Differential Circular RNA Expression Profiles in Umbilical Cord Blood Exosomes from Preeclampsia Patients

Minkai Cao
The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University
Juan Wen
Women's Hospital of Nanjing Medical University
Chaozhi Bu
The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University
Chunyan Li
Women's Hospital of Nanjing Medical University
Yu Lin
Women's Hospital of Nanjing Medical University
Hong Zhang
The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical University
Yanfang Gu
The Affiliated Wuxi Maternity and Child Health Care hospital of Nanjing Medical University
Zhonghua Shi
Women's Hospital of Nanjing Medical University
Yan Zhang
The Affiliated Wuxi Maternity and Child Health Care Hospital of Nanjing Medical Universtiy
Wei Long
Women's Hospital of Nanjing Medical University
Le Zhang (zhle@njmu.edu.cn)
The Affiliated Wuxi Children's Hospital of Nanjing Medical University

Research article

Keywords: umbilical cord blood, exosomes, circular RNA, preeclampsia

Posted Date: February 25th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-271818/v1

Version of Record: A version of this preprint was published at BMC Pregnancy and Childbirth on April 15th, 2021. See the published version at https://doi.org/10.1186/s12884-021-03777-7.

Abstract

Exosomal circular RNAs (circRNAs) are emerging as important regulators of physiological development and disease pathogenesis. However, the roles of exosomal circRNAs from umbilical cord blood in preeclampsia (PE) occurrence remains poorly understood. In this study, we used microarray technology to establish the differential circRNA expression profiles in umbilical cord blood exosomes from PE patients compared with normal controls. According to the microarray data, we identified 143 significantly up-regulated circRNAs and 161 significantly down-regulated circRNAs in umbilical cord blood exosomes of PE patients compared with controls. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) biological pathway analyses demonstrated that circRNA parental genes involved in the regulation of metabolic process, trophoblast growth and invasion were significantly enriched, which are important in PE development. Moreover, pathway network was constructed to reveal the key pathways in PE, such as PI3K-Akt signaling pathway. Further circRNA/miRNA interactions analysis showed that most of the exosomal circRNAs harbored miRNA binding sites, and some miRNAs were associated with PE. Collectively, our results highlight the importance of exosomal circRNAs in the pathogenesis of PE and lay a foundation for extensive studies on the role of exosomal circRNAs in PE development.

Introduction

Preeclampsia (PE) is a gestation-specific syndrome that affects up to 5–7% of pregnancies, characterized by elevated blood pressure and proteinuria after 20 weeks of pregnancy [1, 2]. This multisystem pregnancy disorder is often accompanied by headache, nausea, vomiting, upper abdominal discomfort and other symptoms, and is a leading cause of maternal and neonatal morbidity and mortality worldwide [3]. Due to the multifactorial nature of the disorder, the exact etiology of PE remains largely unknown, and there is currently no effective treatment for PE other than termination of pregnancy. It is reported that PE and its related diseases are responsible for nearly 40% of premature births delivered before 35 weeks of gestation [4]. After giving birth, although most women return to normal blood pressure levels, PE reflects an elevated long-term risk of cardiovascular diseases in both the mother and the child [5–7].

Exosomes have become key mediators of local and systemic intercellular communication by regulating different biological processes between cells [8, 9]. Many studies have reported that exosomes play an important role in the regulation of pregnancy complications such as preeclampsia and gestational diabetes [10, 11]. Specific exosomes derived from placenta can be detected in maternal blood as early as the 6th week of gestation, and concentrations of placenta-derived exosomes increase as pregnancy progresses [12]. In addition, exosomes isolated from maternal blood are biologically active in vitro and can enter target cells through endocytosis [13]. Circular RNA (circRNA) is a novel member of endogenous noncoding RNAs, which is widely distributed and has a variety of cellular functions. Recently, circRNAs have been identified for their enrichment and stability in exosomes [14, 15]. More and more studies have shown that exosomal circrnas are involved in the processes of cell growth, angiogenesis, epithelial mesenchymal transition and targeted therapy [16, 17]. And accumulating evidences suggested that it is possible to identify functional and/or structural differences in umbilical cord blood with risk of PE [18–20]. Therefore, we speculated that exosomal circRNA in umbilical cord blood might play an important role in the regulation of PE development as a new placental derived factor. However, the role of exosomal circRNAs from umbilical cord blood in PE development remains unclear. In this study, we used microarray technology to construct a comparative exosomal circRNA profiling of umbilical cord blood between PE patients and controls, aiming to lay a foundation for further research on the role of exosomal circRNAs in PE development.

Materials And Methods

Ethics statement

This study was approved by the ethics committee of Women's Hospital of Nanjing Medical University (NFKSL2018-107), and all subjects gave written informed consent.

Patients and sample collection

All participants and clinical information were collected at the Nanjing Maternity and Child Health Care Hospital and Wuxi Maternity and Child Health Care Hospital from September 2019 to February 2020. PE was defined as new-onset hypertension (blood pressure ≥ 140/90 mmHg on two separate occasions at least 6 hours apart or blood pressure ≥ 160/110 mm Hg) and proteinuria (> 300 mg/24 hours) after 20 weeks of gestation in previously normotensive women. The pregnant women without PE were included as controls. The controls were matched to PE cases for maternal age. At last, 46 umbilical cord blood samples were collected from the umbilical vein immediately after delivery of fetus during cesarean section (23 PE patients and 23 controls) according to the standard operating procedure. All participants were divided into two sets, 6 participants (3 PE patients and 3 controls) for microarray screening and 40 participants (20 PE patients and 20 controls) for validation.

Exosome purification and analysis

Exosomes were prepared from the umbilical cord blood. Briefly, umbilical cord blood was centrifuged at 3,000 g for 15 min at 4 degree. Supernatants were then centrifuged at 12,000 g for 30 min at 4 degree. Then supernatants were filtered through 0.45 μm polyvinylidene fluoride (PVDF) membrane, and isolated in a final ultracentrifugation at 100,000 g for 180 min at 4 degree. The exosome pellet was resuspended in PBS or lysis buffer. The resulting exosomes were next analyzed with the Nanosight Nano ZS device (Malvern Instruments, UK).

Exosomal RNA extraction and microarray analysis

Total exosomal RNA was extracted using Serum/Plasma Kit (QIAGEN, Germany) according to the manufacturer's instructions and checked for RNA integration by an Agilent Bioanalyzer 2100 (Agilent technologies, USA). Total RNA was amplified and labeled by Low Input Quick Amp Labeling Kit (Agilent technologies, USA), following the manufacturer's instructions. Labeled cRNA was purified by RNeasy mini kit (QIAGEN, Germany). Each slide was hybridized with 1.65μg Cy3-labeled cRNA using Gene Expression Hybridization Kit (Agilent technologies, USA) in Hybridization Oven (Agilent technologies, USA), according to the manufacturer's instructions. After 17 hours hybridization, slides were washed in staining dishes (Thermo Shandon, USA) with Gene Expression Wash Buffer Kit (Agilent technologies, USA), followed the manufacturer's instructions. Slides were scanned by Agilent Microarray Scanner (Agilent technologies, USA) with default settings. Data were extracted with Feature Extraction software 12.0 (Agilent technologies, USA). Raw data were normalized by Quantile algorithm, limma packages in R.

Quantitative real-time PCR (qPCR)

The specific divergent primers were designed using Primer-BLAST to amplify the circular transcripts through head-to-tail splicing. All the primers were synthesized with Realgene (Nanjing, China). After determining the best annealing temperatures, qPCR was performed to measure the relative circRNA expression levels using PowerUP SYBR Green Master Mix (Applied Biosystems, USA) on a Life Tech-ViiA7 system (Applied Biosystems, USA). Glyceraldehyde phosphate dehydrogenase (GAPDH) served as the internal control, the relative expression level of each circRNA was calculated with the $2^{-\triangle\triangle Ct}$ method.

Functional enrichment analyses

The parental gene functions of the differentially expressed circRNAs were analyzed using DAVID Bioinformatics Resources 6.8. Gene Ontology (GO) analysis of the parental genes was performed based on three terms, namely, biological processes, cellular components and molecular functions (http://www.geneontology.org/). The related biological pathways were analyzed by Kyoto Encyclopedia of Genes and Genomes (KEGG, http://www.genome.jp/kegg). We regarded the -log (*P*-value) as the enrichment score that indicated the significance of correlation.

Annotation for circRNA/miRNA interactions

The miRanda (http://www.microrna.org/microrna/home.do/) was used to predict circRNA/miRNA interactions. With the database, we searched miRNA response elements (MREs) on circRNAs, and selected miRNAs based on the seed-match sequences.

Statistical analyses

Statistical analyses were performed with SPSS 18.0 and GraphPad prism 5.0. All the data are displayed as the mean ± standard deviation (SD). Student's *t*-test was used to assess the differences between experimental groups. Differences with *P*-values < 0.05 were considered statistically significant.

Results

circRNA expression profiling in umbilical cord blood exosomes from PE patients compared with controls

To investigate the general characteristics of all the circRNAs in umbilical cord blood exosomes, we performed a preliminary analysis of all the microarray data. A total of 88,371 circRNAs were evaluated, known in circBase (http://circrna.org/). Hierarchical cluster analysis showed the expression levels of circRNA in umbilical cord blood exosomes from PE patients and the control group, and revealed that these levels were distinguishable between the two groups (Fig. 1A). Based on the filter criteria of fold change (FC) ≥ 2 and *P* value < 0.05, a total of 304 circRNAs were screened as differentially expressed circRNAs. Of these, 143 circRNAs were up-regulated, and 161 circRNAs were down-regulated in the PE patients (Fig. 1B). Scatter plot was used to show the differentially expressed circRNAs (Fig. 1C), and the significantly differentially expressed circRNAs are shown in the volcano plot (Fig. 1D). The top 20 up- and down-regulated circRNAs are listed in Table 1. These data suggested that circRNA expression in PE patients was different from that in the normal controls. Additionally, Fig. 1E depicts the chromosome distributions of these differential circRNAs and Fig. 1F shows that the lengths of the differential circRNAs were mostly shorter than 2000bp.

Table 1
The top 20 up-regulated and down-regulated circRNAs between PE patients and controls

circRNA_ID	Chr	Length	Host gene	Normalized signal (log2)						Fold change	*P*	Regulation
				PE-sample1	PE-sample2	PE-sample3	NC-sample1	NC-sample2	NC-sample3			
circ_0077259	6	336	CGA	5.14	5.06	5.15	0.90	2.56	0.88	10.83	2.20E-02	up
circ_0077260	6	521	CGA	3.93	3.91	4.54	1.36	0.91	0.89	8.50	4.22E-04	up
circ_0081930	7	164	LAMB1	4.64	4.74	4.37	2.36	0.89	1.65	7.14	1.52E-02	up
circ_0043597	17	669	KRT19	3.88	4.24	4.08	1.64	1.17	1.09	6.76	4.90E-04	up
circ_0037771	16	166	NAGPA	3.63	3.36	3.87	0.73	0.77	1.11	6.74	1.72E-04	up
circ_0075840	6	995	GPLD1	4.20	4.27	4.31	1.97	2.27	1.20	5.21	1.58E-02	up
circ_0042232	17	126	MPRIP	3.75	4.60	3.99	2.20	1.40	1.75	5.06	2.55E-03	up
circ_0090100	X	525	SAT1	4.38	4.12	4.44	1.64	2.37	1.90	5.00	2.90E-03	up
circ_0013777	1	2640	NOTCH2	3.70	3.51	3.68	1.90	0.88	0.91	4.98	1.64E-02	up
circ_0081910	7	2197	LAMB1	4.51	4.59	4.89	2.45	2.60	2.27	4.68	1.38E-04	up
circ_0036082	15	4057	PAQR5	1.56	0.93	0.74	3.84	4.06	4.67	8.66	8.98E-04	down
circ_0042333	17	416	TOP3A	0.70	0.71	0.72	3.24	3.27	4.26	7.79	1.33E-02	down
circ_0062444	22	4291	PPM1F	0.75	0.76	0.75	3.66	2.88	3.94	6.99	1.32E-02	down
circ_0079385	7	245	ZDHHC4	2.13	1.06	1.52	4.02	3.79	4.66	5.93	3.42E-03	down
circ_0076206	6	1290	MTCH1	0.71	1.26	1.43	3.16	3.27	4.02	5.19	2.88E-03	down
circ_0066266	3	314	FAM116A	0.97	0.94	0.93	3.26	3.25	3.38	5.10	1.12E-04	down
circ_0004552	19	338	CARM1	2.55	0.90	1.42	3.61	3.94	4.66	5.06	1.89E-02	down
circ_0011389	1	1136	EIF3I	0.87	0.74	1.78	3.78	3.24	3.22	4.69	7.77E-03	down
circ_0008808	15	772	ARIH1	0.92	0.92	2.46	3.64	3.56	3.99	4.33	3.92E-02	down
circ_0029899	13	414	USPL1	1.06	1.29	0.98	3.39	3.06	3.20	4.31	9.83E-05	down
circ_0077259	6	336	CGA	5.14	5.06	5.15	0.90	2.56	0.88	10.83	2.20E-02	up
circ_0077260	6	521	CGA	3.93	3.91	4.54	1.36	0.91	0.89	8.50	4.22E-04	up
circ_0081930	7	164	LAMB1	4.64	4.74	4.37	2.36	0.89	1.65	7.14	1.52E-02	up
circ_0043597	17	669	KRT19	3.88	4.24	4.08	1.64	1.17	1.09	6.76	4.90E-04	up
circ_0037771	16	166	NAGPA	3.63	3.36	3.87	0.73	0.77	1.11	6.74	1.72E-04	up
circ_0075840	6	995	GPLD1	4.20	4.27	4.31	1.97	2.27	1.20	5.21	1.58E-02	up
circ_0042232	17	126	MPRIP	3.75	4.60	3.99	2.20	1.40	1.75	5.06	2.55E-	up

											03	
circ_0090100	X	525	SAT1	4.38	4.12	4.44	1.64	2.37	1.90	5.00	2.90E-03	up
circ_0013777	1	2640	NOTCH2	3.70	3.51	3.68	1.90	0.88	0.91	4.98	1.64E-02	up
circ_0081910	7	2197	LAMB1	4.51	4.59	4.89	2.45	2.60	2.27	4.68	1.38E-04	up
circ_0036082	15	4057	PAQR5	1.56	0.93	0.74	3.84	4.06	4.67	8.66	8.98E-04	down
circ_0042333	17	416	TOP3A	0.70	0.71	0.72	3.24	3.27	4.26	7.79	1.33E-02	down
circ_0062444	22	4291	PPM1F	0.75	0.76	0.75	3.66	2.88	3.94	6.99	1.32E-02	down
circ_0079385	7	245	ZDHHC4	2.13	1.06	1.52	4.02	3.79	4.66	5.93	3.42E-03	down
circ_0076206	6	1290	MTCH1	0.71	1.26	1.43	3.16	3.27	4.02	5.19	2.88E-03	down
circ_0066266	3	314	FAM116A	0.97	0.94	0.93	3.26	3.25	3.38	5.10	1.12E-04	down
circ_0004552	19	338	CARM1	2.55	0.90	1.42	3.61	3.94	4.66	5.06	1.89E-02	down
circ_0011389	1	1136	EIF3I	0.87	0.74	1.78	3.78	3.24	3.22	4.69	7.77E-03	down
circ_0008808	15	772	ARIH1	0.92	0.92	2.46	3.64	3.56	3.99	4.33	3.92E-02	down
circ_0029899	13	414	USPL1	1.06	1.29	0.98	3.39	3.06	3.20	4.31	9.83E-05	down

Validation of differentially expressed circRNA by qPCR

Based on relatively high abundance, FC ≥ 4, $P < 0.01$, and their host genes, we selected 12 candidate circRNAs to validate their expression in umbilical cord blood exosomes from additional 20 PE patients and 20 controls, including 6 up-regulated circRNAs (circ_0081910, circ_0037771, circ_0077260, circ_0043597, circ_0042232 and circ_0090100) and 6 down-regulated circRNAs (circ_0029899, circ_0066266, circ_0036082, circ_0076206, circ_0079385 and circ_0011389). In parallel with the microarray data, qPCR results showed that the expression of circ_0077260 and circ_0090100 were increased, and the expression of circ_0076206 were decreased in PE patients (Fig. 2).

GO and KEGG pathway analysis of the circRNA parental genes

To examine the biological functions of these differential circRNAs, GO analysis and KEGG pathways analysis were performed. In the GO analysis, the number of parental genes corresponding to GO entries was determined, and the enrichment score was regarded as the -log (*P*-value): For biological process, the term with the most genes was cellular process (GO:0009987, count = 180) and single-organism process (GO:0044699, count = 163), and the most significantly enriched term was cell-substrate junction assembly (GO:0007044, $P = 1.73E-05$); for cellular component, the term with the most genes was cell (GO:0005623, count = 189) and cell part (GO:0044464, count = 182), and the most significantly enriched term was focal adhesion (GO:0005925, $P = 2.30E-05$); and for molecular function, the term with the most genes was binding (GO:0005488, count = 179) and catalytic activity (GO:0003824, count = 114), and the most significantly enriched term was catalytic activity (GO:0003824, $P = 8.90E-06$) (Fig. 3). All mRNAs annotated involved in these GO terms were listed in **Table S1**.

Furthermore, the KEGG results indicated that differentially expressed circRNAs were involved in ECM-receptor interaction (hsa04512), focal adhesion (hsa04510), glycosaminoglycan degradation (hsa00531), fatty acid metabolism (hsa01212), fatty acid biosynthesis (hsa00061) and Notch signaling pathway (hsa04330) (Fig. 4). All mRNAs annotated involved in these pathways were listed in **Table S2**. Then, we used these pathways to construct a pathway network to investigate the key pathways in PE. As is shown in Fig. 5, the exchanges with these pathways largely depended on the existence of PI3K-Akt signaling pathway.

Prediction of circRNA/microRNA interactions

Recent studies have showed that RNAs regulate each other with miRNA response elements (MREs) and this mechanism is known as "competing endogenous RNA (ceRNA)" hypothesis [21]. CircRNAs can regulate miRNA-targeted gene expression, transcription and protein synthesis as ceRNA molecules or efficient miRNA sponges [21]. Interactions between the differential circRNAs and miRNAs were theoretically predicted by miRanda based on the MREs. We found that 2226 miRNAs could be paired with 302 differentially expressed circRNAs, with the criteria of a max score ≥ 140 and a max energy ≤ -20 (**Table S3**); the lower the max energy is, the more significant the correlation. These results suggest that circRNAs may participate in PE pathogenesis via interactions with PE incidence-related miRNAs.

Discussion

As a rising subject of interest in the field of exosomal contents with distant regulatory potency, exosomal circRNAs have received more and more attention in recent years [15]. Our study is the first to construct circRNA differential expression profiling in umbilical cord blood exosomes of PE patients as a starting point to explore the relationship between exosomal circRNAs and PE development. Based on the microarray data, we identified 304 differentially expressed circRNAs in umbilical cord blood exosomes of PE patients when compared with normal controls, including 143 up-regulated circRNAs and 161 down-regulated circRNAs, which suggested that the exosomal circRNA expression patterns in the PE samples were different from those in controls.

In subsequent validation experiments, the expression of exosomal circ_0077260 and circ_0090100 were significantly increased, and the expression of exosomal circ_0076206 were significantly decreased in PE samples. Growing evidence has shown that circRNAs can regulate parental gene expression through diverse mechanisms, such as transcription and splicing regulation, miRNA sponges, mRNA traps, translational modulation, and post-translational modification [22]. The parental genes of circ_0077260, circ_0090100 and circ_0076206 are CGA, SAT1 and MTCH1, respectively. Of these parental genes, CGA encodes for the common alpha subunit of four glycoprotein hormones, hCG (human chorionic gonadotropin), LH (luteinizing hormone), FSH (follicle-stimulating hormone) and TSH (thyroid-stimulating hormone) [23]. Previous studies have found that α-hCG is correlated with PE [24], and CGA was differentially expressed in placenta tissue among late-onset PE, early-onset PE and healthy controls [25, 26]. CGA is also considered as a novel estrogen receptor response gene in breast cancer and an outstanding candidate marker for predicting response to endocrine therapy [27]. Further studies are needed to determine whether the association among circ_0077260, CGA and estrogen is involved in the pathogenesis of PE.

The biological functions and potential pathways of these differential circRNAs were preliminarily predicted by GO and KEGG pathway analyses. Remarkably, several pathways were found to be significantly enriched, such as focal adhesion, glycosaminoglycan degradation, fatty acid metabolism, fatty acid biosynthesis and Notch signaling pathway. It is well known that focal adhesion is crucial to trigger cell adhesion and many other cellular processes including cell migration, spreading and proliferation [28], which are important in PE development. And localization studies in placental tissues have showed that cytotrophoblasts in all stages of differentiation express focal adhesion kinase [29]. In terms of metabolic process, PE has been demonstrated to be associated with increased insulin resistance, hypertriglyceridemia, high circulating free fatty acids, low high-density lipoprotein particles, and high maternal and fetal plasma amino acid concentrations [30]. These metabolic alterations may contribute to the pathophysiology of the syndrome and may also influence fetal growth. For Notch signaling pathway, defects in this pathway would have adverse effect on placentation. And it has been suggested that Notch pathway down-regulation is associated with PE [31]. Further constructed pathway network showed that the exchanges with these pathways largely depended on the existence of PI3K-Akt signaling pathway. The PI3K-Akt signaling pathway has been demonstrated to be a critical pathway mediating the growth-factor-dependent regulation of trophoblast growth and invasion [32]. The insufficient invasion of trophoblasts is known to be correlated with the development of PE [32]. Together, the altered circRNAs are associated with metabolic process, trophoblast growth and invasion related signaling pathways. Efficient biomarkers underlying these pathways need to be further investigated.

A large amount of evidence have indicated that exosomal circRNAs could act as ceRNA molecules or efficient miRNA sponges to regulate miRNA-targeted gene expression, transcription and protein synthesis [33–35]. The circRNAs may have many miRNA binding sites that competitively bind to miRNAs, and then alleviate the inhibitory effects of miRNAs on target molecules [21]. In our study, through circRNA/miRNA interactions analysis, we found that most of the exosomal circRNAs harbored miRNA binding sites, and some miRNAs were associated with PE. For example, miR-17-3p, miR-197, miR-424, miR-431 and miR-483 were reported to be aberrantly expressed in preeclamptic placenta [36–40]. miR-17-3p and miR-424-5p were matched with circ_0077260, which was verified to up-regulated in the umbilical cord blood exosomes of PE patients; miR-197-5p and miR-431-5p potentially binds to down-regulated circ_0076206; whereas miR-424-5p and miR-483-3p potentially matched with up-regulated circ_0090100. Specifically, exosomal miR-486-1-5p and miR-486-2-5p were reported to be up-regulated in PE pregnancy compared with normal pregnancy [41]. And miR-486-5p was matched with down-regulated circ_0076206. In addtion, miR-885-5p was increased in plasma from PE patients compared with healthy pregnant women, and it was released into circulation mainly inside exosomes [42], whereas miR-885-5p potentially matched with up-regulated circ_0077260. Therefore, we speculate that the role of exosomal circRNAs in PE development may be related to miRNA-mediated effects. The underlying mechanism of the circRNA-miRNA-target gene interaction in PE is worthy of further study.

In summary, our study firstly showed that exosomal circRNAs are aberrantly expressed in the umbilical cord blood of PE patients. Bioinformatics analysis further predicted the potential effects of these differentially expressed circRNAs and their interactions with miRNAs, highlighting the importance of exosomal circRNAs in the pathogenesis of PE and providing a basis for further studies on function and mechanism of exosomal circRNAs in PE development.

Declarations

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the supplementary files.

ETHICS STATEMENT

All subjects gave written informed consent. This study was approved by the ethics committee of Women's Hospital of Nanjing Medical University (NFKSL2018-107), and all methods were carried out from September 2019 until October 2020, in accordance with the 1964 Principles of the Helsinki Declaration and its later amendments.

AUTHOR CONTRIBUTIONS

MC conceived and designed the idea, did data collection, wrote and drafted the manuscript. JW, CB and CL did data collection. YL did literature review. HZ and YG reviewed the manuscript. ZS performed the data analysis. YZ, WL and LZ designed, contributed to the reviewing of the final manuscript. All authors approved the final format of the submitted manuscript.

FUNDING

This work was supported in part by Jiangsu Provincial Key Research and Development Program (BE2020626), Wuxi Key Medical Disciplines (ZDXK12), Jiangsu Maternal and Child Health Research Project (F201827, F201852), Top Talent Support Program for Young and Middle-Aged People of Wuxi Health Committee (BJ2020076, BJ2020089), Wuxi Science and Technology Development Fund (N20192039, N20201006, N20202003) and Project of Wuxi Health Commission (M202003).

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

References

1. Mol BWJ, Roberts CT, Thangaratinam S, Magee LA, de Groot CJM, Hofmeyr GJ: **Pre-eclampsia**. *Lancet* 2016, **387**(10022):999–1011.
2. Redman CW, Sargent IL. Latest advances in understanding preeclampsia. Science. 2005;308(5728):1592–4.
3. American College of O. Gynecologists. Task Force on Hypertension in P: **Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy**. Obstetrics gynecology 2013, 122(5):1122–31.
4. Ananth CV, Keyes KM, Wapner RJ. Pre-eclampsia rates in the United States, 1980–2010: age-period-cohort analysis. Bmj. 2013;347:f6564.
5. Valdiviezo C, Garovic VD, Ouyang P. Preeclampsia and hypertensive disease in pregnancy: their contributions to cardiovascular risk. Clinical cardiology. 2012;35(3):160–5.
6. Bokslag A, van Weissenbruch M, Mol BW, de Groot CJ. Preeclampsia; short and long-term consequences for mother and neonate. Early Hum Dev. 2016;102:47–50.
7. Davis EF, Lazdam M, Lewandowski AJ, Worton SA, Kelly B, Kenworthy Y, Adwani S, Wilkinson AR, McCormick K, Sargent I, et al. Cardiovascular risk factors in children and young adults born to preeclamptic pregnancies: a systematic review. Pediatrics. 2012;129(6):e1552–61.
8. Hessvik NP, Llorente A. Current knowledge on exosome biogenesis and release. Cell Mol Life Sci. 2018;75(2):193–208.
9. Gurunathan S, Kang MH, Jeyaraj M, Qasim M, Kim JH. **Review of the Isolation, Characterization, Biological Function, and Multifarious Therapeutic Approaches of Exosomes**. *Cells* 2019, 8(4).
10. Pillay P, Moodley K, Moodley J, Mackraj I. Placenta-derived exosomes: potential biomarkers of preeclampsia. Int J Nanomed. 2017;12:8009–23.
11. Jayabalan N, Lai A, Ormazabal V, Adam S, Guanzon D, Palma C, Scholz-Romero K, Lim R, Jansson T, McIntyre HD, et al. Adipose Tissue Exosomal Proteomic Profile Reveals a Role on Placenta Glucose Metabolism in Gestational Diabetes Mellitus. J Clin Endocrinol Metab. 2019;104(5):1735–52.
12. Salomon C, Scholz-Romero K, Sarker S, Sweeney E, Kobayashi M, Correa P, Longo S, Duncombe G, Mitchell MD, Rice GE, et al. Gestational Diabetes Mellitus Is Associated With Changes in the Concentration and Bioactivity of Placenta-Derived Exosomes in Maternal Circulation Across Gestation. Diabetes. 2016;65(3):598–609.
13. Mitchell MD, Peiris HN, Kobayashi M, Koh YQ, Duncombe G, Illanes SE, Rice GE, Salomon C. Placental exosomes in normal and complicated pregnancy. American journal of obstetrics gynecology. 2015;213(4 Suppl):173–81.
14. Li Y, Zheng Q, Bao C, Li S, Guo W, Zhao J, Chen D, Gu J, He X, Huang S. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. Cell research. 2015;25(8):981–4.
15. Wang Y, Liu J, Ma J, Sun T, Zhou Q, Wang W, Wang G, Wu P, Wang H, Jiang L, et al. Exosomal circRNAs: biogenesis, effect and application in human diseases. Mol Cancer. 2019;18(1):116.
16. Dai X, Chen C, Yang Q, Xue J, Chen X, Sun B, Luo F, Liu X, Xiao T, Xu H, et al. Exosomal circRNA_100284 from arsenite-transformed cells, via microRNA-217 regulation of EZH2, is involved in the malignant transformation of human hepatic cells by accelerating the cell cycle and promoting cell proliferation. Cell death disease. 2018;9(5):454.
17. Li J, Li Z, Jiang P, Peng M, Zhang X, Chen K, Liu H, Bi H, Liu X, Li X. Circular RNA IARS (circ-IARS) secreted by pancreatic cancer cells and located within exosomes regulates endothelial monolayer permeability to promote tumor metastasis. Journal of experimental clinical cancer research. 2018;37(1):177.
18. Csorba R, Yilmaz A, Tsikouras P, Wieg C, Teichmann A, von Tempelhoff GF. Rheological parameters in the umbilical cord blood in moderate and severe forms of preeclampsia. Clin Hemorheol Microcirc. 2013;55(4):391–401.
19. Kwon JY, Maeng YS, Kwon YG, Kim YH, Kang MH, Park YW. Decreased endothelial progenitor cells in umbilical cord blood in severe preeclampsia. Gynecol Obstet Invest. 2007;64(2):103–8.
20. Yusuf K, Kamaluddeen M, Hasan SU, Al-Awad E, Finch RA, Akierman AR. Ionized calcium levels in umbilical cord blood of women with preeclampsia and normotensive pregnancies. The journal of maternal-fetal neonatal medicine. 2012;25(2):203–5.

21. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, Kjems J. Natural RNA circles function as efficient microRNA sponges. Nature. 2013;495(7441):384–8.
22. Shao T, Pan YH, Xiong XD. Circular RNA: an important player with multiple facets to regulate its parental gene expression. Molecular therapy Nucleic acids. 2021;23:369–76.
23. Bieche I, Parfait B, Nogues C, Andrieu C, Vidaud D, Spyratos F, Lidereau R, Vidaud M. The CGA gene as new predictor of the response to endocrine therapy in ER alpha-positive postmenopausal breast cancer patients. Oncogene. 2001;20(47):6955–9.
24. Moodley D, Moodley J, Buck R, Haneef R, Payne A. Free alpha-subunits of human chorionic gonadotropin in preeclampsia. International journal of gynaecology obstetrics. 1995;49(3):283–7.
25. Liu J, Song G, Meng T, Zhao G. Identification of Differentially Expressed Genes and Signaling Pathways in Placenta Tissue of Early-Onset and Late-Onset Pre-Eclamptic Pregnancies by Integrated Bioinformatics Analysis. Medical science monitor. 2020;26:e921997.
26. Varkonyi T, Nagy B, Fule T, Tarca AL, Karaszi K, Schonleber J, Hupuczi P, Mihalik N, Kovalszky I, Rigo J, Jr. et al: **Microarray profiling reveals that placental transcriptomes of early-onset HELLP syndrome and preeclampsia are similar**. *Placenta* 2011, **32 Suppl**:S21-29.
27. Bieche I, Parfait B, Le Doussal V, Olivi M, Rio MC, Lidereau R, Vidaud M. Identification of CGA as a novel estrogen receptor-responsive gene in breast cancer: an outstanding candidate marker to predict the response to endocrine therapy. Cancer research. 2001;61(4):1652–8.
28. Zhu L, Plow EF, Qin J. **Initiation of focal adhesion assembly by talin and kindlin: A dynamic view**. *Protein science* 2020.
29. Ilic D, Genbacev O, Jin F, Caceres E, Almeida EA, Bellingard-Dubouchaud V, Schaefer EM, Damsky CH, Fisher SJ. Plasma membrane-associated pY397FAK is a marker of cytotrophoblast invasion in vivo and in vitro. Am J Pathol. 2001;159(1):93–108.
30. von Versen-Hoeynck FM, Powers RW. Maternal-fetal metabolism in normal pregnancy and preeclampsia. Frontiers in bioscience. 2007;12:2457–70.
31. Fragkiadaki P, Soulitzis N, Sifakis S, Koutroulakis D, Gourvas V, Vrachnis N, Spandidos DA. Downregulation of notch signaling pathway in late preterm and term placentas from pregnancies complicated by preeclampsia. PloS one. 2015;10(5):e0126163.
32. Zhu X, Cao Q, Li X, Wang Z. Knockdown of TACC3 inhibits trophoblast cell migration and invasion through the PI3K/Akt signaling pathway. Mol Med Rep. 2016;14(4):3437–42.
33. Li Z, Yanfang W, Li J, Jiang P, Peng T, Chen K, Zhao X, Zhang Y, Zhen P, Zhu J, et al. Tumor-released exosomal circular RNA PDE8A promotes invasive growth via the miR-338/MACC1/MET pathway in pancreatic cancer. Cancer letters. 2018;432:237–50.
34. Zhang H, Zhu L, Bai M, Liu Y, Zhan Y, Deng T, Yang H, Sun W, Wang X, Zhu K, et al. Exosomal circRNA derived from gastric tumor promotes white adipose browning by targeting the miR-133/PRDM16 pathway. International journal of cancer. 2019;144(10):2501–15.
35. Xue J, Liu Y, Luo F, Lu X, Xu H, Liu X, Lu L, Yang Q, Chen C, Fan W, et al. Circ100284, via miR-217 regulation of EZH2, is involved in the arsenite-accelerated cell cycle of human keratinocytes in carcinogenesis. Biochimica et biophysica acta Molecular basis of disease. 2017;1863(3):753–63.
36. Han L, Luo QQ, Peng MG, Zhang Y, Zhu XH. miR-483 is downregulated in pre-eclampsia via targeting insulin-like growth factor 1 (IGF1) and regulates the PI3K/Akt/mTOR pathway of endothelial progenitor cells. J Obstet Gynaecol Res. 2021;47(1):63–72.
37. Tang Q, Gui J, Wu X, Wu W. Downregulation of miR-424 in placenta is associated with severe preeclampsia. Pregnancy hypertension. 2019;17:109–12.
38. Yang X, Meng T. MicroRNA-431 affects trophoblast migration and invasion by targeting ZEB1 in preeclampsia. Gene. 2019;683:225–32.
39. Xu P, Zhao Y, Liu M, Wang Y, Wang H, Li YX, Zhu X, Yao Y, Wang H, Qiao J, et al. Variations of microRNAs in human placentas and plasma from preeclamptic pregnancy. Hypertension. 2014;63(6):1276–84.
40. Choi SY, Yun J, Lee OJ, Han HS, Yeo MK, Lee MA, Suh KS. MicroRNA expression profiles in placenta with severe preeclampsia using a PNA-based microarray. Placenta. 2013;34(9):799–804.
41. Salomon C, Guanzon D, Scholz-Romero K, Longo S, Correa P, Illanes SE, Rice GE. Placental Exosomes as Early Biomarker of Preeclampsia: Potential Role of Exosomal MicroRNAs Across Gestation. J Clin Endocrinol Metab. 2017;102(9):3182–94.
42. Sandrim VC, Luizon MR, Palei AC, Tanus-Santos JE, Cavalli RC: **Circulating microRNA expression profiles in pre-eclampsia: evidence of increased miR-885-5p levels**. *BJOG: an international journal of obstetrics and gynaecology* 2016, **123**(13):2120–2128.

Figures

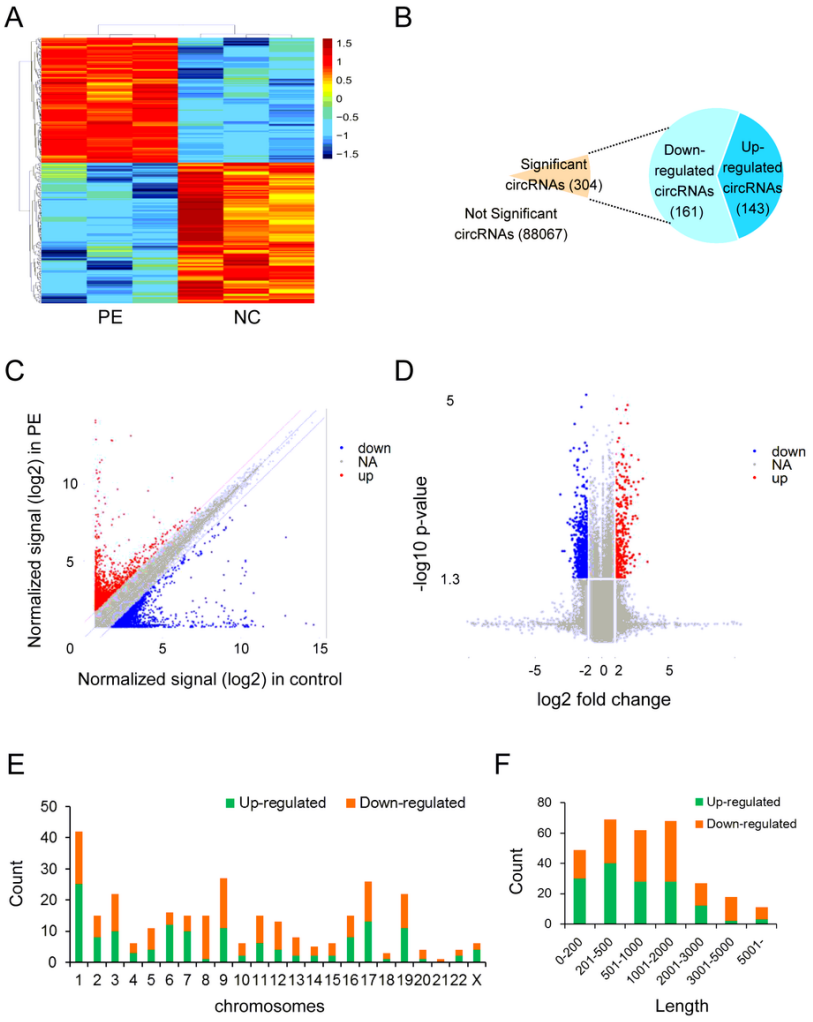

Figure 1

CircRNA expression profiling in umbilical cord blood exosomes from PE patients compared with controls. A: Clustered heat map analysis of differentially expressed circRNAs; B: The total circRNAs detected by microarray and differentially expressed circRNAs between two groups; C: Scatter plots of circRNAs signal values; D: Volcano plots visualizing the differentially expressed circRNAs; E: Chromosome distributions of the differential circRNAs; F: Length distributions of the differential circRNAs.

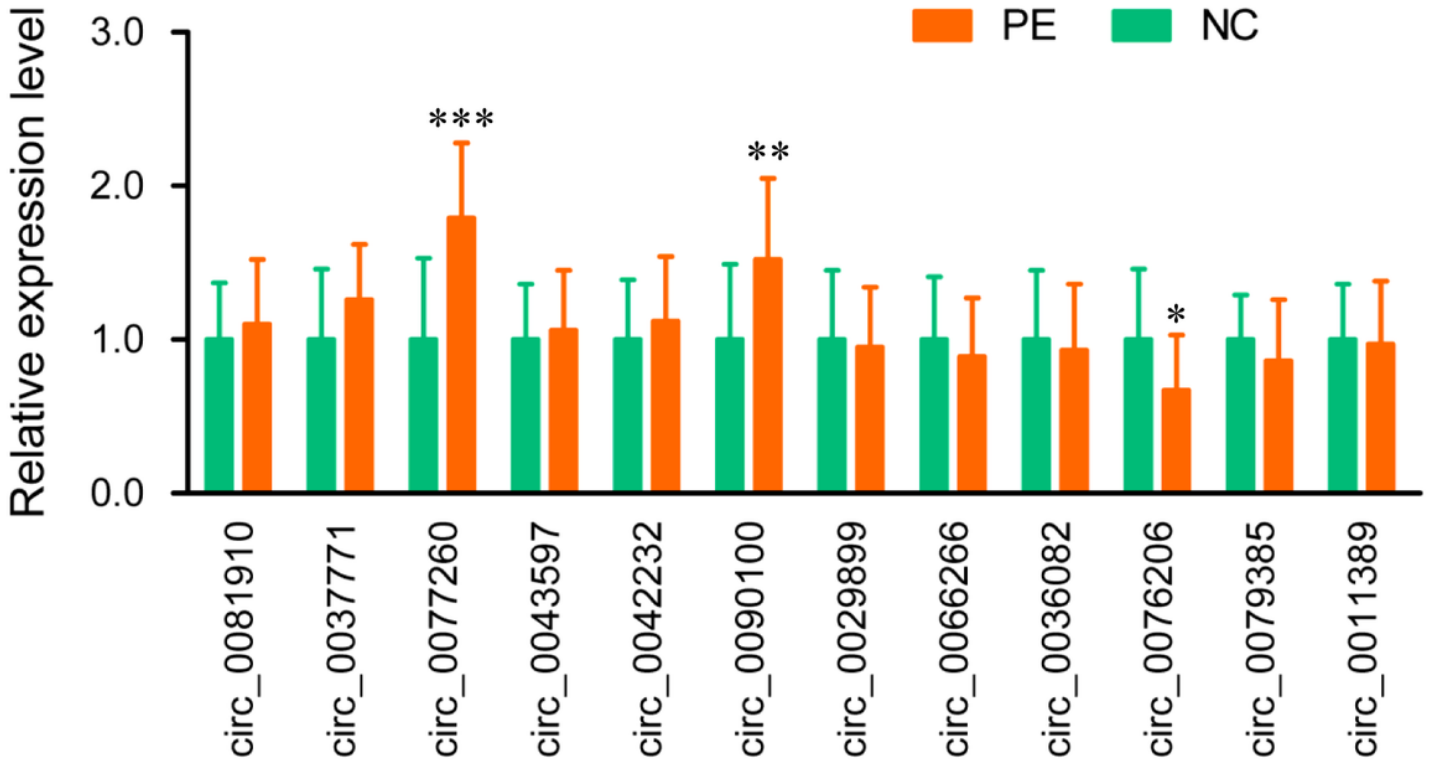

Figure 2

Validation of differentially expressed circRNA by qPCR. * P < 0.05, **P < 0.01 and *** P < 0.001.

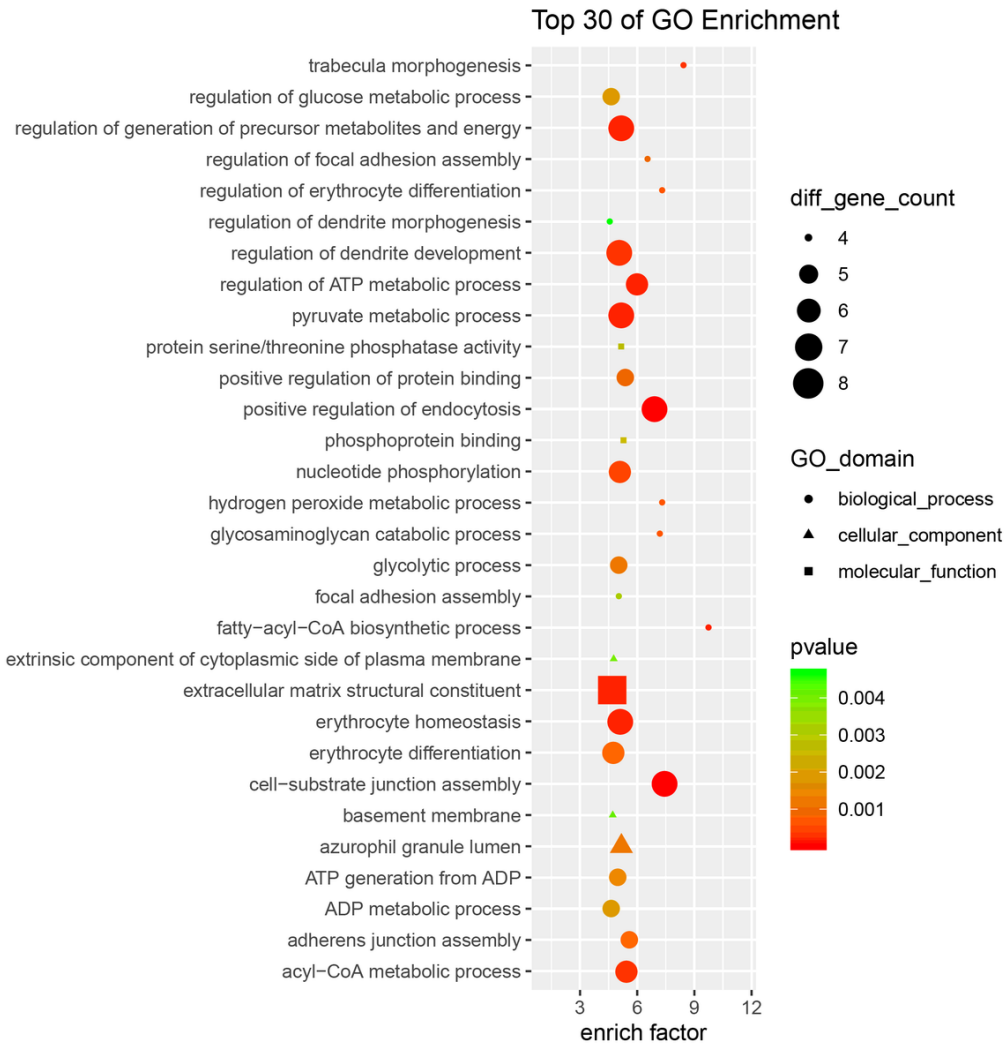

Figure 3

GO analysis of the differential circRNA parental genes.

Figure 4

KEGG pathway analysis of the differential circRNA parental genes.

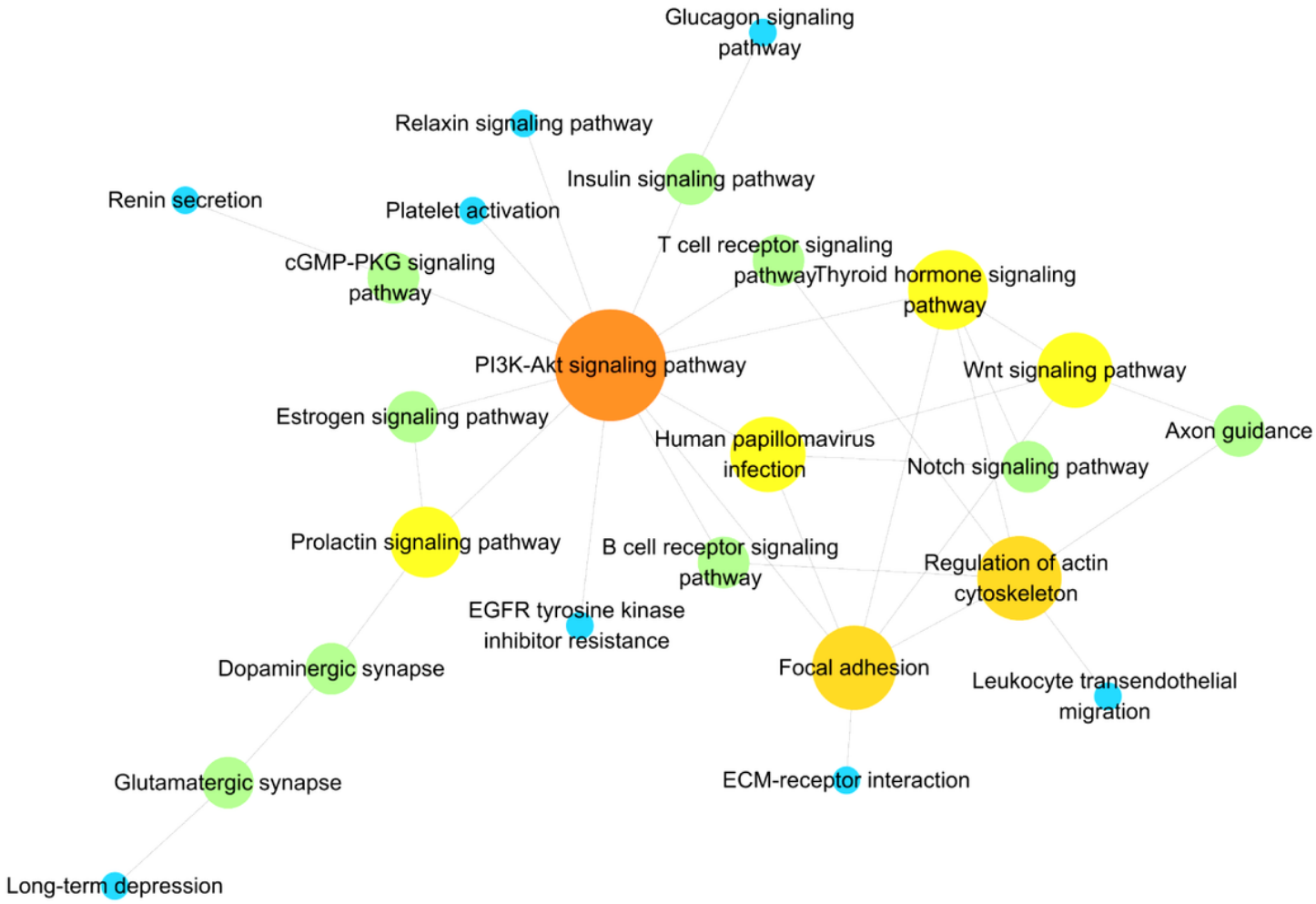

Figure 5

Interaction and overlaps of the differential circRNA parental genes among significantly enriched pathways.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1S3.xlsx