MIR210HG may promote the progression of preeclampsia through CDHR5

Ningxia Sun
The Affiliated Hospital of Qingdao University

Lu Zhang
The Affiliated Hospital of Qingdao University

Jine Xu
The Affiliated Hospital of Qingdao University

Mengmeng Han
The Affiliated Hospital of Qingdao University

Aiping Chen (Chenaiping516@163.com)
The Affiliated Hospital of Qingdao University

Shiguo Liu
The Affiliated Hospital of Qingdao University

Research Article

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Abstract

Introduction: Because early diagnosis and timely detection of preeclampsia (PE) remains a challenge. This study aims to explore the differential expression and pathogenic mechanism of the IncRNA MIR210HG in peripheral blood, which will lay the basis for its use as a biomarker of PE.

Methods: Total RNA was extracted from the peripheral blood of 88 women with preeclamptic pregnancies and 81 women with normal pregnancies. The MIR210HG expression levels were determined by RT-PCR and western blot. SiRNA and MIR210HG overexpression plasmids were constructed and transfected into human trophoblast and vascular endothelial cells.

Results: The lncRNA MIR210HG was significantly upregulated in the peripheral blood of patients with PE compared to the control group. Additionally, the expression level of MIR210HG in patients with early-onset PE was significantly higher than that of the control and late-onset PE groups. CDHR5, identified by bioinformatics predictions after RT-PCR confirmation, was upregulated in placental tissue compared to the control group. Furthermore, cytology experiments confirmed that knockdown of MIR210HG also decreased CDHR5 in human trophoblast and vascular endothelial cells. After knockdown of CDHR5, expression of MIR210HG was also reduced.

Discussion: The IncRNA MIR210HG is highly expressed in the peripheral blood of PE, indicating MIR210HG may become a potential biomarker for the diagnosis and prediction of PE. In addition, MIR210HG may interact with CDHR5 to jointly promote the progression of PE. However, further experiments are needed to confirm the pathogenic mechanism.

Introduction

As a pregnancy-specific disease, preeclampsia (PE) occurs after twenty weeks of gestation and presents with a continuous progression of damage to multiple organs and systems which seriously threatens the lives of mothers and fetuses, and it is one of the main causes of maternal and perinatal death with an incidence rate of 2–8% [1], Although previous studies have confirmed that several key factors are involved in the occurrence and development of PE such as insufficient uterine spiral artery remodeling, endothelial cell damage, massive release of inflammatory factors, and an imbalance of angiogenic and anti-angiogenic factors, the etiology and pathogenesis of PE remain unclear [2]. Therefore, early diagnosis and timely intervention are particularly important for improved PE outcomes. In recent years, previous studies have indicated that there are differential expressions of biomolecules in the placenta and peripheral blood of patients with PE, such as anti-angiogenic factor soluble FMS-like tyrosine kinase-1 (sFlt-1), vascular endothelial growth factor (VEGF), and placental growth factor (PLGF), among which hypoxia-inducible factor-1α (HIF-1α), endothelin-1 (ET-1) and inducible nitric oxide synthase (iN-OS) are essential in vascular remodeling, endothelial cell damage, and even placental development and formation [3–5]. However, most of the identified biomarkers for PE diagnosis were derived from placental tissues, and their lagging and invasive properties lead to low accuracy and specificity. Therefore, biomarkers in peripheral blood have higher clinical significance for the diagnosis of PE due to the feasibility and non-invasive nature of peripheral blood samples.

Long non-coding RNAs (lncRNAs) consist of more than 200 nucleotides and participate in a variety of biological processes including histone modification, chromatin remodeling, regulation of transcriptional and post-
transcriptional gene expression affecting cell proliferation, and migration and invasion [6]. Luo et al. [7] randomly selected twelve lncRNAs that were differentially expressed in placentas and detected their levels of expression in peripheral blood. NR-027457, AF085938, G36948, and AK002210 were found to be differentially expressed compared with the normal controls, and affected cell proliferation, migration, and invasion abilities. Previous studies [8] by our group have screened the differentially expressed lncRNA profiles by analyzing the RNA extracted from five placentla tissues in PE and control groups using RNA-seq technology. We found that upregulated MIR210HG inhibited proliferation and migration of HTR8/SVneo cells, suggesting that it may be a regulator of the biological behavior of trophoblasts. Highly expressed in various cancers, such as hepatocellular carcinoma, non-small cell lung cancer, and osteosarcoma [9–11], the lncRNA MIR210HG may promote various biological processes such as proliferation, migration, and invasion of tumor cells. In our present study, we describe for the first time the differential expression of the lncRNA MIR210HG in the peripheral blood of patients with PE, and revealed its potential co-regulating factor CDHR5 with the aim to provide a reliable basis for MIR210HG to become a potential biomarker for the diagnosis and prediction of PE.

Materials and methods

Subject

A total of 88 patients with PE were selected as the case group while during the same period, 81 women with normal pregnancies were selected as the control group from the obstetrics department of the Affiliated Hospital of Qingdao University from 2020 to 2021. PE was diagnosed according to the criteria of the International Society for the Study of Hypertension in Pregnancy [12]; Exclusion criteria are as follows: (1) pregnancies with chronic hypertension; (2) multiple pregnancies; (3) pregnancy-related complications such as abnormal liver and renal function, diabetes, and heart disease; (4) developmental abnormalities of fetuses. This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (approval number see the additional materials), and all participants provided informed consent.

Basic information was collected from all pregnancies such as age, gestational age, pregnancy time, fetal birth weight, hemoglobin, liver and renal function indexes (creatinine, urine protein, alanine aminotransferase, aspartate aminotransferase, and albumin globulin ratio), systolic blood pressure, diastolic blood pressure, mean arterial pressure, and body mass index (BMI). A total of 88 cases were divided into two subgroups according to the gestational age at onset: early-onset PE group (n = 13) gestational age at onset 28–33 weeks and late-onset PE group (n = 75) gestational age at onset: 34–39 weeks. The cases were also divided into patients with PE (n = 47) and patients with severe PE (n = 41) according to disease severity [13].

Peripheral blood and placental tissue collection

The upper plasma and buffy coat were separated by centrifugation at 3000 rpm for 10 min immediately after 5 mL of venous blood was drawn from patients before childbirth. Trizol reagent (TaKaRa, Japan) was added to the plasma and buffy coat, and then stored at -80°C until further use.

After delivery of the placentae in both groups, the tissue attached to the root of the umbilical cord (1.0 × 1.0 × 1.0 cm) was immediately obtained, and both the fetal and maternal sides were preserved. The placental tissues were washed with pre-cooled saline at 4 °C until there was no blood and immersed in RNA store solution (Kangwei
Century, China) overnight at 4 °C. The RNA store solution was discarded and the placentae were stored at -80 °C until further use.

Cell culture and transfection

Endothelial cells (Eahy) and human trophoblasts cell lines (Bewo/ JEG-3) were purchased from the Chinese Academy of Sciences, and were cultured with DMEM (Procell, USA) containing 12% fetal bovine serum (FBS; Biological Industries, Israel), 1% 100× penicillin-streptomycin mixture (Procell, USA); Bewo special medium (Procell, USA) and MEM (Procell, USA) containing 15% FBS at 37°C in a 5% CO₂ cell incubator, respectively. Lipofectamine 3000 (Lipo3000TM) was purchased from Invitrogen (America), siRNA was purchased from Shanghai Jima Company, and the culture media were changed according to the growth of the cells. When the cell densities reached more than 80%, trypsin (Procell, America) was used to dissociate adherent cells for passaging. The cells were inoculated into 24-well plates and cultured until the cell aggregation rate reached more than 70%. Then, the cells were transfected with si-NC (si-NC group), si-MIR210HG (si-MIR210HG group), pcDNA3.1-NC (pcDNA3.1-NC group), pcDNA3.1-MIR210HG (pcDNA3.1-MIR210HG group), and si-CDHR5 (si-CDHR5 group). RNA was isolated and purified after 48 h. The cells were inoculated into 12-well plates and cultured for 72 h. Protein was extracted from the cells and the concentration was measured. Biological experiments were performed in triplicates. The sequence of the siRNA was as follows:

<table>
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<th></th>
<th>sense</th>
<th>antisense</th>
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</thead>
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<td>siRNA1 5'-CACCUGCAGAGCUAACUUATT-3'</td>
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<tr>
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<tr>
<td></td>
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<td>5'-UUAUAGAAGUGUUGGGCTT-3'</td>
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<tr>
<td>CDHR5</td>
<td>siRNA1 5'-GAGGACACGAAAGUGAACUTT-3'</td>
<td>5'-AGUUCACUUCUGGUCCCTT-3'</td>
</tr>
<tr>
<td></td>
<td>siRNA2 5'-GCCCAACAUCAUCGAAUATT-3'</td>
<td>5'-UAUUCGAGAUGUGGCTT-3'</td>
</tr>
<tr>
<td></td>
<td>siRNA3 5'-GUGGUGAGUACCCAUCAATT-3'</td>
<td>5'-AUGUAGGAGUACCCACTT-3'</td>
</tr>
</tbody>
</table>

RNA isolation and real-time quantitative PCR (RT-qPCR) and western blot

Total RNA was isolated from the peripheral blood of pregnant patients and cells using Trizol reagent. The reverse transcription reaction was performed using HiScript® II Q RT SuperMix for qPCR (+ gDNA wiper) kit (Vazyme, China). Real-time qPCR was performed using the ChamQ Universal SYBR qPCR Master MIX kit (Vazyme, China). The reaction conditions were as follows: pre-denaturation at 95°C for 30 s, denaturation at 95°C for 5 s, and extension at 60°C for 30 s. All samples were normalized to the RNA of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data were analyzed using the 2^{-\Delta Ct} method, where \triangle Ct = Ct gene-Ct control. All reactions were performed in triplicate. The following primers were used:
Total protein was isolated using RIPA lysis buffer. Protein concentrations were determined using the BCA Protein Assay Kit (Beyotime, China). Equal amounts of protein (50 µg) were separated by 10% SDS-PAGE and transferred onto a PVDF membrane (Solarbio, America). After blocking with 10% non-fat dry milk dissolved in tri-buffered saline Tween (TBST, Solarbio, USA), the membranes were placed in diluted primary antibody and incubated overnight at 4°C, and then incubated in diluted goat anti-rabbit IgG. The Omni-ECL™ Femto Light Chemiluminescence kit (Epizyme, China) was used to detect the proteins. The gray values of the bands were analyzed with Image J software, and the relative expression of CDHR5 was calculated using GAPDH as the internal reference. (Antibodies were purchased from Proteintech).

### Prediction of MIR210HG function

AnnoInc2 (http://annoinc.gao-lab.org) was used to predict upstream regulatory genes, miRNAs, and proteins interacting with the lncRNA MIR210HG. The encyclopedia of RNA interactomes (ENCORI) database was used to predict the RNAs and RNA-binding proteins (RBPs) that interact with MIR210HG to perform gene ontology (GO) functional annotation. The functional analysis of MIR210HG was enriched by the Kyoto encyclopedia of gene and genomes (KEGG) and BioCarta pathways.

### Statistical analysis

The experimental data were statistically analyzed using IBM SPSS Statistics 20.0 and GraphPad Prism 8.4.3 software. Each group had three independently replicated experiments, and the description of the measurement data was expressed as mean ± standard deviation (x ± s). A two-tailed Student’s t-test was used for comparison of measurement data satisfying normal distribution with homogeneous variance between two groups while measurement of data not satisfying normal distribution was performed using the Wilcoxon rank sum test. One-way ANOVA was used for comparison among multiple groups. The count data were described in the form of frequency or rate (%), and the \( \chi^2 \) test was used; the Mann-Whitney test was used for comparison between multiple groups; the correlation between the relative expression of lncRNA and related indexes was analyzed using the Pearson correlation or Spearman correlation method, and the diagnostic value of lncRNA for PE was analyzed by ROC. The difference was considered statistically significant at \( P < 0.05 \).

### Results

The analysis of clinical characteristics between PE group and control group.

The clinical characteristics of these pregnancies are shown in Table 1. There was no statistical difference in age, creatinine, and International Normalized Ratio (INR) between the PE group and the control group (\( P > 0.05 \)). The gestational age, gravida, and fetal birth weight of the patients were lower than those of the control group, while
the systolic blood pressure, diastolic blood pressure, mean arterial pressure, BMI, hemoglobin, alanine aminotransferase, aspartate aminotransferase, and albumin globulin ratio were higher than those of the control group. The difference was statistically significant. (P < 0.05).

**IncRNA MIR210HG expression between PE and control group**

Previous studies by our group have found that a total of twenty-six differentially expressed IncRNAs were obtained by RNA-seq, of which twenty-one were upregulated and five were downregulated. Among the twenty-one upregulated IncRNAs, the differences in expression of maternally expressed gene 3 (MEG3), RP11-416N2.3, KB-1572G7.2, AC110619.2, and MIR210HG were greater, and among the five downregulated IncRNAs, RP11-161H23.5 had the greatest difference in expression. Experiments have confirmed that the expression level of MIR210HG in maternal placental tissue was higher in the PE group than in the control group [8]. In present study, we further investigated the expression of IncRNA MIR210HG in the peripheral blood of patients with PE.

The qPCR results confirmed that MIR210HG was significantly upregulated in the peripheral blood of the PE group (n = 88) compared to the control group (n = 81) (Fig. 1a, t = 2.314, P = 0.02 0.05).

By analyzing the expression of MIR210HG in 88 patients in the PE group and 81 patients in the control group, we found the area under the curve (AUC) value of IncRNA MIR210HG in peripheral blood for the diagnosis of PE was 0.641, which had a significant difference (95% CI: 0.558 ~ 0.724, p = 0.0015 0.01, Fig. 1b). MIR210HG had 44% sensitivity and 80% specificity at a cutoff value of < 0.2150.

To investigate the effect of MIR210HG expression on clinical indices, we analyzed the correlation between MIR210HG and clinical indices. The results showed that the expression level of MIR210HG was significantly positively correlated with BMI, systolic blood pressure, diastolic blood pressure, and mean arterial pressure (the correlation coefficients were 0.172, 0.153, 0.162, and 0.165, respectively, p-values were 0.026, 0.047, 0.036, and 0.033, respectively), and was significantly negatively correlated with gestational age (p = 0.01, the correlation coefficient was −0.199) (Fig. 2).

PE occurs after twenty weeks of pregnancy, and if no intervention occurs, it will further develop into severe PE, eclampsia, or even hemolysis, elevated liver enzymes, and low platelets (HELLP) syndrome [2, 14]. Furthermore, according to its onset time PE can also be divided into early-onset (before 34 weeks) and late-onset (34 weeks or later).

By studying the different onset time and severities of the case group, we found that MIR210HG was upregulated in patients with PE when compared to the control group (p = 0.034 0.05). There was no significant difference between patients with severe PE [13] and the control group (p = 0.105 > 0.05), or patients with PE and severe PE (P = 0.705 > 0.05). (Fig. 3a, Table 2)

MIR210HG in patients with early-onset PE was significantly upregulated when compared to the control (p = 0.000 0.01) and late-onset PE groups (P = 0.001 < 0.01). However, there was no statistical significance between the late-onset PE patient group and control group (p = 0.171 0.05) as shown in Fig. 3a, Table 2.

Next, we analyzed the proportion of severe PE in early-onset and late-onset PE. The proportion of severe PE with early-onset PE was higher than that of patients with late-onset PE (p 0.002,Table 3,Fig. 3b).
Prediction of possible downstream target genes

A total of 399 transcription factors, 57 miRNA families and 237 proteins that may interact with the lncRNA MIR210HG were identified by Annolnc2 (http://annolnc.gao-lab.org/), 28 proteins by UV cross-linking immunoprecipitation followed by high-throughput sequencing [15], and 38 RNAs by The ENCORI database including processing pseudogene, ribosomal RNA (rRNA), small nuclear RNA, transcriptional processing pseudogene, mRNA, lncRNA in the intergenic region, and transfer RNA (tRNA). KEGG pathway enrichment revealed that MIR210HG may be involved in eight signaling pathways, including type I diabetes, autoimmune thyroid disease, graft-versus-host disease, viral myocarditis, antigen processing and presentation, allograft rejection, natural killer cell-mediated cytotoxicity, and cell adhesion molecules, of which the first four are more typical. In addition, GO functional annotation analysis revealed that MIR210HG may be involved in twenty-seven signaling pathways, including nineteen pathways involving biological processes, six pathways involving cellular components, and two pathways involving molecular functions. BioCarta pathway enrichment also showed that MIR210HG may be involved in eight signaling pathways.

CDHR5 may be a downstream target gene of MIR210HG

Next, we found MIR210HG possible downstream target genes including IRF7, RNH1, DEAF1, HRAS, DRD4, LRRC56, PHRF1, SCT, LMNTD, CDHR5, PTDSS2, and RASSF7 using bioinformatics prediction. We examined whether the expression of these genes was changed by qPCR after MIR210HG knockdown or overexpression in vascular endothelial cells, and found that there was a significant difference in the expression of CDHR5. Therefore, we speculated that CDHR5 may be a downstream target gene of MIR210HG. (Fig. 4a).

To further investigate the role of CDHR5, we verified the expression of CDHR5 protein in placental tissues. The expression level of CDHR5 protein in the PE group was higher than that of the control group (PE vs. Control: 3.341 ± 1.032, n = 4 vs. 1.000 ± 0.000, n = 4, p = 0.004, Fig. 4b/c).

To verify the correlation between MIR210HG and CDHR5, we knocked down or overexpressed MIR210HG in vascular endothelial and trophoblast cells. RT-qPCR showed that after transfection with si-MIR210HG in Eahy/Bewo/JEG3 cells, the relative expression of MIR210HG and CDHR5 were all significantly lower than in those transfected with si-NC (Fig. 4d/e).

After transfecting Bewo/JEG3 cells with the pcDNA3.1-MIR210HG and negative control plasmids, RT-PCR showed that the relative RNA expression levels of MIR210HG and CDHR5 in Bewo/JEG3 cells transfected with the pcDNA3.1-MIR210HG plasmid were significantly higher than those in the negative control plasmid group (Fig. 8f/g). There was no statistical difference in Bewo cells.

The results of western blotting showed that the expression level of CDHR5 protein also decreased (p = 0.019, 0.006 0.05, Fig. 4h/i) in Bewo/JEG3 cells after the knockdown of MIR210HG.

Validation experiment of the relationship between MIR210HG and CDHR5

To verify the association of MIR210HG with CDHR5, we performed further validation experiments. We transfected si-CDHR5 and negative control si-NC in Bewo/JEG3 cells, and RT-PCR showed that the relative RNA expression
levels of Bewo/JEG3 cells transfected with si-CDHR5 were significantly lower than those transfected with si-NC (p \textless 0.0001, Fig. 4j). Next, we detected the RNA expression of MIR210HG in Bewo/JEG3 cells in the successful knockdown group. The study found that the relative RNA expression levels of MIR210HG in Bewo/JEG3 cells successfully transfected with si-CDHR5 were lower than those in Bewo/JEG3 cells transfected with si-NC (p = 0.023 < 0.05, p = 0.083 < 0.05, Fig. 4h), but there was no statistical difference in JEG3 cells.

**Discussion**

Our previous studies have found that the expression level of MIR210HG in PE placental tissue was significantly higher than that of the control group, and the proliferation and migration of trophoblast cells were significantly enhanced after knockdown of MIR210HG, suggesting that MIR210HG may promote the development of the pathological placenta. In this study, we demonstrated that the expression of IncRNA MIR210HG in peripheral blood of patients with PE was significantly upregulated. Cytology experiments confirmed that the expression of MIR210HG was positively correlated with CDHR5, suggesting that MIR210HG may act by regulating the expression of CDHR5 in PE.

As a new type of IncRNA, MIR210HG is the host gene of miRNA MIR210 and can work together to participate in the regulation of hypoxia and the proliferation of tumor cells [16]. Overexpressed in cervical, endometrial, colon, breast, and lung cancers [10, 16–21], MIR210HG plays an important role in the occurrence and development of various tumors. Wang et al. [17] demonstrated the oncogenic role of MIR210HG in cervical cancer for the first time. As a ceRNA, overexpressed MIR210HG can inhibit the expression of miR-503-5p and upregulate TRAF4, thereby promoting the progression of cervical cancer, which was associated with advanced clinical features and poor prognosis. Furthermore, through the TGF \( \beta / \text{Smad3} \) and Wnt/\( \beta \)-catenin signaling pathways, MIR210HG targets the miR-337-3p/137-HMGA2 regulatory axis to promote the malignant biological behavior of endometrial cancer cells. The high expression of MIR210HG is also positively correlated with the late clinicopathological stage of endometrial cancer and the high positive rate of lymph node metastasis [18]. Although MIR210HG has been shown to be an independent prognostic factor associated with poor prognosis in cervical cancer [17], endometrial cancer [18], osteosarcoma [11], liver cancer [9], and breast cancer [21], the role and underlying mechanism of MIR210HG in PE remain unclear. Blood pressure and urine protein are mainly used to assess the severity of PE patients and most patients already have severe PE at the time of diagnosis. At present, symptomatic treatments such as antihypertensive treatment and albumin supplementation are often used to relieve symptoms. Here, we revealed that the expression of MIR210HG in the peripheral blood of PE patients was up-regulated and positively correlated with systolic, diastolic, and mean arterial pressures, suggesting that in addition to monitoring the blood pressure and urine protein of patients, monitoring the expression of MIR210HG in peripheral blood of patients is helpful for early clinical diagnosis of PE. We found that MIR210HG has high diagnostic values with a ROC curve AUC value of 0.642. Chronic hypoxic placental injury is more common in patients with early-onset PE than in patients with late-onset PE [22]. We also showed the expression level of MIR210HG in the peripheral blood of patients with early-onset PE was higher than those of normal controls and late-onset PE groups, indicating that MIR210HG may be involved in cell homeostasis and proliferation under hypoxic conditions [16]. Our further study found that the proportion of severe PE with early-onset PE was higher than that of patients with late-onset PE, and the difference was statistically significant. This suggested that the IncRNA MIR210HG may be utilized as a novel biomarker to help determine the onset and prognosis of PE patients by detecting their expression levels in peripheral blood; thus, providing a reliable basis for MIR210HG to become a potential diagnostic biomarker for the diagnosis and prediction of PE in clinical practice.
CDHR5, a member of the cadherin-related family, encodes a protein that is a developmentally regulated cell adhesion molecule expressed in various epithelial tissues, and is a candidate tumor suppressor for colon cancer [23], pancreatic duct adenocarcinoma [24], clear cell renal carcinoma [25], and delayed intracerebral hemorrhage [26]. Losi et al. [27] found that nuclear translocation of β-catenin may be involved in the expression of CDHR5, and that they may work together to promote the progression of colon cancer. Bersuder et al. also revealed the role of anti-oncogenic β-catenin/MUCDHL (mucin and cadherin-like) negative feed-back loop in colorectal cancer [23]. In addition, aberrant methylation of the CDHR5 promoter downregulates CDHR5, which is beneficial to abnormal cell proliferation [28]. However, some studies have different opinions that the high expression of CDHR5 can significantly promote the invasion and migration of pancreatic duct adenocarcinoma (PDAC) cells. In this study, our results indicated that the expression of CDHR5 protein in placental tissue was significantly higher than that of the control group, which was positively correlated with the expression of the IncRNA MIR210HG. Previous studies on this subject have found that highly expressed MIR210HG can inhibit the proliferation and migration of trophoblast cells, resulting in pathological implantation of the placenta, insufficient remodeling of spiral arteries, and promotion of PE [8]. Our study further found that the IncRNA MIR210HG may interact with CDHR5 to jointly inhibit the proliferation and migration of trophoblast cells, thereby promoting the progression of PE. However, further experiments are needed to confirm its pathogenic mechanism.

Our study found that MIR210HG is highly expressed in the peripheral blood of PE, and has a high diagnostic value, which provides a reliable basis for MIR210HG to become a potential diagnostic biomarker for the diagnosis and prediction of PE. The advantage of easy access to the material has promoted MIR210HG as a biomarker. Therefore, improving the diagnosis of PE by detecting the expression level of the IncRNA MIR210HG in peripheral blood is promising. However, further experimental confirmation remains necessary.

Declarations

Acknowledgements

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Competing interests

The authors declare that they have no competing interests

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Affiliated Hospital of Qingdao University (QYFY WZLL 27435), and all participants provided informed consent.

Consent for publication

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed.
Availability of data and material

Datasets are available through the corresponding author upon reasonable request.

Code availability

Not available.

References

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Sequencing. mSystems, 2019. 4(6).


Tables

Table 1 Clinical characteristics of PE and normal pregnancies
<table>
<thead>
<tr>
<th>Variable</th>
<th>PE group n=88</th>
<th>Control group n=81</th>
<th>P value</th>
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<tr>
<td>Maternal age/year</td>
<td>29.8462±5.1575 19-40</td>
<td>30.3827±5.2525 18-43</td>
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<td>Gestational age/week</td>
<td>37.1616±2.3142(28.57-42.00)</td>
<td>39.2360±1.1906(36.29-42.30)</td>
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<td>Gravida/time</td>
<td>2.5000±1.2865 1-6</td>
<td>2.9630±1.5037 1-9</td>
<td>0.033*</td>
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<td>SBP/mmHg</td>
<td>155.1136±13.3644 138-210</td>
<td>118.9500±9.5836 100-139</td>
<td>0.000***</td>
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<td>DBP/mmHg</td>
<td>99.7500±10.7887 75-140</td>
<td>77.3000±7.0700 65-89</td>
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<tr>
<td>Mean arterial pressure/mmHg</td>
<td>118.2045±10.4923 100.00-163.33</td>
<td>91.1833±6.8253 76.67-103.67</td>
<td>0.000***</td>
</tr>
<tr>
<td>Fetal birth weight/g</td>
<td>3091.5663±772.3333 1500-4800</td>
<td>3520.6410±476.0343 2000-4500</td>
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<td>BMI/kg/m²</td>
<td>31.2983±4.0924 19.53-41.62</td>
<td>29.1385±2.8877 22.58-37.34</td>
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<td>Hb/g/L</td>
<td>119.3793±14.9116 73-147</td>
<td>114.0694±13.0905 80-152</td>
<td>0.019*</td>
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<td>Creatinine/umol/L</td>
<td>56.9455±11.8294 28.09-83.90</td>
<td>54.3220±12.3758 34.23-105.12</td>
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<td>ALT/U/L</td>
<td>15.8280±14.4883 3.65-90.50</td>
<td>9.1840±5.1561 3.10-36.00</td>
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<td>19.8597±14.8204 8.91-111.98</td>
<td>14.8040±6.7730 7.00-52.10</td>
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<td>A/G</td>
<td>1.1479±0.2934 0.40-1.80</td>
<td>1.3073±0.1268 1.09-1.60</td>
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<td>INR</td>
<td>0.9762±0.0848 0.79-1.16</td>
<td>0.9677±0.0799 0.82-1.29</td>
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Data are presented as the mean ± SD. ***P < 0.001, **P < 0.01, *P < 0.05. Abbreviations: SD: standard deviation. BMI, body mass index. SBP, Systolic blood pressure. DBP, Diastolic blood pressure. Hb, Hemoglobin. ALT, alanine aminotransferase. AST, aspartate aminotransferase. A/G, albumin globulin ratio. INR, international normalized ratio.

Table 2 LncRNA expression levels in different subtype of PE

<table>
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<th>Grades/Types</th>
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<th>P value</th>
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<td>Severe preeclampsia</td>
<td>3.156±5.948</td>
<td>0.705</td>
</tr>
<tr>
<td>Control group</td>
<td>1.659±3.168</td>
<td>0.034*</td>
</tr>
<tr>
<td>Early-onset preeclampsia</td>
<td>7.478±9.606</td>
<td>-</td>
</tr>
<tr>
<td>Late-onset preeclampsia</td>
<td>2.693±4.754</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD. ***P < 0.001, **P < 0.01, *P < 0.05.
Table 3 Relation of different subtype of PE

<table>
<thead>
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<th>Grades</th>
<th>PE</th>
<th>Severe PE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Types</td>
<td>Early-onset PE</td>
<td>2 (8.33%)</td>
</tr>
<tr>
<td></td>
<td>Late-onset PE</td>
<td>45 (42.86%)</td>
</tr>
</tbody>
</table>

Figures

Figure 1

MIR210HG was overexpressed in peripheral blood of PE. (a) the levels of MIR210HG were examined using RT-qPCR in peripheral blood isolated from patients with PE and healthy pregnant women. Data are expressed as the mean ± SD. *P < 0.05. (b) ROC curve of IncRNA MIR210HG
Figure 2

Correlation between MIR210HG relative expression and clinical characteristics in peripheral blood of 169 pregnancies

(a) correlation analysis evaluated the interaction between MIR210HG and BMI. (b) correlation analysis evaluated the relationship between MIR210HG and systolic blood pressure. (c) correlation analysis evaluated the interaction between MIR210HG and diastolic blood pressure. (d) correlation analysis evaluated the interaction between MIR210HG and mean arterial pressure. (e) correlation analysis evaluated the interaction between MIR210HG and gestational age.
Figure 3

The relationship between MIR210HG relative expression in peripheral blood and subtype of PE (a) Correlation between MIR210HG relative expression and subtype in peripheral blood of PE group. (b) The proportion of severe PE with different PE type.
Figure 4

Knockdown or overexpression of MIR210HG and consequent decrease or increase of CDHR5. (a) Screening for possible target genes of MIR210HG by RT-qPCR. (b/c) Western blot revealed that CDHR5 was upregulated in placental tissues. (d/e/f/g/h/i) The relative expression level of RNA (d/e/f/g) and protein (h/i) of MIR210HG and CDHR5 transfected with si-MIR210HG and pcDNA3.1-MIR210HG plasmid in different cells. (j/k) Reverse validation of the correlation between MIR210HG and CDHR5.