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Original Paper

Potential Significance of Circular RNA in Human Placental Tissue for Patients with Preeclampsia

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Key Words
CircRNAs • Preeclampsia • CircRNA microarray • MicroRNA sponge

Abstract
Aims: This study aimed to identify the different expression of circular RNAs (circRNAs) in the placental tissues of pregnant women with preeclampsia (PE) and to provide a new avenue of research regarding the pathological mechanisms of PE. ***Methods:*** In this study, we collected 40 placental tissues from PE patients and 35 placental tissues from gestational age-matched patients who gave premature birth. Arraystar circRNA Microarray Technology (KANGCHEN, Shanghai, China) was used to analyze the differential expression of circRNAs. According to the basic content of circRNAs in the two groups and their fold changes and due to the practicability of the designed divergent primers of each candidate circRNA, we selected three up-regulated circRNAs, hsa_circRNA_100782, hsa_circRNA_102682 and hsa_circRNA_104820, to validate the data. Real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was utilized to estimate the Ct values in both groups. We further evaluated the differences with a paired t-test and a receiver operating characteristic (ROC) curve. ***Results:*** Many circRNAs were found to be differentially expressed in PE placental tissues versus their controls; of these, 143 circRNAs were up-regulated and 158 were down-regulated. The expression levels of hsa_circRNA_100782 ($p < 0.05$), hsa_circRNA_102682 ($p < 0.05$), and hsa_circRNA_104820 ($p < 0.0001$) were validated as significantly up-regulated in the experimental group compared with the controls. Finally, we performed a literature comparison to forecast the possible mechanisms of circRNA function during PE. ***Conclusion:*** circRNA expression significantly differed in placental PE tissues compared with controls. According to the circRNA microarray results and the existing papers, circRNAs may contribute to the pathogenesis of PE by acting as miRNA sponges; this possibility requires additional investigation in future studies.

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Introduction

Preeclampsia (PE), a specific complication of pregnancy, mainly causes injuries to the blood vessels and kidneys as well as long-term injuries, and remains the most important cause of maternal and neonatal death [1]. This complicated and serious pregnancy-related diseases is characterized by hypertension and proteinuria. Some cases of PE can uaually co-occur with gestational diabetes mellitus (GDM), for they may share common pathogrnrsis [2, 3] and cause damage to multiple organs in the human body even in cases when prior to pregnancy, both blood pressure or blood glucose and renal function were normal in these patients.

As PE proceeds during pregnancy, blood pressure may continue to increase to more than 160/110 mmHg, and the level of proteinuria may reach greater than 5000 mg/24 h, developing into severe preeclampsia (sPE). Additional symptoms may develop as well, including HELLP syndrome (hemolysis, elevated liver enzymes and low platelets syndrome), persistent headache, and even a choked optic disc, resulting in vision loss [4]. Including poor placental vascular invasion, decreased 2-methoxyestradiol level, abnormal proteomics expression, many factors have been identified to participate in the pathogenesis of PE [5-8], but no more detailed or exact mechanisms were found. Due to the unknown pathogenesis of PE, prompt delivery of the fetus and placenta may be the only effective treatment in the later stages of the disease. Unfortunately, preterm delivery can result in further maternal and infant health problems. As a result, PE, and especially sPE, exists as one of the most common causes of mortality during pregnancy, with an increasingly higher incidence rate. Additional research is urgently needed to foster a better understanding of the pathogenic mechanisms involved in PE.

From the previous papers, non-coding RNAs, such as miRNAs, play a vital role in PE. Circular RNAs (circRNAs) are a special type of non-coding RNA in mammalian cells that interact closely with miRNAs; thus, circRNAs have attracted increasing attention from researchers due to their distinctive ring frame, which is in contrast the more common linear structures. As circRNAs have no 5' to 3' polarity or polyadenylated tails [9, 10], they are immune to RNase and are expressed stably. circRNA expression is concentrated in some specific tissues or organs, particularly in the brain [11]. Most of the known circRNAs are produced from the back-splicing [12, 13] of exons through three main mechanisms, including lariat-driven circularization, intron-pairing-driven circularization and the self-circularization of an intron to form circRNAs (ciRNAs) [14-16]. These features contribute to the performance of crucial physiological functions by circRNAs. Some specific circRNAs may have regulatory effects on gene expression [17] and human diseases, and some are correlated with the RNA binding protein Quaking [18]. The most well-known circRNA functions as a microRNA sponge by interacting with miRNA-7 [11, 19]. As research has progressed, circRNAs have been demonstrated to be associated with atherosclerosis, neurological disorders, diabetes and cancer [20-23]. However, few studies in the field of gynecology and obstetrics have identified their potential significance in the onset of PE.

In this study, we quantified the expression level of circRNAs and identified the role that circRNAs played in placental tissue during the development of PE to provide a new avenue of research regarding the pathological mechanisms of PE.

Materials and Methods

Patients and sample collection

All human placental tissue samples were obtained from the Nanjing Medical University Affiliated Nanjing Maternal and Child Health Hospital from February 2014 to January 2015 (Table 1). Overall, the study included 75 patients. To form the experimental group, 40 samples were collected from patients with severe preeclampsia at gestational weeks ranging from 30 to 34 and with levels of proteinuria ranging from 5000 mg/24 h to 11,700 mg/24 h. The control group consisted of samples acquired from 35 healthy

but premature births at 32.3 to 34 weeks gestation. Immediately following acquisition, the fresh placental tissue samples were placed in sterile, RNase-free 2.0 ml centrifuge tubes. Then, the samples were minced and allowed to soak in TRIzol. After these steps, all 75 samples were stored at -80 °C.

Six placental tissue samples, including three PE samples and three control samples, were sent to KANGCHEN (Shanghai, China) for the Arraystar circRNA Microarray analysis. On the basis of the chip results, we picked out several circRNAs as the candidate validation genes due to their high levels in both groups and their significant fold changes. The final choice of the validation genes was determined by the practicability of the designed divergent primers, which are described in greater detail below.

Total RNA extraction and reverse transcription

According to the instructions, we extracted total RNA from the samples using TRIzol reagent (Invitrogen, Karlsruhe, Germany) and the RNAprep pure tissue kit (TIANGEN) (DP431) in a step-by-step manner. The integrity of the extracted RNA was tested via 1% agarose gel electrophoresis using the following criteria: there could be up to three bands; the ratio of 28 S rRNA/18 S rRNA should be 2; and the 5 S rRNA should not be too bright. The purity of the extracted RNA was measured by a UV spectrophotometer using the following criteria: the 260/280 nm absorbance ratio of the qualified sample should be between 1.8 and 2.1, with 2.0 being considered best. According to the concentration of each sample, we added 1000 ng to the 20 μl reverse transcription system and then examined each sample using reverse transcription with random primers following the recommendations of the Thermo Scientific RevertAid First Strand cDNA Synthesis Kit.

Regular-PCR (R-PCR) and annealing temperature determination

Considering the unique ring structure of circRNAs, we designed corresponding divergent primers using Primer 3.0 according to the sequences provided by the microarray analysis results. These primers were synthesized by the Realgene Company (Nanjing, China). The integral internal reference gene we used was glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The primers were designed to amplify a target sequence with a length of approximately 200 bp. Based on the instructions for Taq DNA Polymerase, we utilized 3 different temperature gradients (56, 59 and 62 °C) in a 25μl reaction system.

The thermal cycling conditions were as follows: start at 94 °C for 5 mins; 30 cycles of 94 °C for 30 s, a pre-selected annealing temperature for 30 s, and 72 °C for 30 s; and maintenance at 72 °C for 10 mins for

Table 1. General features of the pregnant women in the two groups. NS, non-significant difference

	Preeclampsia (n = 40)	Control (n = 35)	p-value
Age (years)	30.79 ± 3.68	30.30 ± 3.44	0.1853
Height (cm)	161.47 ± 4.86	162.18 ± 4.41	0.7585
Weight (kg)	79.11 ± 13.53	78.77 ± 7.23	0.3373
Gestational week	33.53 ± 1.56	33.15 ± 0.89	0.2615
Proteinuria level (mg/24 h)	8412.63 ± 1793.27	1382.23 ± 873.45	0.0034
Systolic pressure (mmHg)	165.55 ± 19.84	113.27 ± 6.17	0.0002
Diastolic pressure (mmHg)	105.36 ± 10.87	70.91 ± 6.35	0.0026
ALT / AST	0.66 ± 0.27	0.65 ± 0.14	0.9843
PLT (10^9/L)	158.81 ± 59.25	201.23 ± 72.03	0.9321
Mode of delivery	C-sect	C-sect	NS
Neonatal weight (g)	2292.50 ± 781.44	3300.00 ± 324.04	0.0003
Neonatal Apgar score	9.68 ± 0.69	10	0.0885

Table 2. Sequence of the internal reference and the paired primers

	5'-3' (sense)	5'-3' (antisense)
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC
hsa_circRNA_100782	TCATGCTGATCTCAAGCCAGA	ACCAAGACTTGTGAGGCCAT
hsa_circRNA_102682	GTTTCTCTGAGTCCTGCCCT	GGATTGCTGCAGGTTCGAAT
hsa_circRNA_104820	CCGTTGCTGACTATGTACGC	TCATACGCAACCAAGCCATG

full extension. The products of R-PCR were examined using 1.5% agarose gel electrophoresis under the following criteria: there should be only one band in the lane of the specific annealing temperature, and if there was one band in more than one lane, the brightest band was chosen; no existence of primer dimers or by-products; and compared to the DNA marker, the molecular weight must be equal to the size of the target fragment (approximately 200 bp). Finally, combining all the conditions above, three ideal up-regulated genes, hsa_circRNA_100782 (FC = 3.71), hsa_circRNA_102682 (FC = 3.59) and hsa_circRNA_104820 (FC = 5.96) were selected as the validation genes. Table 2 contains the sequences of the paired primers we designed. The best annealing temperatures were 59 °C for hsa_circRNA_100782 and hsa_circRNA_102682 and 56 °C for GAPDH and hsa_circRNA_104820.

qRT-PCR detection of target genes

Once the annealing temperature of each gene was identified, qRT-PCR was performed with SYBR. To eliminate the experimental random error, samples were loaded in triplicate and each well was treated identically. The data were analyzed using the $2^{-\Delta\Delta t}$ method; to guarantee the accuracy of the results, all data are represented as the means ± SD of three independent experiments.

Statistical analyses

To analyze the complex and tedious data, both Statistical Program for Social Sciences Version 22 (SPSS) and GraphPad Prism 5.0 were utilized simultaneously. In addition, a *t*-test was also used to estimate the data, and $p \leq 0.05$ was used to denote statistical significance. To determine the significance of these data for preeclampsia, we also established a receiver operating characteristic (ROC) curve for each circRNA. The area under the curve (AUC) was calculated for each respective circRNA.

Results

Characteristics of the study population

In total, 40 placenta samples of patients with PE and 35 placenta samples from corresponding premature births were collected in our study. The patients' characteristics are summarized in Table 1. No differences were observed between the two groups regarding age, height, weight, gestational week, ALT/AST, PLT, mode of delivery or the neonatal Apgar score ($p > 0.05$). However, the 24 h proteinuria, blood pressure and neonatal weight were

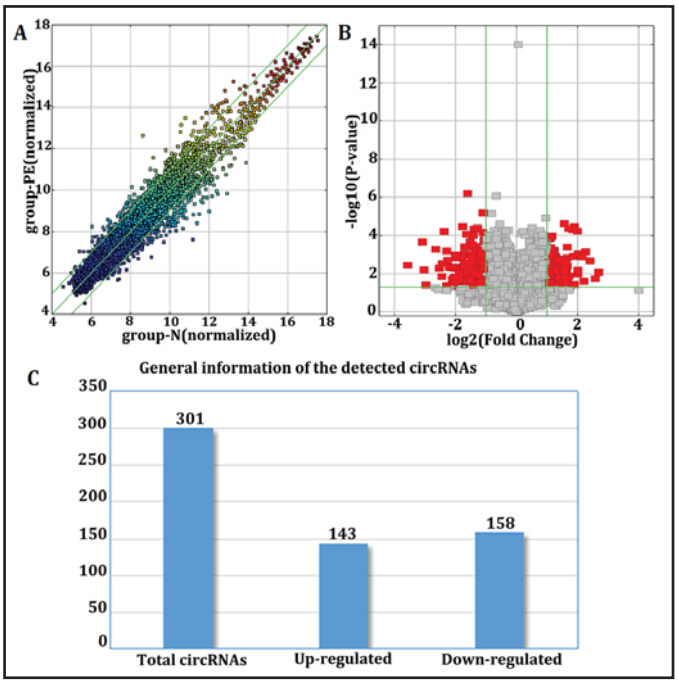

Fig. 1. Detection results of all circRNAs. a. Scatter Plots The values for the X and Y axes are normalized signal values (log2 scaled). The green lines represent fold change lines. circRNAs above the top green line and below the bottom green line indicated more than a 2.0-fold change of circRNAs between the two groups. b. Volcano Plots The red point in the plot represents the differentially expressed circRNAs that were statistically significant. c. Histogram Considering the fold change and p-value, a total of 301 circRNAs were detected, of which 143 were up-regulated and 158 were down-regulated.

Table 3. List of circRNAs with significant differences between two groups (FC>3.0)

circRNA	Regulation	Fold Change	p-value	chrom	strand	txStart	txEnd
hsa_circRNA_104893	up	3.00	0.00715887	chr9	+	118969734	119033695
hsa_circRNA_102650	up	3.01	0.017272762	chr2	+	26587169	26598016
hsa_circRNA_104891	up	3.02	0.017547647	chr9	+	118969734	118989831
hsa_circRNA_103667	up	3.02	0.011978023	chr4	-	77055327	77065626
hsa_circRNA_104682	up	3.05	0.002392826	chr8	-	125332326	125343033
hsa_circRNA_104892	up	3.06	0.005049701	chr9	+	118969734	118997916
hsa_circRNA_101900	up	3.08	0.033676024	chr16	+	88008653	88017865
hsa_circRNA_001695	up	3.08	0.03159226	chr20	+	58755107	58755971
hsa_circRNA_103618	up	3.10	0.027353647	chr4	-	36230203	36231267
hsa_circRNA_102680	up	3.11	0.007328049	chr2	+	36623756	36706837
hsa_circRNA_100921	up	3.15	0.032709009	chr11	-	85707868	85718626
hsa_circRNA_102677	up	3.15	0.001446147	chr2	+	36623756	36623930
hsa_circRNA_104547	up	3.16	0.000403266	chr7	-	158552176	158557544
hsa_circRNA_103489	up	3.16	0.003944009	chr3	+	149563797	149613347
hsa_circRNA_102649	up	3.21	0.016766036	chr2	+	26587169	26596497
hsa_circRNA_103487	up	3.26	4.17515E-05	chr3	+	149563797	149570383
hsa_circRNA_103492	up	3.30	0.012977941	chr3	+	149563797	149639014
hsa_circRNA_103116	up	3.34	0.046724496	chr21	+	30693541	30702014
hsa_circRNA_102682	up	3.59	4.83021E-05	chr2	+	36623756	36749456
hsa_circRNA_102681	up	3.62	0.001261817	chr2	+	36623756	36744685
hsa_circRNA_100782	up	3.71	0.036767421	chr11	+	33307958	33309057
hsa_circRNA_102679	up	3.72	3.41004E-05	chr2	+	36623756	36691798
hsa_circRNA_102678	up	3.97	5.75085E-05	chr2	+	36623756	36669878
hsa_circRNA_103493	up	4.00	0.010305698	chr3	+	149613259	149639014
hsa_circRNA_104823	up	4.01	0.006571296	chr9	+	96238537	96259881
hsa_circRNA_104700	up	4.07	0.000914974	chr8	-	141710989	141716304
hsa_circRNA_104822	up	4.23	0.000895222	chr9	+	96233422	96278551
hsa_circRNA_104824	up	4.43	0.001274347	chr9	+	96238537	96261168
hsa_circRNA_104981	up	4.60	0.024418528	chrX	-	10534927	10535643
hsa_circRNA_104819	up	4.80	0.00069232	chr9	+	96233422	96238620
hsa_circRNA_103474	up	5.31	0.002076334	chr3	+	130851588	130852800
hsa_circRNA_104820	up	5.96	0.018264734	chr9	+	96233422	96259881
hsa_circRNA_104821	up	6.47	0.008384202	chr9	+	96233422	96261168
hsa_circRNA_104075	down	3.03	0.024352363	chr6	-	17669523	17669777
hsa_circRNA_100021	down	3.04	6.2365E-07	chr1	-	5987708	6022009
hsa_circRNA_101722	down	3.05	0.008152406	chr16	+	16146580	16150152
hsa_circRNA_100476	down	3.09	0.004771015	chr1	+	230795209	230807386
hsa_circRNA_001216	down	3.09	0.000382127	chr10	+	53459000	53459207
hsa_circRNA_400027	down	3.11	8.48125E-05	chr15	+	25325262	25326442
hsa_circRNA_104099	down	3.13	0.024218314	chr6	+	35195356	35201078
hsa_circRNA_100117	down	3.17	0.015660628	chr1	-	28362054	28384605
hsa_circRNA_103039	down	3.18	0.033397141	chr20	-	33954359	33962059
hsa_circRNA_101803	down	3.22	0.001025761	chr16	-	47143393	47165936
hsa_circRNA_100750	down	3.25	0.000882698	chr11	+	3988781	4080626
hsa_circRNA_104109	down	3.25	0.000375716	chr6	+	42819829	42821476
hsa_circRNA_000799	down	3.28	0.002706406	chr11	+	65266845	65267149
hsa_circRNA_102958	down	3.28	0.02506972	chr2	-	239184383	239186596
hsa_circRNA_100860	down	3.28	0.004513648	chr11	+	68115314	68115711
hsa_circRNA_100660	down	3.37	0.038814527	chr10	+	99967857	99969656
hsa_circRNA_102550	down	3.40	3.37516E-05	chr19	+	41089303	41089623
hsa_circRNA_103781	down	3.47	0.001295906	chr5	+	896779	901546
hsa_circRNA_101861	down	3.65	0.021670739	chr16	-	70294946	70302282
hsa_circRNA_400066	down	3.65	0.009461681	chr22	-	19965129	19965469
hsa_circRNA_104872	down	3.79	0.019917377	chr9	-	114860749	114864565
hsa_circRNA_000993	down	3.80	0.015349172	chr9	-	125930467	125936121
hsa_circRNA_102195	down	3.92	0.001464989	chr17	+	73808192	73809959
hsa_circRNA_101740	down	3.98	0.004539618	chr16	+	19619499	19628130
hsa_circRNA_104423	down	4.00	0.001072232	chr7	+	90355880	90356126
hsa_circRNA_001950	down	4.00	0.04480486	chr11	-	3177371	3177731
hsa_circRNA_102774	down	4.01	0.026505596	chr2	-	85625142	85626408
hsa_circRNA_101846	down	4.03	0.005101387	chr16	+	68893786	68896964
hsa_circRNA_400095	down	4.11	0.014954005	chr9	+	116764513	116764813
hsa_circRNA_103307	down	4.30	0.004687443	chr3	-	18456602	18462483
hsa_circRNA_100876	down	4.31	0.018936472	chr11	+	71668272	71671937
hsa_circRNA_101877	down	4.61	0.004592684	chr16	-	74670243	74671868
hsa_circRNA_001109	down	4.80	0.001979339	chr2	-	100721965	100754399
hsa_circRNA_101945	down	4.85	0.044180868	chr17	-	2195845	2196271
hsa_circRNA_104551	down	4.88	0.00063567	chr8	+	9437668	9437893
hsa_circRNA_000031	down	4.91	0.046623197	chr1	+	2225564	2225807
hsa_circRNA_104126	down	5.17	5.99233E-05	chr6	-	53365044	53365148
hsa_circRNA_100269	down	5.40	0.002952801	chr1	+	82302569	82372915
hsa_circRNA_104873	down	5.47	0.013811499	chr9	-	114860749	114875148
hsa_circRNA_102533	down	5.76	0.005009807	chr19	+	34942885	34957919
hsa_circRNA_100477	down	6.25	0.000525415	chr1	+	230798886	230807386
hsa_circRNA_104342	down	7.83	0.038947537	chr7	+	33185853	33217203
hsa_circRNA_104166	down	8.18	0.006226957	chr6	-	105563560	105573453
hsa_circRNA_104916	down	8.47	0.000217748	chr9	+	127064214	127089724
hsa_circRNA_103519	down	11.86	0.003573195	chr3	+	179045348	179046139

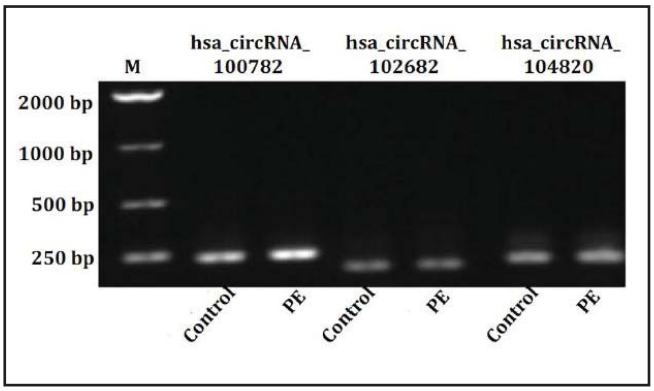

Fig. 2. Image showing testing of qRT-PCR products on a 1.5% agarose gel. The molecular weight of the marker is 10,000. According to the markers, the molecular weights of the target genes were approximately 200 - 250 bp. Only one band was present in each lane. In comparison, the bands in the control samples were not as bright as their corresponding PE samples, which shows the differential circRNA expression levels between PE samples and controls.

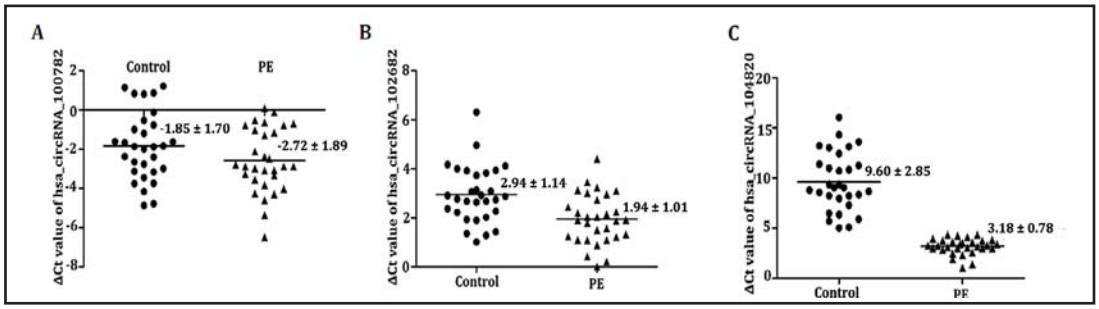

Fig. 3. The expression levels of circular RNAs in patients with PE and patients who delivered prematurely. The expression levels of hsa_circRNA_100782, hsa_circRNA_102682 and hsa_circRNA_104820 in each patient were compared. Higher ΔCt values indicate lower levels of expression. The expression levels of each gene were significantly higher versus their controls; all p-values < 0.05.

significantly different between the two groups ($p < 0.01$).

Results of the microarray analysis

We accounted for the fold change (FC ≥ 2.0) and p-values (≤ 0.05) in this analysis. The expression of specific circRNAs was significantly different between groups. The general information pertaining to the detected circRNAs is shown in Figure 1. Altogether, 301 differentially expressed circRNAs were identified, of which 143 were up-regulated and 158 were down-regulated. Certain circRNAs with highly differential levels of expression are shown in Table 3 (FC ≥ 3.0).

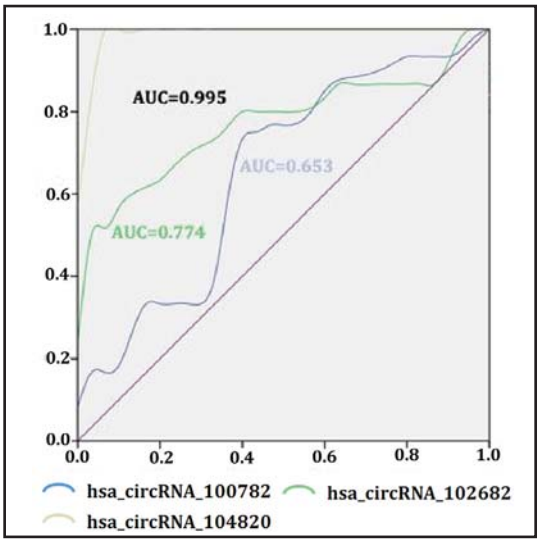

Fig. 4. The ROC curves for the three circRNAs.

Evaluation of primers and products

To increase the rigor of the study and to enhance the efficacy of the primers that we designed, we measured both the specificity and sensitivity of the amplification products. After qRT-PCR, 1.5% agarose gel electrophoresis was used to test the uniqueness of the products and the relative amounts between the experimental and control groups (Fig. 2). According to the electrophoresis bands, only the targeted products and no primer dimers or non-specific amplification products were present, indicating that the divergent primers utilized for the circRNAs were effective and appropriate. The data in Figure 3 and the band brightness in Figure 2 show that the circRNA levels were significantly higher in PE group.

Values of circRNA in PE

These validation results agreed with the ROC curves (Fig. 4). The potential role of circRNAs in the pathogenesis of PE is of great importance. Furthermore, the area under the ROC curve for hsa_circRNA_100782, hsa_circRNA_102682 and hsa_circRNA_104820 were 0.653, 0.774 and 0.995, respectively.

Discussion

circRNAs differ substantially from conventional linear RNAs and have recently become an important research topic due to their stable structures and high degrees of tissue specificity. circRNAs were first accidently observed in RNA viruses and were regarded as splicing errors or by-products during the onset of splicing [9, 12] despite having been observed for decades in eukaryotic cells. While previously limited by existing technology, circRNA research has emerged as an important research topic only in recent years. circRNAs have highly conserved sequences and stable expression in different individuals [15]; these futures hint at their potential to mediate the occurrence of specific diseases in the absence of an external environment influence.

Exonic circRNAs might have extraordinary effects in cellular physiology, including miRNA binding, translational regulation, protein interactions, and even protein translation (found only in viruses) [23]. Additional studies have noted that circRNAs can function as miRNA sponges, which is to say circRNAs have many miRNA binding sites that competitively bind to miRNAs. Thus, circRNAs may alleviate the inhibitory effects of miRNAs on target molecules, thereby regulating gene expression levels. The most widely studied molecular sponge is antisense to the cerebellar degeneration-related protein1 transcript (CDR1as), which is located in the brains of humans and mice. CDR1as has approximately 74 miR-7 binding sites, and CDR1as over-expression can down-regulate miR-7 expression level [11, 14, 16]. This finding revealed a new therapeutic strategy for Alzheimer's disease.

The circRNAs isolated from the placental tissues of PE patients in our study also have several miRNA binding sites [24], and some even were associated with two different diseases like PE and gestational diabetes mellitus[25]. As shown in Table 4, many circRNAs have miRNA-17 binding sites, suggesting that these circRNAs can regulate the expression level of miRNA-17 in human placental tissues. miRNA-17 has been identified as one of the angiogenesis-associated miRNAs in the human placenta and was found to be highly expressed in PE placentas [30]. In the work of Chen and Wang [24] and Wang et al. [30], up-regulated miRNA-17 in the placenta could advance the process of PE by targeting the ephrin-B2/Eph receptor B4 (EPHB4) system, a classical pathway involved in trophoblast invasion; notably, the disorganization of this system exacerbates the process during PE. The differential expression of circRNAs could possibly up-regulate the expression levels of miRNA-17 through miRNA sponges, thereby contributing to the pathogenesis of PE. This possibility warrants further investigation.

The fact that the miRNA binding sites of miRNA-17 are strongly related to the onset of PE is not a peculiar phenomenon, and from Table 4, we can tell that many other MREs of other circRNAs also play an important role in PE. These results suggested that the sponge functions of circRNAs may be highly significant and thus deserve further investigation. Despite the miRNA sponge functions, the interaction of proteins in blood corpuscles should also be mentioned. Recently, the work of Zhang et al. [31] showed that one plasma protein factor, endoglin (ENG), can combine with the up-regulated circRNA_101222 in blood corpuscles of preeclampsia patients; this combination of circRNA and ENG may be a potential biomarker for the early prediction and diagnosis of PE. While this finding reveals new information for researchers, further study is still warranted in the future.

It is believed that when PE occurs, the deficiency of placental trophoblast invasion may cause reduced placental flow and, eventually, placental ischemia, which is the initiating agent for subsequent disorders. Ischemia accompanied by hypoxia raises a series of complications

Table 4. List of PE incidence-related miRNAs in placenta. miRNAs in bold were shown to participate in the pathogenesis of PE via different pathways in the placenta. Their references are given in brackets

miRNA Binding Sites	circRNA	Regulation	Fold Change	Target
hsa-miR-17-3p[24]	hsa_circRNA_101289	up	2.0245	VEGFA, EFNB2, EPHB4
	hsa_circRNA_102390	up	2.0368	
	hsa_circRNA_101608	up	2.1259	
	hsa_circRNA_101611	up	2.1303	
	hsa_circRNA_102685	up	2.5299	
	hsa_circRNA_102683	up	2.8964	
	hsa_circRNA_100782	up	3.7106	
	hsa_circRNA_102679	up	3.7227	
	hsa_circRNA_102678	up	3.9735	
	hsa_circRNA_103285	down	2.1379	
	hsa_circRNA_101568	down	2.1406	
	hsa_circRNA_101695	down	2.2970	
	hsa_circRNA_104166	down	8.1805	
hsa-miR-152-3p[24]	hsa_circRNA_100181	down	2.5581	HLA-G
hsa-miR-155-5p[24-26]	hsa_circRNA_102719	up	2.0041	Cyclin D1
hsa-miR-210-5p[14,24-26]	hsa_circRNA_400068	up	2.1154	ISCU, EFNA3, homeobox-A9, HSD17B1
	hsa_circRNA_001096	down	2.2375	
hsa-miR-222-5p[25,26]	hsa_circRNA_101784	down	2.2515	P57, FOXO, TIMP3, MMP1, SOD2, KIT
hsa-miR-29a-3p[25,26]	hsa_circRNA_100291	up	2.1439	Insig1, PCK2
	hsa_circRNA_400059	down	2.5877	
hsa-miR-29a-5p[25,26]	hsa_circRNA_102719	up	2.0041	Insig1, PCK2
	hsa_circRNA_100225	up	2.0162	
	hsa_circRNA_102823	up	2.1090	
	hsa_circRNA_103689	down	2.0472	
hsa-miR-519d-3p[25,26]	hsa_circRNA_101462	up	2.0475	CDKN1A/p21, PTEN, AKT3, TIMP2
	hsa_circRNA_101463	up	2.5327	
hsa-miR-15b-3p[25,26]	hsa_circRNA_101611	up	2.1303	CCNE1
	hsa_circRNA_100845	down	2.6807	
hsa-miR-15b-5p[25,26]	hsa_circRNA_104681	up	2.9562	CCNE1
	hsa_circRNA_104682	up	3.0469	
	hsa_circRNA_101852	down	2.3583	
hsa-miR-223-3p[25,26]	hsa_circRNA_103039	down	3.1797	GZMB, STAT3, E2F1, FOXO1
hsa-miR-223-5p[25,26]	hsa_circRNA_104810	up	2.6023	GZMB, STAT3, E2F1, FOXO1
	hsa_circRNA_100181	down	2.5581	
hsa-miR-26a-1-3p[25,26]	hsa_circRNA_100660	down	3.3707	Bcl-2, Mcl-1, CCND, MMP2
hsa-miR-26a-2-3p[25,26]	hsa_circRNA_104563	up	2.6390	Bcl-2, Mcl-1, CCND, MMP2
hsa-miR-26a-5p[25,26]	hsa_circRNA_104823	up	4.0147	Bcl-2, Mcl-1, CCND, MMP2
	hsa_circRNA_104824	up	4.4342	
	hsa_circRNA_104819	up	4.7952	
hsa-miR-26b-3p[25,26]	hsa_circRNA_103116	up	3.3351	SLC7A11
	hsa_circRNA_104126	down	5.1660	
	hsa_circRNA_103149	down	2.3110	
hsa-miR-136-3p[25,26]	hsa_circRNA_100750	down	3.2472	AEG-1, Bcl-2
hsa-miR-136-5p[25,26]	hsa_circRNA_104556	up	2.6705	AEG-1, Bcl-2
	hsa_circRNA_100720	down	2.0446	
	hsa_circRNA_103149	down	2.3110	
	hsa_circRNA_101803	down	3.2246	
	hsa_circRNA_100876	down	4.3141	
hsa-miR-16-2-3p[25,26]	hsa_circRNA_104075	down	3.0258	BCL2
hsa-miR-16-1-3p[25,26]	hsa_circRNA_104075	down	3.0258	BCL2
hsa-miR-16-5p[25,26]	hsa_circRNA_101852	down	2.3583	BCL2
	hsa_circRNA_101853	down	2.3850	
hsa-miR-181a-5p[25,26]	hsa_circRNA_101766	down	2.7519	BCL-2, Tcl1
hsa-miR-181a-2-3p[25,26]	hsa_circRNA_001416	down	2.8290	BCL-2, Tcl1
hsa-miR-181a-3p[26]	hsa_circRNA_104253	up	2.0196	BCL-2, Tcl1
	hsa_circRNA_101066	up	2.3439	
hsa-miR-21-3p[26]	hsa_circRNA_103205	up	2.1452	PTEN, TPM1, BCL2
hsa-miR-21-5p[26]	hsa_circRNA_104574	up	2.0468	PTEN, TPM1, BCL2
hsa-miR-26a-2-3p[26]	hsa_circRNA_101618	up	2.4874	Bcl-2, Mcl-1, CCND, MMP2
hsa-miR-1-3p[26]	hsa_circRNA_104822	up	4.2287	JCN
	hsa_circRNA_002042	down	2.4405	
hsa-miR-584-3p[27]	hsa_circRNA_400066	down	3.6520	eNOS
hsa-miR-584-5p[27]	hsa_circRNA_100477	down	6.2461	eNOS
hsa-miR-335-3p[27]	hsa_circRNA_100039	up	2.0422	eNOS
	hsa_circRNA_000799	down	3.2752	
	hsa_circRNA_102219	down	2.0709	
hsa-miR-335-5p[27]	hsa_circRNA_101320	down	2.5180	eNOS
hsa-miR-141-5p[28]	hsa_circRNA_100431	up	2.3484	JEG-3, HTR-8/SVneo
	hsa_circRNA_104700	up	4.0670	
	hsa_circRNA_102599	down	2.3641	
	hsa_circRNA_101706	down	2.6574	
	hsa_circRNA_103456	down	2.8941	
hsa-miR-193b-3p[29]	hsa_circRNA_104939	down	2.5029	HTR-8/SVneo
	hsa_circRNA_400066	down	3.6520	
	hsa_circRNA_102195	down	3.9196	

regarding placental function, and some virulence factors are released into the blood. Therefore, the placenta is both the start and center of all the mechanisms responsible for PE. As the expression of circRNAs may differ between the placenta and the peripheral blood [32], we utilized placental tissues rather than serum in our study for a more circumstantial research.

Our study has some limitations. First, the sample selection was limited by a small number of PE samples, which was not large enough to establish definitive conclusions. Moreover, the choice of samples may not be generalizable to the general population. In future works, more samples should be collected to perform a detailed study. Equally important is that the samples were all collected from one hospital in a single year, which may have resulted in regional differences. Second, the study of circRNAs in PE has just started, and the functional analysis is imperfect; more work should be done to improve this shortcoming in the future. Through further study regarding the functions of circRNAs, our understanding of circRNA-related mechanisms of diseases could be improved, and the diagnostic accuracy and development of alternative prevention methods could be enhanced. Third, the expression of circRNAs in peripheral blood is essential for finding a suitable biomarker for earlier diagnoses of PE. In future studies, we also plan to analyze blood samples.

PE is an agnogenic disease occurring in pregnant women that primarily causes renal damage. PE cannot be identified until clinical manifestation becomes apparent, which is usually too late for clinical intervention measures. Thus, there is an urgent need to discover an ideal biomarker for PE before the condition progresses beyond the point of treatment. We hope that our research will open up new research directions regarding the pathogenesis of PE and will foster the development of effective breakthroughs.

To conclude, to identify the role that circRNAs play in PE placental tissues, our study analyzed the content of circRNAs in both patients with PE and patients who delivered prematurely. The results indicated that circRNAs expression differed significantly between the two groups and that circRNAs may have an important function as miRNA sponges. Our research represents a new breakthrough in the pathogenesis of PE. Of course, more work is needed to further uncover the molecular mechanisms of circRNAs and to reveal their deeper involvement in disease pathogenesis.

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Disclosure Statement

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