blank group, the miR-335 inhibitor group, the CRIM1 group, and the miR-335 inhibitor + CRIM1 group exhibited increased mRNA and protein expressions of CRIM1, TGF-β1, and Bcl-2 and decreased Bax expression (all P<0.05); the changes of these indicators were most significant in the miR-335 inhibitor + CRIM1 group. Compared with the blank group, the miRNA-335 mimic group exhibited increased miRNA-335 expression, decreased CRIM1, TGF-β1, and Bcl-2 expressions, and increased Bax expression (all P<0.05). There were no significant differences in any of the indexes between the blank and NC groups (all P>0.05) (Figure 4).

miR-335 inhibition or CRIM1 overexpression promotes the proliferation of placental trophoblast cells

Compared with the normal group, the proliferation of the trophoblast cells in the other groups decreased at each time point (all P<0.05). Compared with the blank group, the cell proliferation in the miR-335 inhibitor group, the CRIM1 group, and the miR-335 inhibitor + CRIM1 group increased significantly (all P<0.05), and the changes in the miR-335 inhibitor + CRIM1 group were most significant. Compared with the blank group, the cell proliferation in the miR-335 mimic group decreased significantly (P<0.05). These results indicate that inhibiting miRNA-335 expression can promote CRIM1 expression and the proliferation of trophoblast cells in preeclamptic rats (Figure 5).

miR-335 inhibition or CRIM1 promotion can inhibit the apoptosis of trophoblast cells

Compared with the normal group, the apoptosis in other groups increased significantly (all P<0.05). Compared with the blank group, the miR-335 inhibitor group, the CRIM1 group, and the miR-335 inhibitor + CRIM1 group exhibited significantly decreased apoptosis (all P<0.05), with the miR-335 inhibitor + CRIM1 group exhibiting the greatest change. In contrast, the miR-335 mimic group exhibited increased apoptosis (P<0.05). There was no significant difference between the blank and NC groups (P>0.05). These results indicate that inhibiting
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Figure 4. The miR-335, CRIM1, TGF-β1, Bcl-2, and Bax expressions in the rat placental trophoblast cells after the transfection. A: The miR-335 expressions and the mRNA in CRIM1, TGF-β1, Bcl-2, and Bax; B: The CRIM1, TGF-β1, Bcl-2 and Bax protein bands; C: A quantitative diagram of the CRIM1, TGF-β1, Bcl-2, and Bax protein expressions. Compared with the normal group, *P < 0.05; compared with the black group, #P < 0.05; compared with the miRNA-335 inhibitor + CRIM1 group, %P < 0.05. TGF-β1, transforming growth factor.

Figure 5. The cell viability of the rat placental trophoblast cells after transfection. Compared with the normal group, *P < 0.05; compared with the black group, #P < 0.05; compared with the miRNA-335 inhibitor + CRIM1 group, %P < 0.05.

Discussion

In this study, we explored the role of miRNA-335 in the pathogenesis of preeclampsia and its potential mechanism. Our results suggest that inhibiting the expression of miRNA-335 can promote the expression of CRIM1, thereby promoting the viability and reducing the apoptosis of trophoblast cells, and thus playing a protective role in the pathogenesis of preeclampsia.

As a complication during pregnancy, the most common characteristics of preeclampsia are hypertension, proteinuria, and renal dysfunction, of which hypertension and proteinuria are important criteria for assessing the severity of preeclampsia and the other organs involved [18-21]. First, we measured the hypertension and proteinuria indexes and found that the systolic blood pressure, diastolic blood pressure, and urinary protein levels in the model rats were significantly higher than the corresponding levels in normal rats, indicating that the preeclamptic model was successfully established. Next, we found a significant increase in the miRNA-335 expression and a decrease in the CRIM1 expression in the extracted trophoblast cells. In recent years, more and more small RNAs have been found to play a regulatory role in the pathogenesis of preeclampsia [7, 8]. In addition, a study reported that the expression of miRNA-155 in the serum of patients with advanced eclampsia was significantly increased, and its expression level was closely related to the urinary protein content [22]. The expression of miRNA-210 was significantly up-regulated in preeclampsia placenta, and the over-expression of miRNA-210 was able to inhibit the invasion of trophoblast cells and accelerate the progress of preeclampsia [23]. Studies have shown that miR-335 can
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Figure 6. The apoptosis rate of the rat placental trophoblast cells after the transfection. A: The apoptosis after the transfection in each group; B: The apoptosis rate in each group. Compared with the normal group, *P<0.05; compared with the black group, ^P<0.05; compared with the miRNA-335 inhibitor + CRIM1 group, %P<0.05.
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accelerate renal injury and renal aging by up-regulating the mitochondrial antioxidant enzyme SOD2 [24]. Preeclamptic patients also often have kidney damage, so we speculated that miR-335 may play a role in the progress of preeclampsia. One study confirmed that the over-expression of miRNA-335 can inhibit the migration of trophoblast cells and accelerate the progress of severe preeclampsia [9]. CRIM1 is thought to be a key gene in placental development, and a previous study confirmed that CRIM1 plays an important role in the differentiation and regulation of placental trophoblasts [12]. Based on previous studies, this study further confirmed that CRIM1 expression is regulated by miRNA-335, both of which play an important role in the pathogenesis of preeclampsia.

The expression of TGF-β1 in normal cultured placental trophoblast cells is significantly up-regulated [25, 26]. TGF-β1 can enhance the adhesion and infiltration abilities of trophoblast cells by up-regulating the expression of E-cadherin and plays a protective role in preeclampsia [27]. Previous studies on metastatic cancer cells found that the expression of E-cadherin decreased significantly after the knock-out of CRIM1, suggesting that CRIM1 can promote the adhesion and infiltration abilities of cancer cells [13]. Our study found that the TGF-β1 and Bcl-2 expression in trophoblast cells in preeclampsia were significantly down-regulated, but the Bax expression was up-regulated. After inhibiting miRNA-335 expression or overexpressing CRIM1, the above changes were reversed, and the combined effect of the two interventions was even more significant. These results suggest that inhibiting the expression of miRNA-335 can promote the expression of CRIM1 and thus inhibit apoptosis and promote the proliferation and infiltration of trophoblast cells in preeclampsia placenta. MTT and flow cytometry further confirmed that inhibiting miR-335 expression or promoting CRIM1 expression can enhance the viability and reduce the apoptosis of trophoblast cells, and play a significant protective role in preeclampsia. In addition, miR-335 inhibition combined with CRIM1 overexpression further enhanced the above results. This may be due to the loss of the post-transcriptional regulation of CRIM1 expression after miRNA-335 inhibition, while CRIM1 overexpression further enhances its role and augments its effect.

This study explored the role of miRNA-335 and CRIM1 in the progression of preeclampsia at the level of placental trophoblast cells, elucidating the molecular mechanisms of preeclampsia and contributing to its treatment. However, the effects of miRNA-335 and CRIM1 and their related pathways on preeclampsia still warrant future studies.

Inhibiting the expression of miRNA-335 can promote the expression of CRIM1, which in turn inhibits apoptosis and promotes the viability of trophoblast cells and thus plays a protective role in the pathogenesis of preeclampsia.

Disclosure of conflict of interest

None.

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