ORIGINAL RESEARCH

Screening and analysis of plasma differential lncRNA/mRNA in early pregnancy of preeclampsia

Xin ZHANG[1,2,4]#, Ning YANG[5#], Jie LU[1,3#], Yan-yan TAO[6], Xiulong NIU[2], Wei CAI[2,4], Shaobo CHEN[2,4]*, Yuming LI[1]*

1. Clinical School of Cardiovascular Disease, Tianjin Medical University, Tianjin, 300457, China
2. Department of Cardiology, Characteristic Medical Center of the Chinese People's Armed Police Force. Tianjin,300162, China
3. Department of Cardiopulmonary Function, Tianjin Cancer Hospital Airport Hospital, Tianjin 300308, China
4. Tianjin Key Laboratory of Cardiovascular Remodeling and Target Organ Injury, Tianjin,300162, China
5. Department of Hypertension, Tianjin Kanghui Hospital, Tianjin 300380, China
6. Department of Clinical Laboratory, Haihe Hospital, Tianjin University. Tianjin,300350, China

*** Correspondence:** Xin ZHANG, Ning YANG and Jie LU contributed to this article equally as first authors; chensbwj@126.com & cardiology2023@126.com

Abstract

Objectives

This study aimed to characterise the expression profiles of long non-coding RNA (lncRNA) and messenger RNA (mRNA) in plasma samples from individuals with preeclampsia (PE) during early pregnancy (7–14 weeks of gestation). We sought to identify key signalling pathways and biological functions linked to these transcripts and evaluate their potential for early PE diagnosis and therapeutic intervention.

Methods

From January to June 2019, we analysed frozen plasma samples from eight PE patients and eight normotensive pregnant women matched for gestational age. Transcriptome sequencing was performed using the Illumina HiSeq 4000 platform. Differentially expressed lncRNAs and mRNAs were identified with thresholds of $|\text{fold change (FC)}| \geq 2$ and $P \leq 0.05$. Functional enrichment analyses (Gene Ontology [GO] and Kyoto Encyclopedia of Genes and Genomes [KEGG]) were conducted to elucidate associated pathways, and a lncRNA-mRNA coexpression network was constructed to explore regulatory interactions.

Results

We identified 361 significantly dysregulated lncRNAs (171 up- and 190 down-regulated) and 3,798 mRNAs (3,320 up- and 478 down-regulated). Top dysregulated transcripts included ENST00000440816 (lncRNA, $\log_2$FC = +175.29) and TEX35 (mRNA, $\log_2$FC = +8.70). Gene Ontology analysis revealed enrichment in inflammatory response, cell adhesion, and transferase activity, while Kyoto Encyclopedia of Genes and Genomes pathways implicated phosphoinositide 3-kinase-protein kinase B, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and mechanistic target of rapamycin (mTOR) signalling. Coexpression networks highlighted strong associations between dysregulated transcripts and oxidative stress/inflammatory processes.

Conclusion

Early-pregnancy plasma lncRNAs and mRNAs are markedly dysregulated in PE and correlate with pathogenic pathways. Notably, lncRNAs with $|\log_2\text{FC}| \geq 5$ and mRNAs with $|\log_2\text{FC}| \geq 6$ may serve as novel biomarkers for early PE prediction.

Keywords: preeclampsia; lncRNA; mRNA; screening; analysis; pregnancy; women health

Introduction

About 5–8% of all pregnancies are afflicted by the condition known as preeclampsia (PE), which is characterised by high blood pressure (greater than 140/90 mmHg) and proteinuria after 20 weeks of gestation. The placental embolism (PE) was one of the most serious obstetric problems. It can damage many organs and lead to a higher probability of coronary artery disease in both the mother and the foetus[1,2]. It has not yet been entirely recognised that the pathogenesis of PE until now, and investigations have demonstrated that the condition could involve a range of variables, including inadequate trophoblast cell infiltration[3], improper regulation of immunological function[4], damaged endothelium function[5], diet[6], and genetics[7]. At this point, the pathogenesis of PE is still not entirely understood. In most cases, termination of the pregnancy would alleviate the manifestations of PE as well as any difficulties it may have caused. In the past few years, as studies regarding the pathogenesis and epidemiology of PE have progressed more comprehensively, more academic attention has been drawn to biological variables. Amongst these variables, the aberrant expression of genes could be associated with the pathogenesis of PE. The subclass of noncoding RNAs, also known as long noncoding RNAs (lncRNAs), has a sequence length of over 200 nucleotides, does not include any identifiable open reading frames, and controls gene expression at both the transcriptional and post-transcriptional levels[8]. There is evidence that long non-coding RNA (lncRNA) is associated with the emergence and development of several diseases and that it plays a significant role in tumours[9], cardiovascular disease[10], cerebrovascular disease[11], as well as other disorders. Additionally, long non-coding RNAs appear differently in the placenta of PE

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patients in comparison to a normal pregnancy. It has been demonstrated that these Long non-coding RNAs expressed differently in PE patients' placentas are strongly associated with the onset and progression of PE[12]. For example, LncRNA STOX2-IT3 can down-regulate the differentiation and invasion of trophoblast cells by regulating STOX2, thus affecting the PE process at the cellular level[13], Lnc-DCs up-regulate the expression of Th1 cells by stimulating dendritic cells in the decidua of the uterus, thus affecting the occurrence and development of PE on the immune level[14]; lncRNA H19 rs217727 polymorphism and h19-IGF2 domain affect PE at the epigenetic level[15]. Some irregularly expressed Long non-coding RNAs may be employed as an early indicator of PE and may disclose the pathophysiology of PE from a novel perspective, according to a theory. The researchers discovered that long non-coding RNAs may play important roles in biological processes via multiple purposes, including signals (regulating transcription and translation), antisense decoys (interfering with complementary mRNAs and miRNAs), folds (forming functional complexes via binding proteins and other RNAs), and generating miRNAs[16,17]. The studies discovered that long non-coding RNAs may have essential roles in physiological processes via their various functions, such as signals (regulating transcription and translation). A recent study concluded that differences in the gene expression of cell-free RNA (cfRNA) between normotensive mothers and preeclamptic mothers are pronounced and stable early in gestation, which is well before the onset of symptoms. They even uncovered 18 genes that have predictive value when assessed between weeks 5 and 16 of pregnancy, which could recognise moms at risk of preeclampsia even before clinical signs reveal themselves[18]. When examined during weeks 5 and 16 of pregnancy, these genes have prognostic significance. In the present investigation, the technology of transcriptional sequencing was utilised to establish the differential lncRNA/mRNA expression profile of the PE group and healthy pregnant women in the beginning stages of pregnancy. The Gene Ontology (GO) and the Kyoto Encyclopaedia of Genes and Genomes (KEGG) were utilised to investigate the involvement of differentially expressed lncRNA/mRNA in the signalling cascade and biological function associated with PE. Additionally, a lncRNA/mRNA co-expression network was built to investigate this mechanism's potential role in the development and progression of PE.

Materials and methods

Materials

This investigation was conducted as a prospective cohort study, focusing on singleton normotensive pregnant women in Tianjin, China, starting in November 2016. The study received approval from the Medical Ethics and Human Clinical Trial Committee at the Characteristic Medical Centre of PAP (Approval No. PJHEC-2015-R1) and registration number ChiCTR-EOC-15007644. The people who participated were chosen from a total of 19 community hospitals. The participants satisfied the established criteria for inclusion. (1) A pregnancy involving a single fetus. (2) The age at which an individual is enrolled. Participants at least 18 years old and who had a gestational age of 13+6 weeks or less at registration were included in the study. There is no evidence of persistent hypertension, as indicated by a systolic blood pressure (SBP) measurement at the time of enrolment that is below 140 mmHg and a diastolic blood pressure (DBP) upon enrolment below 90 mmHg. All participants in the study provided their written consent. The participants provided informed consent and were then monitored by the established protocol. The prenatal screening method typically commences between 6 and 13+6 weeks of gestation. Pregnancy is assessed at enrolment, followed by subsequent assessments conducted at four-week intervals until the 28th to 30th week. The gestational period typically spans from 2 to 36 weeks, followed by an additional week leading up to the delivery. Plasma samples were obtained from a cohort of pregnant women at gestational ages ranging from 7 to 14 weeks over the period from January 2019 to June 2019, who subsequently experienced a pregnancy outcome. The study consisted of two cohorts: a normal group and a physical exercise (PE) group comprising pregnant women aged between 30 and 40 years. All participants were experiencing their first pregnancy and had comprehensive medical information available. Individuals with pre-existing conditions such as hypertension, diabetes, hyperlipidemia, and significant organ abnormalities were excluded from the study. The results showed no statistically significant differences in age, gestational age, and number of primiparas between the two groups ($P > 0.05$).

Methods

Plasma Collection

A total of 5 mL of peripheral blood samples were obtained from pregnant women during the preliminary prenatal assessment. Subsequently, the specimens were centrifuged at 3,000 ×g for 10 min at 25°C. This process separated plasma, serum, and blood cells, subsequently preserved in a biobank at -80 °C unless they were considered ready for utilisation.

Analytical Methods

The plasma samples from 8 individuals with preeclampsia and 8 healthy pregnant women were subjected to high-through transcriptome sequencing using the Illumina HiSeq 4000 sequencer. The resulting data consisted of dual-end reads. The Q30 metric was employed for quality control, while the procedure for coupling was carried out using Cutadapt software version 1.9.3. The investigation acquired high-quality literature and analysed differential long non-coding RNA (lncRNA) and messenger RNA (mRNA) using software and databases.

Differential lncRNA/mRNA clustering

Using cuffdiff software computation expression, variations among the two sets of samples, and testing of lncRNA, with fold change ($|FC|$) ≥ 2, $P \leq 0.05$ and 2.0 or fragments per kilobase of transcript per million mapped reads (FPKM) ≥ 0.05 was considered as a differential screening criterion. The cluster analysis of differentially expressed RNA using standardised readings was conducted using the R package heatmap2. In a broader context, it can be observed that samples belonging to the identical group tended to cluster together within a particular branch.

GO function enrichment analysis of different lncRNA and mRNA

The Gene Ontology (GO) framework encompasses three fundamental components, namely Molecular Function (MF), Biological Process (BP), and Cell Component (CC). In the context of analysing differentially expressed long non-coding RNA (lncRNA) and messenger RNA (mRNA), the GO Function enrichment analysis is employed[19,20]. To

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elucidate the biological roles associated with distinct regions abundant in long non-coding RNA (lncRNA) and messenger RNA (mRNA), a significance level of $P<0.05$ was employed to determine statistical significance.

KEGG signalling pathway analysis of different lncRNA and mRNA

The KEGG Pathway refers to the systematic method of correlating molecular data sets from genomics, transcriptomics, proteomics, and metabolomics with the KEGG signalling pathway map. This correlation aims to elucidate the underlying biological functions of these molecules [19,20]. The involvement of differentially expressed long non-coding RNA (lncRNA) and messenger RNA (mRNA) in the signalling pathway of the pathogenesis of preeclampsia (PE) was inferred using pathway analysis. Subsequently, their role in the pathophysiological process of PE was deduced. A significance level of $P<0.05$ was employed as the threshold for determining significant enrichment.

Statistical analysis

This study examined the expression profiles of long non-coding RNA (lncRNA) and messenger RNA (mRNA) by utilising the Comprehensive GEO gene expression database, the KEGG pathway database, and the GO gene ontology database. The statistical analysis of the data was conducted using SPSS 22.0. The measurement results were presented as mean ± standard deviation (□□±□), and a One-way analysis of variance was employed to compare the groups. A significance level of less than 0.05 was deemed to be statistically significant.

Result

Identification of lncRNA

The screening criteria for the plasma samples were determined based on the sequencing data. A threshold of $|FC| \geq 2$, equivalent to $|\log_2 FC| \geq 1$, and a significance level of $P \leq 0.05$ were used. To identify the differently expressed long non-coding RNA (lncRNA), a comparison was conducted among the gene expression levels in plasma samples from women with preeclampsia (PE) and plasma samples from normal pregnant women. It was observed that the expression of 171 long non-coding RNAs exhibited a substantial increase, whereas the expression of 190 Long non-coding RNAs showed a significant reduction. These differences were confirmed to be statistically significant, as depicted in Figure 1. The three elements, A, B, and C, are being discussed. Among the identified Long non-coding RNAs, ENST00000440816 exhibits the highest up-regulation, with a fold shift log2FC of 175.286. Conversely, ENST00000608904 has the most pronounced down-regulation, with a fold change log2FC of -82.8757. Table 1 displays the top 10 long non-coding RNAs with up-regulated expression and the top 10 Long non-coding RNAs with down-regulated expression in the preeclampsia (PE) group, compared to the control group.

(A/D) Heatmaps showing expression patterns of all significantly dysregulated Long non-coding RNAs (A) and mRNAs (D) in PE (n=8) versus control (n=8) plasma samples collected at 7-14 weeks of gestation. Each row represents a transcript, and each column represents a sample. Red indicates upregulation (log2FC ≥1), and green indicates downregulation (log2FC ≤1). (B/E) Scatter plots of transcript expression with red points indicating significantly upregulated (log2FC ≥1, P≤0.05) and green points indicating downregulated (log2FC ≤1, P≤0.05) Long non-coding RNAs (B) and mRNAs (E). (C/F) Volcano plots displaying magnitude (log2FC) versus significance (-log10P value) of lncRNA (C) and mRNA (F) differential expression. Grey points: non-significant; red points: significantly upregulated; left green points: significantly downregulated.

Identification of mRNA

In a similar manner, we employed screening criteria of $|FC| \geq 2$, where $|\log2FC| \geq 1$ and $P \leq 0.05$, and compared the gene expression levels of plasma samples from the group with preeclampsia (PE) with the group with normal pregnancy. The objective was to identify differentially expressed mRNAs. The study revealed that 3320 mRNAs exhibited substantial up-regulation in their expression levels, whereas 478 mRNAs were considerably down-regulated. These observed changes were deemed statistically significant, as Figure 1.D, E, and F. Further details may be found in the above figures.

Among the identified genes, TEX35 had the highest up-regulation in mRNA expression, with a fold change of 8.70. Conversely, SMPDL3B displayed the most significant down-regulation, with a fold change of -10.39. The group engaged in physical education exhibited the highest levels of up-regulated expressions compared to the control group. Table 2 displays the up-regulated and down-regulated expression of the top 10 mRNAs in the PE group in comparison to the control group.

GO enrichment analysis

To conduct a more comprehensive investigation into the biological activities of the differentially expressed long non-coding RNA (lncRNA) and messenger RNA (mRNA) in the plasma samples of both the preeclampsia (PE) group and the control group, we carried out an analysis of gene ontology (GO) function enrichment. The findings indicate that the presence of differential long non-coding RNA (lncRNA) exhibits significant improvements in multiple biological functions. These functions primarily encompass protein metabolism, transferase activity, protein demethylation and alkylation, and the growth and differentiation of striated muscle cells and cardiomyocytes. A significant increase is observed in the signal transduction of protein kinase A. The cellular constituents include organelles, microtubule assembly, myosin, actin, spindle microtubules, and cell adhesion. The molecular functions encompass many activities such as protein binding, serine/threonine kinase activity, transmembrane receptor protein tyrosine phosphatase activity, Transforming Growth Factor-β receptor activity, phospholipase C activity, and others (see Figure 2 for further information). The primary roles of differential mRNA high enrichment encompass several biological activities, specifically the breakdown and transportation of macromolecular organics, such as cells, nucleic acids, and proteins. Cellular constituents primarily encompass the regulation of organelles, cytoplasm, nucleus, and other anatomical entities. Molecular functionalities encompass growth factor binding, smooth muscle cell migration, cell adhesion molecule binding, antioxidant activity, tyrosine phosphatase activity, MAPK phosphatase activity, and so forth (refer to Figure 3 for further information). According to reports, the secretion of Transforming Growth Factor-β1 by activated platelets can modulate trophoblasts' invasion, proliferation, and differentiation. This process is believed to contribute to the

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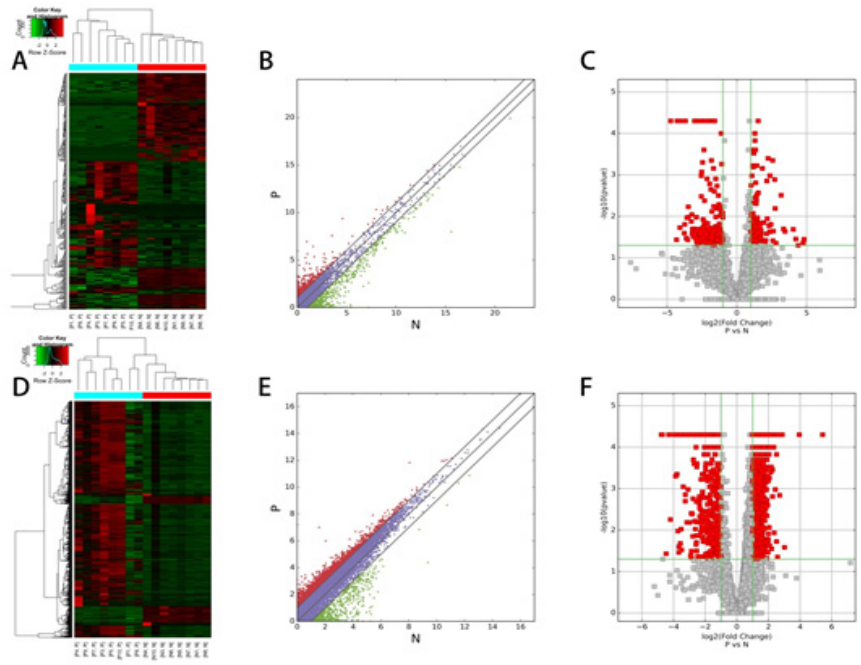

Figure1. Differential expression profiles of Long non-coding RNAs and mRNAs in preeclampsia (PE) versus normal pregnancy plasma samples

Table 1. Compared with the control group, the top 20 Long non-coding RNAs have the largest multiples of difference in the PE group

Transcript ID	Gene ID	Log_2FC	*P* value	Regulation	Chrome
ENST00000440816	ENSG00000244625	175.286	0.044	up	chr22
ENST00000452864	ENSG00000235703	108.169	0.050	up	chrX
uc022bdg.1	DQ580140_2	30.5159	0.030	up	chr9
uc001ksz.2	BTRC	20.7782	0.025	up	chr10
uc021sss.1	LOC161527	19.6967	0.032	up	chr15
uc011bax.1	PTPN23	17.6918	0.047	up	chr3
uc011lpu.1	PAX5	16.593	0.029	up	chr9
NR_045035	APH1A	15.7987	0.018	up	chr1
TCONS_00027184	XLOC_013222	13.0249	0.041	up	chr19
ENST00000413904	ENSG00000236859	7.24596	0.027	up	chr2
ENST00000608904	ENSG00000229227	-72.8757	0.049	down	chr10
uc010blo.1	FAH	-66.0984	0.049	down	chr15
ENST00000471502	ENSG00000272679	-7.26527	0.027	down	chr9
ENST00000604666	ENSG00000270823	-6.50265	0.003	down	chr7
ENST00000522963	ENSG00000249859	-6.18567	0.037	down	chr8
ENST00000548204	ENSG00000257271	-5.86104	0.048	down	chr11
uc003tnq.3	IGFBP3	-5.83653	0.026	down	chr7
ENST00000600831	ENSG00000233355	-5.41082	0.047	down	chr1
ENST00000496780	ENSG00000244003	-5.1821	0.030	down	chr16
NR_024433	LINC00926	-5.03774	0.047	down	chr15

development of preeclampsia by stimulating endothelial cell pathways or regulating systemic inflammation[21]. The down-regulation of Placental Growth Factor (PlGF) expression in Pre-eclampsia (PE) is a notable phenomenon. Additionally, it has been observed that Protein Kinase A is an efficient regulator of both PlGF gene expression and protein release in trophoblast cells. Hence, it may be inferred that protein kinase A plays an indirect role in the regulation of placental growth factor (PlGF), thereby potentially influencing the process of placental endothelium (PE) development[22]. The infiltration of trophoblast cells is directly affected by the aberrant adhesion of cells. The activation of MAPK can potentially enhance the communication between actin and myosin, resulting in increased contractions of the smooth muscle of the vascular system. This, in turn, may contribute to the development of placental ischemia and exacerbate the advancement of preeclampsia (PE)[23]. In brief, the present study employed GO functional enrichment analysis to identify differentially expressed long non-coding RNA (lncRNA) and messenger RNA (mRNA) molecules. These molecules were discovered to have a role in the onset and progression of preeclampsia (PE) by modulating numerous biological functions, including protein kinase A, Transforming Growth Factor-β, MAPK signalling pathways, and aberrant adherence of cells.

(A1/B1/C1) Top 10 enriched biological processes (BP), cellular components (CC), and molecular functions (MF) for upregulated Long non-coding RNAs in PE plasma. (A2/B2/C2) Top 10 enriched terms for downregulated Long non-coding RNAs. Analysis was performed using plasma samples from 8 PE patients and 8 controls (7-14 weeks gestation) with $|FC| \geq 2$ and $P \leq 0.05$. Key findings include enrichment in protein metabolism (BP), organelle components (CC), and protein binding (MF) for upregulated Long non-coding RNAs.

(A1/B1/C1) Top 10 enriched terms in BP, CC, and MF categories for upregulated mRNAs in PE plasma. (A2/B2/C2) Top 10 enriched terms for downregulated mRNAs. Analysis criteria as in Figure 2. Notable findings include macromolecule transport (BP), cytoplasmic components (CC), and growth factor binding (MF) for upregulated mRNAs.

KEGG pathway analysis

The findings of the KEGG pathway enrichment analysis indicated that the differentially expressed long non-coding RNA (lncRNA) is mostly enriched in several pathways, including the P53 signalling pathway (P=0.0417) (Figure 4A), Cell Adhesion Molecules (CAMs) (P=0.0085), and Inflammatory Bowel Disease (IBD) (P=0.0059) (Figure 4B), among others. The analysis revealed that there is a significant enrichment of differentially expressed mRNAs in several pathways, including the PI3K-Akt signalling route (P=0.0048), P53 signalling pathway (P=0.0383), cell cycle (P=0.0188), and adhesion (P=0.0468) (Figure 4C). The NF-ϰB signalling route (P=0.0007), mTOR signalling pathway (P=0.0082), apoptosis (P=0.0037), B cell receptor signalling pathway (P=0.0001), and inflammatory bowel disease Inflammatory Bowel Disease (P =0.0095) were observed to have statistically significant associations (Figure 4D) among other factors. The characteristic that sets preeclampsia (PE) apart is the heightened occurrence of programmed cell death in placental villous trophoblast cells, known as apoptosis. The regulatory role of p53 in cell apoptosis is of particular significance in this context. The overexpression of p53 has resulted in the initiation of cell cycle arrest and autophagy. According to reports, there has been a notable alteration in the expression of p53 and the downstream proteins of the PE placental villous trophoblast. This shift in expression has been observed to elicit apoptosis

Table 2. Compared with the control group, the top 20 mRNAs with the largest fold difference were in the PE group

Gene	Log2FC	P Value	Regulation	Chrome
TEX35	8.70057	0.000	Up	chr1
LRFN4	7.60837	0.001	Up	chr11
ACVRL1	6.02958	0.000	Up	chr12
HBG2	6.00833	0.000	Up	chr11
CTC-429P9.4	5.82173	0.000	Up	chr19
BOLA2B	5.68722	0.037	Up	chr16
GPR21	5.46462	0.026	Up	chr9
CERKL	5.44682	0.000	Up	chr2
FAM72B	5.42734	0.000	Up	chr1
EDNRB	5.36293	0.001	Up	chr13
SMPDL3B	-10.3904	0.021	Down	chr1
STEAP2	-10.0684	0.000	Down	chr7
SEZ6	-7.93806	0.000	Down	chr17
GNG4	-7.75744	0.044	Down	chr1
VTN	-7.70617	0.000	Down	chr17
TRIM6	-7.41756	0.006	Down	chr11
HSD11B1	-6.96447	0.000	Down	chr1
LAMC3	-6.8133	0.046	Down	chr9
TNFRSF12A	-6.58709	0.001	Down	chr16
RP11-455G16.1	-6.42067	0.005	Down	chr4

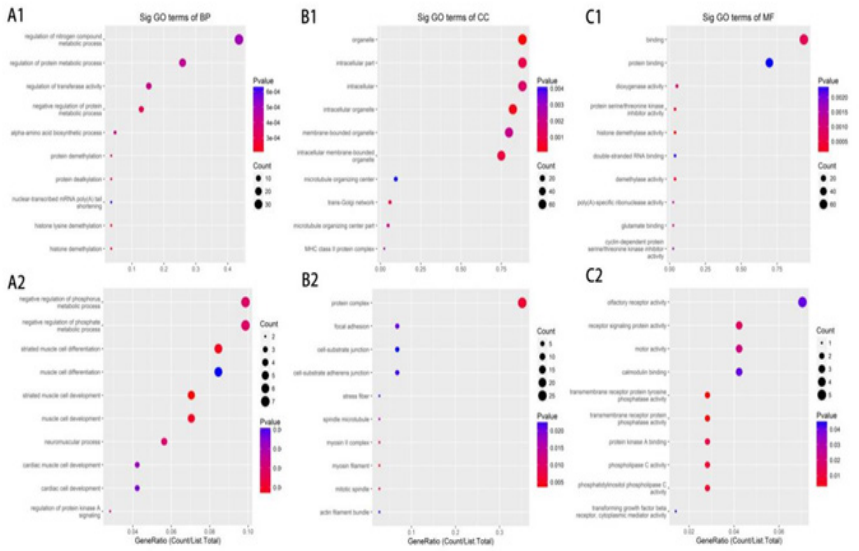

Figure 2. Gene Ontology (GO) enrichment analysis of differentially expressed Long non-coding

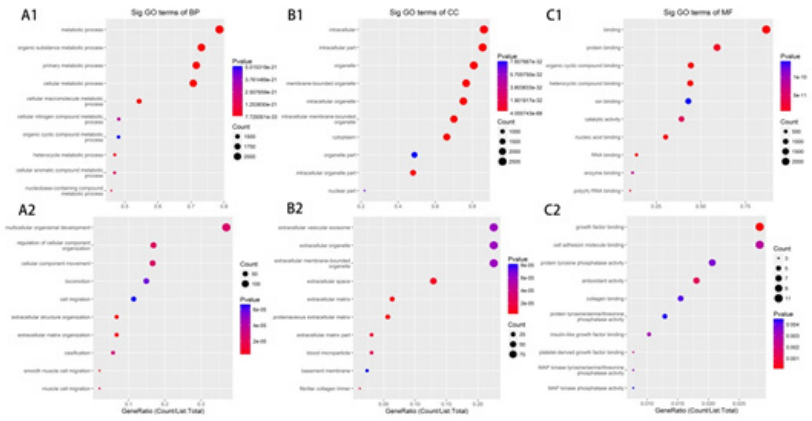

Figure 3. Gene Ontology (GO) enrichment analysis of differentially expressed mRNAs

and autophagy in human trophoblasts through activating the p53 pathway[24]. The PI3K/AKT signalling pathway significantly influences various cellular processes, including but not limited to trophoblast cell development, proliferation, migration, and invasion. RBP4 mediates the regulation of trophoblast cell proliferation and invasion through the activation of the PI3K/AKT pathway. The down-regulation of RBP4 expression in the placenta leads to a decrease in the invasion ability of trophoblasts, thereby facilitating the progression of preeclampsia[25]. The up-regulation of NF-κB expression and activation has been confirmed in the placenta affected by preeclampsia (PE), resulting in excessive and persistent inflammation in this condition. Hence, it may be inferred that the NF-κB signalling pathway directly influences PE's pathophysiological progression, as indicated by previous research[26]. The enrichment analysis of the KEGG pathway conducted in this work reveals that the differentially expressed long non-coding RNA (lncRNA) and messenger RNA (mRNA) identified play a role in the pathogenesis of preeclampsia (PE) through the regulation of the p53, PI3K/AKT, RBP4, and NF-κB signalling pathways.

Network diagram showing interactions between the top 20 dysregulated Long non-coding RNAs (Table 1) and top 60 mRNAs (Table 2) from PE (n=8) vs control (n=8) plasma samples. Nodes represent transcripts (red: upregulated; green: downregulated), and edges represent correlation pairs (|coefficient| >0.7). Key hub genes include PI3K-Akt pathway members (LAMA4, PIK3AP1), NF-κB signalling components (CARD11, TRADD), and MAPK pathway genes (DUSP1, MAP4K3).

Discussion

Organisms possess a diverse array of RNA molecules. RNA molecules can be classified into coding RNA and non-coding RNA (ncRNA) based on their ability to encode proteins. Coding RNA refers to messenger RNA (mRNA), which encodes the genetic information necessary for protein synthesis. On the other hand, non-coding RNA encompasses various forms, including microRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA), among others. In recent years, there has been a progressive discovery of the potential activities of long non-coding RNA (lncRNA) in cardiovascular illnesses through further research. For instance, studying long non-coding RNAs is crucial in investigating coronary atherosclerosis and other cardiovascular disorders. Additionally, specific Long non-coding RNAs hold potential as novel targets for treatment and diagnostic markers for atherosclerosis[27].

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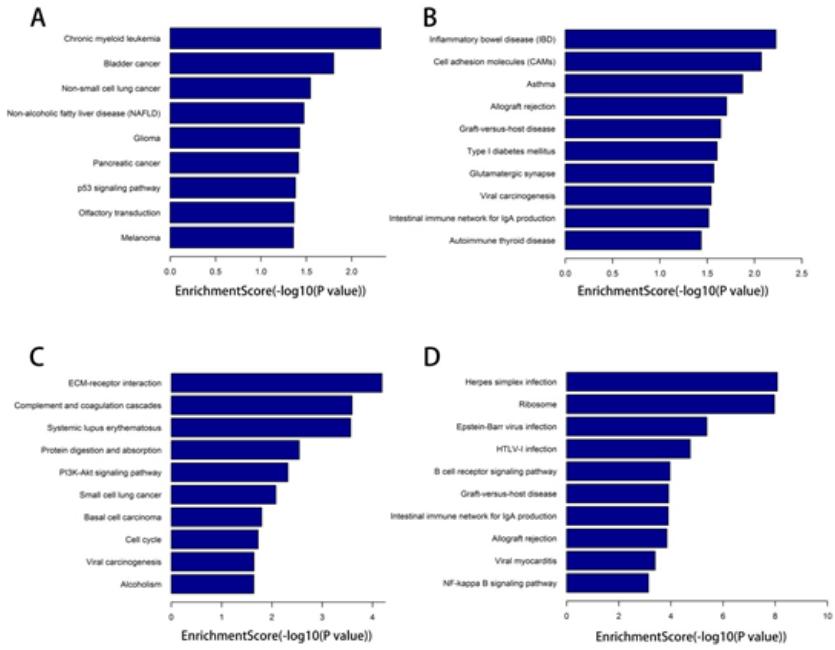

Figure 4. KEGG pathway enrichment analysis of differentially expressed transcripts

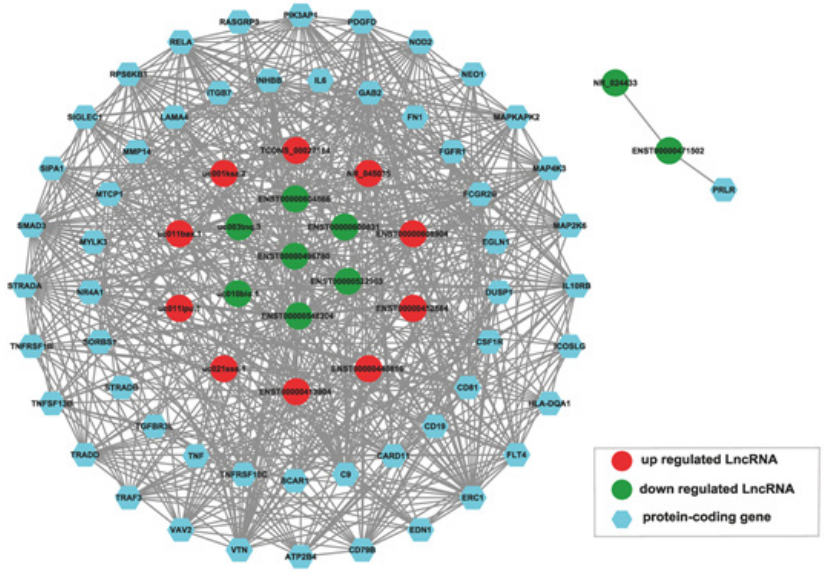

Figure 5. lncRNA-mRNA coexpression network in preeclampsia plasma

The aberrant proliferation or migration of smooth muscle cells is a well-established factor in the process of vascular remodelling, which serves as the underlying pathogenic mechanism for developing target organ damage in individuals with hypertension[28]. The upregulation of a specific long non-coding RNA (lncRNA), XR007793, was confirmed in an in vitro study conducted on vascular smooth muscle cells affected by hypertension. In a reciprocal manner, the inhibition of XR007793 reduced the proliferation and migration of vascular smooth muscle cells. Therefore, it is evident that specific long non-coding RNAs have a significant impact on the onset and progression of cardiovascular disorders, including hypertension[29]. The long non-coding RNA (lncRNA) NR_002794 exhibited significant upregulation in the context of preeclampsia (PE). According to the paper, the overexpression of NR_002794 was found to have a suppressive effect on the proliferation and migration of trophoblast cells while concurrently enhancing the induction of apoptosis. The gene NR_002794 is believed to significantly impact various aspects of trophoblast cell function, including the remodelling of the uterine spiral artery and the proper formation of the placenta. Hence, it may be inferred that NR_002794 holds the potential as a viable target for the diagnostic and therapeutic intervention of PE, as indicated by previous research[30].

Preeclampsia (PE) is a frequently encountered prenatal condition clinically distinguished by elevated mortality rates. PE also significantly increases fetal morbidity and mortality due to iatrogenic preterm because there is no recognised treatment other than delivery[31]. The current medical consensus is that hypoxia/reperfusion contributes to the pathogenic alteration of excessive oxygen free radicals and inflammatory factors present in PE. The disruption of the equilibrium between oxidation/antioxidation and pro-inflammatory/anti-inflammatory processes in maternal individuals results in an overabundance of oxidative stress and an inflammatory reaction[32]. Many different components, cells, tissues, and organs may be involved in this disease process, all of which may undergo structural and functional changes. A growing body of research has recently emphasised investigating the involvement of long non-coding RNA (lncRNA) in cardiovascular disease. Nevertheless, literature about the prognostic significance and underlying mechanism of long non-coding RNA (lncRNA) in the early stages of pregnancy complicated by preeclampsia (PE) is scarce. In this study, we assess the findings of sequencing the transcriptomes of plasma samples taken from PE patients and normal pregnant women between 7 and 14 weeks of their pregnancies. The study demonstrates the differential expression patterns of long non-coding RNA (lncRNA) and messenger RNA (mRNA) in two distinct groups. It successfully identifies the differential expression profiles of both lncRNA and mRNA and further identifies 171 up-regulated Long non-coding RNAs and 190 down-regulated Long non-coding RNAs. Through the process of GO enrichment analysis, it has been determined that the differentially expressed long non-coding RNA (lncRNA) and messenger RNA (mRNA) mostly participate in many cellular functions such as binding, transport, and metabolism of cells, proteins, and nucleocytoplasmic substances. Furthermore, these entities play a crucial role as integral constituents of organelles, cytoplasm, and nucleoplasm. The potential impact of these molecules extends to the modulation of protein, DNA, and RNA binding and the regulation of kinase activity. According to reports, a positive correlation exists between the severity of maternal sickness and the proliferation, migration, and invasion capabilities of placental trophoblast cells. The insufficient infiltration of trophoblast cells can easily lead to excessive apoptosis, cell cycle arrest, and uterine spiral artery remodelling disorder. These issues can easily lead to developmental errors in the placenta, contributing to PE occurrence and progression[33]. Recent research indicates that preeclampsia (PE) is not only an autonomous risk factor for cardiovascular health in mothers, but it also has detrimental effects on the cardiovascular system of their children. A study

has found a widespread reduction in DNA methylation levels in placentas affected by preeclampsia (PE). The modified methylation pattern of foetal endothelium colony-forming cells might have existed before clinical signs of preeclampsia. Hence, it may be inferred that epigenetic modifications have the potential to serve as indicators for assessing the susceptibility of cardiovascular disease in offspring with a history of preeclampsia, specifically at the epigenetic level[34]. Based on the results of the KEGG pathway analysis, our findings indicate that the differentially expressed long non-coding RNA (lncRNA) and messenger RNA (mRNA) are primarily enriched in key signalling pathways such as the p53 signalling system, PI3K-Akt signalling pathway, and NF-κB signalling pathway. These pathways involve several cellular activities, including cell cycle regulation, adhesion, and death. Ischemia and hypoxia of the placenta will produce a wide variety of biological activity factors, such as factors that inhibit angiogenesis, promote inflammation, and are induced by hypoxia, amongst others. These variables influence diverse biological processes through their involvement in numerous signalling pathways. As an illustration, the inhibition of human trophoblast cell proliferation, migration, and invasion in preeclamptic placenta can be attributed to the downregulation of receptor tyrosine kinase-like orphan receptor 1 (ROR1), which operates through the regulation of the PI3K/Akt/mTOR network[35]. The transcription factor NF-κB is critical in the signalling cascade associated with inflammation. This factor's expression levels and activation have been observed in the placenta and the systemic vasculature in women diagnosed with preeclampsia. It has been suggested that this activation is involved in trophoblast death and the development of systemic endothelial dysfunction. Hence, inhibiting this signalling pathway enhances PE[26]. In summary, the abovementioned sequencing indicates a potential correlation between differentially expressed long non-coding RNA (lncRNA) and messenger RNA (mRNA) and immunological response, inflammatory response, and oxidative stress. These relationships align with the believed underlying mechanisms of preeclampsia (PE). It has been postulated that differences in the expression of long non-coding RNA (lncRNA) and messenger RNA (mRNA) may have a role in the development of preeclampsia (PE).

Ultimately, our investigation revealed that 17 long non-coding RNAs (exhibiting significant variations were found to be strongly associated with 55 messenger RNAs (mRNAs) through establishing a co-expression network between Long non-coding RNAs and mRNAs. The co-expression network comprised various signalling pathways, including PI3K-Akt, NF-κ B, MAPK, TNF, and B cell receptor signalling pathways. Nevertheless, the aforementioned signalling pathways have been verified to be implicated in the aetiology of pulmonary embolism[36]. For instance, it has been documented that the PI3K-Akt signalling pathway plays a role in the pathogenesis of pulmonary embolism (PE) by suppressing angiogenesis and facilitating inflammatory response[37]. Oxidative stress and an inflammatory reaction were observed in the placenta of individuals with preeclampsia (PE). The pro-inflammatory cytokines TNF-α and IL-1β were found to contribute to this response by activating the NF-κB signalling pathway. The NF-κB signalling system has demonstrated efficacy as a therapeutic target for managing inflammatory disorders. The user has provided a numerical reference without any accompanying text. The activation of the MAPK signalling pathway can be induced by excessive oxidative stress and pro-inflammatory substances. This activation has been observed to impact various cellular processes, including cell proliferation, differentiation, apoptosis, and embryonic development. Consequently, this phenomenon resulted in the aberrant functioning of trophoblast cells, thereby triggering the onset of preeclampsia[39]. Most of the 55 messenger RNA (mRNA) molecules examined had a significant association with the development and progression of preeclampsia (PE). One study has indicated that Nuclear NR4A1 regulates intracellular oxidative stress and mitigates ROS-dependent ER stress in pancreatic cancer cells. This is achieved by modulation of thioredoxin domain-containing 5 (TXNDC5) expression, which regulates cellular ROS production[40]. Moreover, several inflammatory cytokines can facilitate the fast induction of NR4A1 in monocytes and macrophages by activating the NF-κB pathway[41]. FCGR2B is a member of a diverse receptor family that can impede the spread of early antigen determinants, promote the generation of IgG aAb, hinder the infiltration of early neutrophils, and actively engage in both inflammatory and immunological responses[42]. The downregulation of EGLN1 leads to an upregulation of HIF-1α and vWF expression, resulting in a significant rise in platelet count and an increased susceptibility to thrombosis[43]. The overexpression of CARD11 results in the activation of the NF-κB and JNK signalling pathways, causing an imbalance in B cell differentiation and ultimately leading to lymphoma and other malignant disorders[44]. These results suggested that the lncRNA and mRNA that were expressed variably influenced the occurrence and progression of PE via modulating the PI3K-Akt, NF-B, and MAPK signalling pathways directly or indirectly. It is possible that its upstream or downstream targets could help improve the pregnancy outcome of patients with PE. This would allow both the mother and the child to receive the full clinical benefits of the treatment. Unfortunately, Our study also has some limitations, First, as our work is focused on the screening and analysis of plasma differential lncRNA / mRNA, so still have not yet conducted further verification the genes we have found; Second, this study is only a preliminary screening of differential genes with possible research value at present, and it still needs to be verified and confirmed in the later study of large samples.

Conclusion

This study aimed to analyse the differential expression of long non-coding RNA (lncRNA) and messenger RNA (mRNA) in the peripheral plasma of two groups: the preeclampsia (PE) group and the regular pregnant group during early pregnancy. This analysis used Gene Ontology (GO), the Kyoto Encyclopaedia of Genes and Genomes (KEGG), and co-expression network creation. Our study revealed that the differentially expressed long non-coding RNA (lncRNA) and messenger RNA (mRNA) have the potential to modulate several signalling pathways, including Protein Kinase A, Transforming Growth Factor-β, MAPK, p53, PI3K-Akt, RBP4, and NF-κB. The significance of these biological processes in the pathophysiology of PE has been substantiated. Hence, our study has substantiated that the identification of differentially expressed long non-coding RNA (lncRNA) and messenger RNA (mRNA) in the peripheral blood during early pregnancy could potentially serve as a novel approach for the early prediction, diagnosis, and treatment of preeclampsia (PE). This finding establishes a theoretical and empirical foundation for future investigations into the involvement of lncRNA and mRNA

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in the pathogenesis of PE.

Author Contributions

YM. L. SB.C. W.C. XL.N. and N. Y. conceived the project. X.Z. Y.T. and.J.L. performed the experiments. X.Z., J.L. and Y.T. analysed the results. X.Z. N. Y. and.J.L. wrote the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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