



Original Article

The expression profile of circRNA and its potential regulatory targets in the placentas of severe pre-eclampsia

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ABSTRACT

Objective: To determine the expression profiles of circular RNAs (circRNAs) of women with severe pre-eclampsia (sPE group) versus normal pregnancies (normal control group).

Materials and methods: RNA-sequencing (RNA-seq) was conducted to characterize differentially expressed circRNAs and mRNAs in the placental tissues of women with sPE versus normal pregnancies. circRNA functions were predicted by Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database analysis. The backsplicing junctions of circRNAs were validated with the use of divergent primers. Relative expression levels of circRNAs were verified by quantitative real-time PCR (qPCR). A circRNA-miRNA-mRNA interaction network was constructed to outline the regulatory network of the differentially expressed circRNAs.

Results: A total of 49 differentially expressed circRNAs were found in the placental tissues of women with sPE. Several differentially expressed mRNAs were also observed in the sPE patients. KEGG analysis revealed that the most enriched pathway of the circRNAs was the MAPK signaling pathway, while the differentially expressed mRNAs were primary enriched in pathways in cancer. Among these circRNAs, hsa_circ_0001438, hsa_circ_0001326, and hsa_circ_32340 were upregulated in the sPE patients and the circRNA-miRNA-mRNA interaction network generated with these three circRNAs revealed a broad regulatory network that might be involved in the pathogenesis of sPE.

Conclusion: circRNAs are differentially expressed in sPE. The upregulation of hsa_circ_0001438, hsa_circ_0001326, and hsa_circ_32340 has a potential role in the regulation of miRNA and mRNA expression. Changes to the expression profiles of the circRNAs might be linked to the pathogenesis of sPE and could function as biomarkers.

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Introduction

Pre-eclampsia (PE) is a common and severe disease for both the mother and fetus during pregnancy, affecting 3%–5% of all pregnancies and leading to severe morbidity and mortality during pregnancy [1]. PE is considered a multisystem placentally mediated disease that is classically diagnosed by the combined presentation of high blood pressure and proteinuria [2]. In patients with severe

PE (sPE) whose blood pressure may reach higher than 160/110 mmHg, PE may progress to more severe forms, such as eclampsia or HELLP (hemolysis elevated liver enzymes, and low platelets) syndrome, which poses a greater risk of increased morbidity and mortality to both the mother and fetus [3]. Currently, the exact pathology of PE remains largely unknown. Failure of vascular remodeling of the maternal spiral arteries, endovascular extravillous trophoblast differentiation, and defects in trophoblast invasion are thought to be important pathological events in PE development [4]. Due to the lack of biomarkers to clinically predict the onset of PE, expedited delivery becomes the only effective curative treatment [5]. Hence, a better understanding of the pathological relevant molecular pathways in sPE is urgently needed.

Circular RNA (circRNA) is a type of RNA that is processed from linear precursor mRNA (pre-mRNA) by non-canonical splicing [6] and is characterized by a covalently closed continuous loop with no canonical 5' cap or 3' polyadenylated tail, which renders these

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molecules more stable than linear RNA even in the presence of RNAase [7]. Previously, circRNAs were believed to be of low abundance or even disregard as by-products and splicing errors of transcription [8]. To date, numerous circRNAs have been successfully identified in diverse cell types and different contexts of disease [9]. Many circRNAs have been proposed as candidate biomarkers for clinical diagnosis and prognosis of PE [10].

In the context of PE, microarray analysis have revealed groups of differentially expressed circRNAs in the placental tissues of PE patients [11]. Several of these circRNAs have been proposed as biomarkers for re-eclampsia [12–14]. However, the global profiles of circRNAs in sPE patients, as detected by RNA-sequencing, remain unavailable, as most have not been validated. Therefore, the aim of the current study was to assess the expression profiles of circRNAs to gain an overview of the expression patterns of circRNAs in sPE. In addition, as the involvement of circRNAs in regulating tumor behaviors by functioning as a miRNA sponge has been confirmed [8], we further constructed a circRNA-miRNA-mRNA interaction network to reveal the potential functions of these differentially expressed circRNAs.

Materials and methods

Clinical specimens

A total of 60 placental tissues obtained from Sun Yat-sen Memorial Hospital were collected immediately after elective cesarean section. The placental tissue that near the maternal surface was taken and the size was as large as soybean, then stored in liquid nitrogen for subsequent total RNA isolation. The study cohort included women with normal pregnancies and no disease during pregnancy over 28 weeks, while the diagnostic criteria for sPE was systolic blood pressure ≥ 140 mmHg and diastolic blood pressure ≥ 90 mmHg after gestational week 20, accompanied by one of the following: urine protein ≥ 0.3 g/24 h, urine protein/creatinine ratio ≥ 0.3 , or random urine protein $\geq (+)$ [15]. Of the 60 patients included for analysis, 30 (50%) had normal pregnancies and 30 (50%) were diagnosed with sPE. Women with normal pregnancies were matched with those with sPE according to age and gestational age. The demographics of the patients in these two groups are shown in Table 1. All patients gave informed consent prior to inclusion in this study.

RNA isolation and RNA-sequencing (RNA-seq)

The collected placental tissues were stored in cryotubes containing liquid nitrogen. Total RNA was isolated from the placental tissues of women with sPE and normal pregnancies (normal and sPE groups) using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) following the standard protocol. The quality of the isolated total RNA was determined using an Agilent 2100 Bioanalyzer pico-

RNA chip (Agilent Technologies, Santa Clara, CA, USA). To prepare the RNA-seq library, ribosomal RNA was separated from total RNA using the Epicentre Ribo-zero™ rRNA Removal Kit (Epicentre, Madison, WI, USA). Then, a library was constructed using the RNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA) according to the manufacturer's instructions. After quantification of the library of each group with the Agilent 2100 Bioanalyzer (Agilent Technologies), RNA-seq was performed using the HiSeq 2000 platform (Illumina, Inc., San Diego, CA, USA).

Validation of back-splice junctions of circRNAs

Total RNA was reverse transcribed into complementary DNA (cDNA) using PrimeScript RT Master Mix (TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China) by polymerase chain reaction (PCR) with random primers. Convergent primers were designed for linear mRNA production while divergent primers targeting the predicted backspliced region were designed for the circular form. cDNA was amplified using convergent and divergent primers, respectively, to confirm whether the circRNA was a transcription product of pre-mRNA. Genomic DNA was isolated using the QIAamp® DNA Mini Kit (Qiagen France SAS, Les Ulis, France). The fragments amplified by PCR were separated by 1% agarose gel electrophoresis and confirmed by Sanger sequencing. The primers of hsa_circ_0001438, hsa_circ_0001326, and hsa_circ_32340 for circular form identification are listed in Table 2.

Quantitative real-time PCR (qPCR)

To quantify the expression of the circRNAs, cDNA was amplified using the SYBR Real-time PCR Master Mix kit (Toyobo Co., Ltd., Osaka, Japan) with divergent primers. The amplification reaction was performed using an ABI 7500 real-time PCR system (Applied Biosystems, Carlsbad, CA, USA). The relative expression profiles of circRNAs were determined using the $2^{-\Delta\Delta Ct}$ method.

Bioinformatics analysis

Reads sequenced with the HiSeq 2000 platform were used for circRNA prediction, circRNA expression, and mRNA expression profile analysis. Clean reads were aligned to the human reference genome GRCh37/hg19 using Burrows-Wheeler Alignment Tool 0.7.13 followed by CIRI software for circRNA prediction and annotation (<https://omictools.com/ciri-tool>). The mRNA reads were mapped to GRCh37/hg19 genome using TopHat v2.1.0 (<https://ccb.jhu.edu/software/tophat/index.shtml>). CircRNAs and mRNAs that meet the criteria of \log_2 fold change, as calculated with PE/Normal > 1 and an adjusted probability (p) value of < 0.005 , were identified as differentially expressed. The fold change of each circRNA/mRNA was calculated based on the “reads per kilobase per million mapped

Table 1
Patient demographics.

Characteristics	Pre-eclampsia	Normal control	<i>p</i> value
case number	30	30	–
Age (years)	30.87 \pm 5.49	31.72 \pm 5.3	0.562
Systolic pressure (mmHg)	144 \pm 18.12	114.03 \pm 8.92	< 0.0001
Diastolic pressure (mmHg)	85.93 \pm 12.95	74.8 \pm 8.04	< 0.0001
PLT	194.39 \pm 63.42	221.47 \pm 58.85	0.089
Gestational week at delivery	36.42 \pm 2.17	39.09 \pm 0.55	< 0.001
Gestational week at diagnosis of preeclampsia	33.52 \pm 1.86	–	–
Neonatal Apgar score	8.88 \pm 0.65	10	< 0.0001
Neonatal weight (Kg)	2.74 \pm 0.83	3.25 \pm 0.29	0.0021

Table 2
Primer sequences.

Primer name	Primer sequence
hsa_circ_0001438 convergent	ATTGGCCAACACCAAGTGAA; GCCTCATCCTCACTGACGTTT
hsa_circ_0001438 divergent	ACAGCAAAGAAAACCGGAAAC; TTTGTCTTGAGCTTTCCTGCCT
hsa_circ_0001326 convergent	AGTCTGTGTTTGAGGAAGCCC; GTGCTCTGGGGGTAAAGAA
hsa_circ_0001326 divergent	ACCGGAAAATAGCTGAAGTGG; GGTGGTCCCAGTAGATAAGCG
hsa_circ_32340 convergent	GAATTCACACCAACACCGGC; ATCAAGAAGTCCCCGGTCTCT
hsa_circ_32340 divergent	GACTTCTTGATTGAGTACTGC; TCITCAATGGGTGTGTCAGC
Hsa GAPDH convergent	GAGTCAACGGATTGGTCGT; GACAAGCTTCCCCTTCTCAG
Hsa GAPDH divergent	TCCTCACAGTTGCCATGTAGACCC; TCGGGGTCAATTTATAGAAACCGGG

reads" algorithm. The *p* values were adjusted using Benjamini and Hochberg's approach to control the false discovery rate.

An unsupervised clustering heat map and Volcano plot were created using R package (<https://cran.r-project.org/web/packages/pheatmap/>) to obtain an overview of the expression profiles of circRNAs and mRNAs. The Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (<http://www.genome.jp/kegg/>) database (adjusted *p* value <0.05; gene count ≥2) analysis were conducted to provide clues into the functions of these differentially expressed circRNAs. The analysis of the interactions among circRNAs, miRNAs, and mRNAs were based on the complementary pairing principle. The interactions among the circRNAs and miRNAs were predicted using the MiRanda algorithm (<https://omictools.com/miranda-tool>). The interactions between the miRNAs and mRNAs were predicted using the miRTarBase database (<https://bio.tools/mirtarbase>). miRNAs that target to the differentially expressed circRNAs and mRNAs were predicted by R package and the common targets shared by circRNAs and mRNAs were used to construct a circRNA-miRNA-mRNA interaction network and visualized with Cytoscape-V2.8.3 (<https://www.innatedb.ca/cytoscape-v2.8.3/plugins/>).

Statistical analysis

All data analysis were conducted using SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA). The relative expression levels of circRNAs in the sPE group were compared with those in the normal group using the *t*-test. A *p* value of <0.05 was considered statistically significant.

Results

Expression profiles of circRNAs and mRNAs in the placental tissues of sPE patients

RNA-seq was performed for the sPE and normal control groups. Most of the circRNA reads were concentrated within 700 nt, with the largest proportion within 250 nt (Fig. 1D). A total of 49 circRNA were differentially expressed in sPE patients, of which 29 (59%) were upregulated and 20 (41%) were downregulated. A clustering heat map showed that the differentially expressed circRNAs of the sPE group had different expression patterns from those of the normal control group (Fig. 1A). The Volcano Plot map showed that there were no significant differences in the expression patterns of most of the circRNAs between the sPE group and normal control group (black dots), although some of the circRNAs were downregulated (green dots), while others were upregulated (red dots) (Fig. 1C). Analysis of the genomic distributions of the differentially expressed circRNAs showed that the upregulated circRNAs were distributed other than on chromosomes 6, 16, 18, 21, and 22, while the downregulated circRNAs were mainly distributed on

chromosomes 1–5, 8, 10, 12, 14–17, and 19–21 (Fig. 1E). Most of these differentially expressed circRNAs were distributed in exons, while relatively few were distributed in introns. In the intergenic regions, almost no circRNAs were distributed in gene intervals (Fig. 1F).

In response to changes in the expression profiles of circRNAs, differences in mRNA profiles between the sPE and normal groups were investigated in order to identify mRNAs with potential regulatory relationships. As shown in Fig. 1B, there were differences in the expression profiles of many mRNAs, as 251 were upregulated and 1003 were downregulated in the sPE group. The mRNA expression profiles differed between the groups.

GO and KEGG analysis

GO and KEGG analysis were conducted to predict the potential functions of the differentially expressed circRNAs and mRNAs in the sPE group. As shown in Fig. 2, the differentially expressed circRNAs and mRNAs fell into the categorical terms of biological processes, cellular components, and molecular functional blocks, with the greatest enrichment in terms of biological processes (Fig. 2A and B). KEGG pathway enrichment analysis revealed that the differentially expressed circRNAs were mainly enriched in ubiquitin-mediated proteolysis, the TNF signaling pathway, the MAPK signaling pathway, human T-lymphotropic virus (HTLV-1) infection, apoptosis, Chagas disease, the NF-κB signaling pathway, and the RIG-I-like receptor signaling pathway. Of these, most circRNAs were associated with the MAPK signaling pathway (Fig. 2C). The differentially expressed mRNAs were mainly enriched in cancer pathways, the PI3K-Akt signaling pathway, proteoglycans in cancer, the cAMP signaling pathway, and the spliceosome pathway, with most associated with cancer pathways (Fig. 2D).

Hsa_circ_0001438, hsa_circ_0001326, and hsa_circ_32340 were upregulated in the placental tissues of sPE patients

To identify the backsplicing of circRNAs predicted by the bio-informatic pipeline, five circRNAs (i.e., hsa_circ_0000239, hsa_circ_0001326, hsa_circ_32340, hsa_circ_0001438, and hsa_circ_0001801) were selected for qPCR analysis. Both divergent and convergent primers were designed to amplify fragments containing backsplicing regions or linear mRNAs. As shown in Fig. 3, with the divergent primers, fragments containing the backsplicing regions of these five candidate circRNAs were amplified from the cDNA sample, while the products were absent in the genomic DNA (gDNA) samples. In contrast to the divergent primers, product amplified by the convergent primers were present in both the cDNA and gDNA samples (Fig. 3A), indicating the products amplified by divergent primers were derived from cDNA only. Furthermore, Sanger sequencing confirmed that the backsplicing junctions were consistent with the predicted sites (Fig. 3B, D, and F).

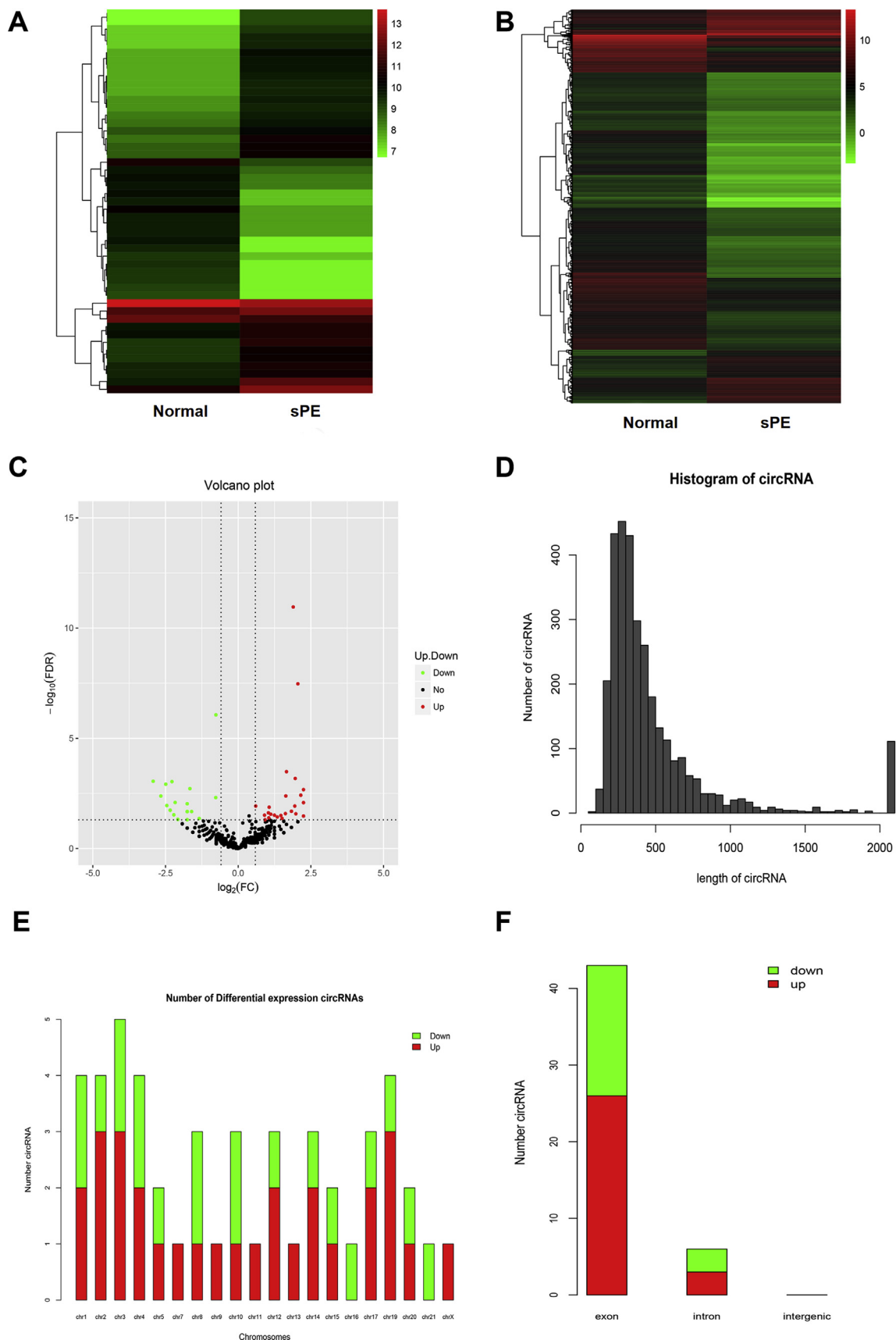
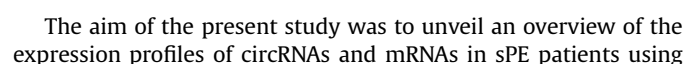


Fig. 1. The expression profiles of circRNAs and mRNAs in the placental tissues of sPE patients. (A) An unsupervised clustering heat map showing differentially expressed circRNAs generated with RNA-seq in the placental tissues of normal control (Normal) and sPE patients (sPE). (B) An unsupervised clustering heat map displaying the overview of the differentially expressed mRNAs in the placental tissues of the normal control group vs. sPE patients, as determined by RNA-seq analysis. (C) A Volcano Plot diagram showing an overview of the proportion of upregulated (red dots) and downregulated (green dots) circRNAs in the placental tissues of sPE patients. The circRNA length (D), chromosome distribution (E), and genomic location distribution (F) of the differentially expressed circRNAs in the placental tissues of sPE patients.



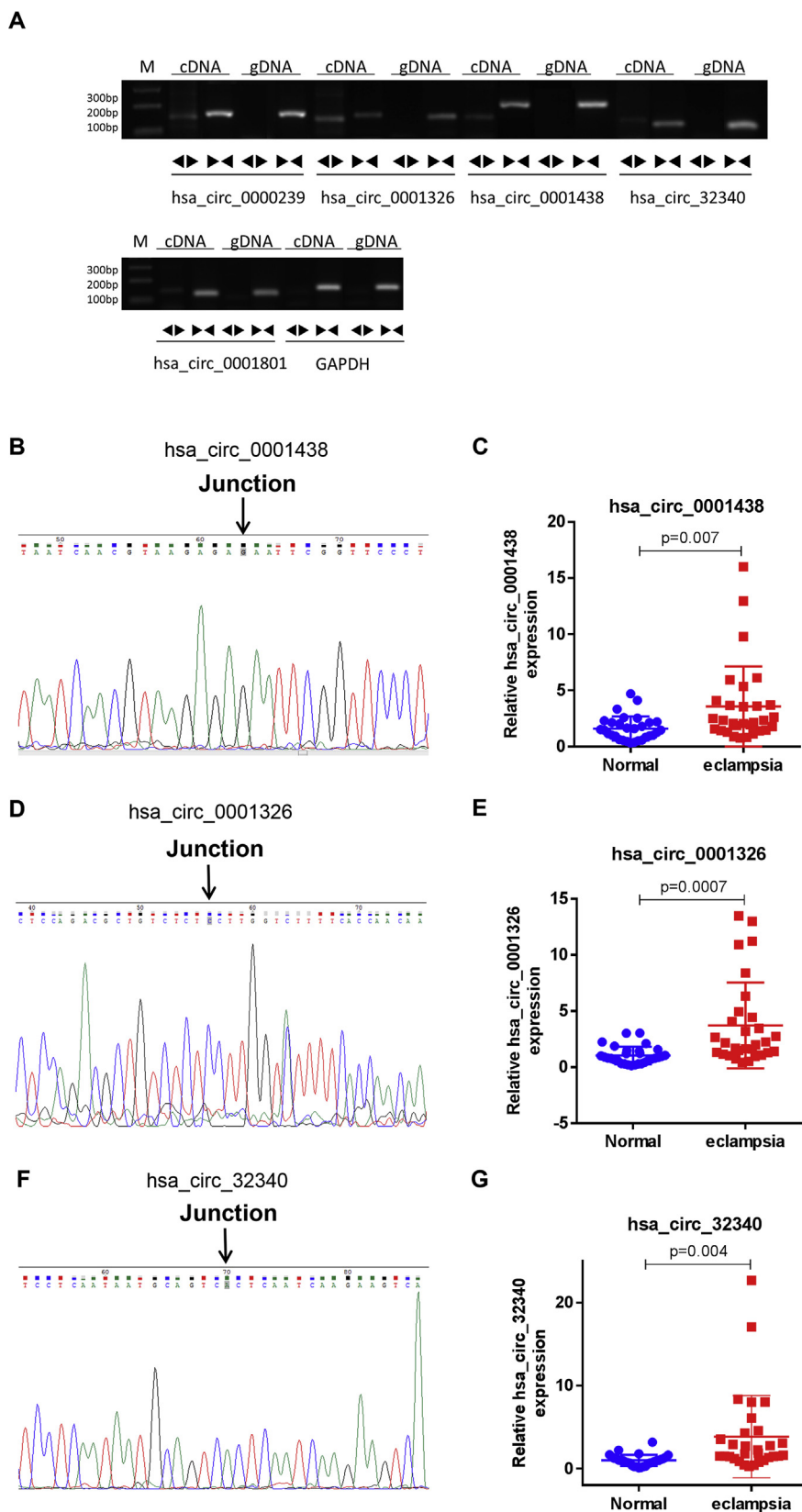


Fig. 3. Validation and expression profiles of circRNAs. (A) The backsplicing sites of hsa_circ_0001438, hsa_circ_0001326, and hsa_circ_32340 were confirmed by qPCR. The backsplicing sites of hsa_circ_0001438 (B), hsa_circ_0001326 (C), and hsa_circ_32340 (D) were validated by Sanger sequencing. (C, D, and E) The circRNAs hsa_circ_0001438, hsa_circ_0001326, and hsa_circ_32340 were differentially expressed in the placental tissues of sPE patients, as determined by qPCR analysis.

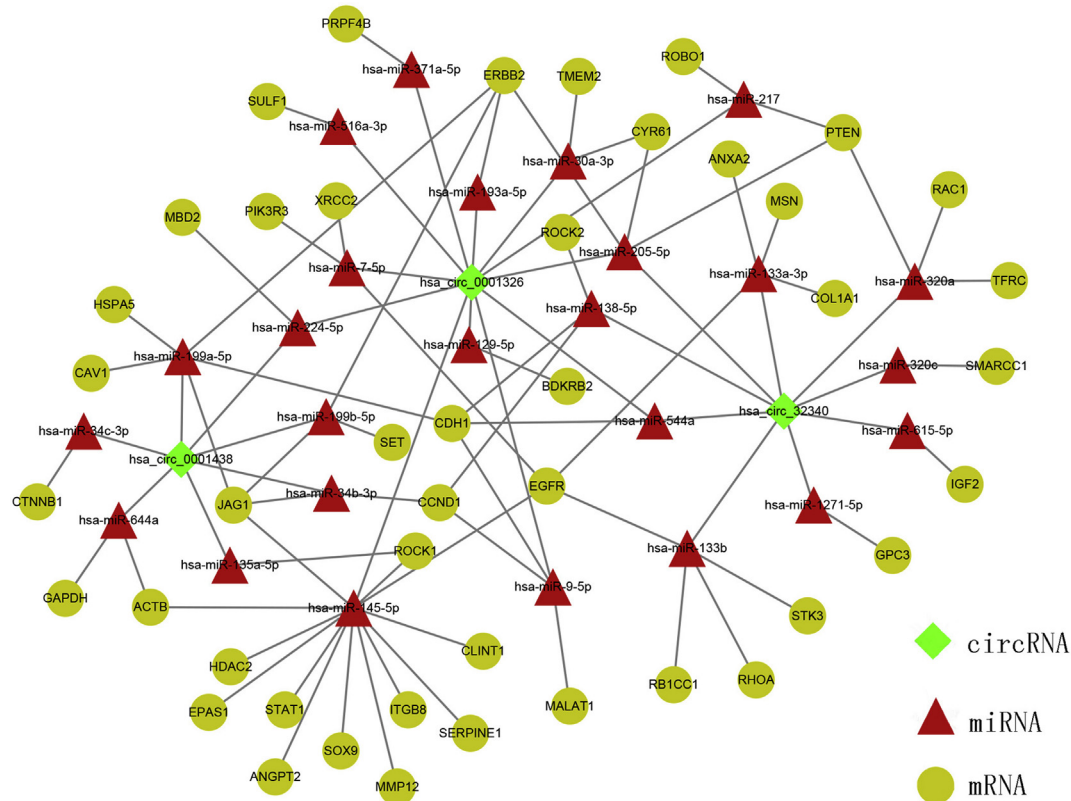


Fig. 4. The circRNA-miRNA-mRNA interaction network constructed with cytoscape. The network consists of three circRNAs (i.e., hsa_circ_0001438, hsa_circ_0001326, and hsa_circ_32340) (green nodes), miRNAs (red), and their target genes (yellow).

RNA-seq analysis. The 29 upregulated and 20 downregulated circRNAs were enriched in the GO terms of biological processes and pathways of ubiquitin-mediated proteolysis, the TNF signaling pathway, the MAPK signaling pathway, HTLV-1 infection, apoptosis, Chagas disease, the NF- κ B signaling pathway, and the RIG-1-like receptor signaling pathway. Further analysis revealed that the differentially expressed mRNAs were primarily enriched in cancer pathways, the PI3K-Akt signaling pathway, proteoglycans in cancer, the cAMP signaling pathway, and spliceosome pathway. In particular, the results confirmed the upregulation of hsa_circ_0001438, hsa_circ_0001326, and hsa_circ_32340 in sPE patients for the first time, and predicted a theoretical circRNA-miRNA-mRNA interaction network.

We confirmed that the expression levels of hsa_circ_0001438 (derived from LARP1B), hsa_circ_0001326 (PHLDB2), and hsa_circ_32340 (SHANK2) were upregulated in the placental tissues of sPE patients. In previous reports, none of the three circRNAs had been validated with no evidence of functions in human diseases. However, a previous study indicated that the parental genes were involved in placental growth and development, which may be linked to complications during pregnancy and/or abnormal infant development. As a member of the LARP family, LARP1B plays an important role in transcription and/or mRNA translation, as well as the promotion of cellular proliferation, migration, and invasion [16]. In macrosomia, placental expression of PHLDB2 is upregulated [17], which is associated with growth abnormalities of the infant. Differentially methylated CG-cites of PHLDB2 have been identified in neonatal cord blood and are predicted to be included in the single nucleotide polymorphism networks of placental abruption [18]. In addition, SHANK2 in the placenta is associated with infant neurobehavioral development [19]. Since the circRNAs were the product of backsplicing of pre-mRNAs, their function may be

closely related with the functions of the parental genes. Indeed, a previous study revealed that circZKSCAN1 and its linear isoform ZKSCAN1 mRNA were downregulated and both function in hepatocellular carcinoma growth and metastasis inhibition [20].

circRNAs are a type of endogenous RNAs that regulate gene expression by functioning as miRNA sponges. An extreme case of a miRNA sponge is CDR1as (also termed ciRS-7), which contains more than 70 conventional binding sites for miR-7 [21]. The binding of CDR1as to miR-7 results in relief of the decrease in miR-7-regulated mRNAs, such as CCNE1, PIK3CD, p70S6K, and Myrip [22–24]. This regulatory relationship is also known as a competitive endogenous RNA (ceRNA) mechanism. In the present study, RNA-seq was conducted in order to obtain an overview of the expression profiles of potential competitive mRNAs of circRNAs. Theoretically, the differentially expressed circRNAs may cluster in pathways that at least partially cover differentially expressed mRNAs. Interesting, the global expression patterns of differentially expressed circRNAs and mRNAs are not stringently parallel. The most enriched pathway of the circRNAs was the MAPK signaling pathway, while the most enriched pathway of the differentially expressed mRNAs was the cancer pathway. We supposed that this non-parallel change between circRNAs and mRNAs may due to the complicated regulatory networks in the human body. The expression profiles of numerous molecules, including those observed in the current study, had changed during sPE. However, the ceRNA mechanism is only one of the possible mechanisms that affect disease outcome, as other possible pathways involving peptides and proteins that are directly produced by circRNAs [25] may also contribute to cell fate and sPE progression.

Despite the differences in the primary functional enriched pathways between the group of circRNAs and mRNAs, the functions of the identified signaling pathways were all associated with PE. For

instance, invasion and differentiation of trophoblast cells are hallmark biological events during placental development [26]. The MAPK signaling pathway is reported to function in the regulation of trophoblast invasion, a key event in the pathogenesis of PE [27]. Pathways in cancer, such as apoptosis, hypoxia, and the HIF pathway have been suggested to contribute to placental differentiation [28–30]. Therefore, our findings support the idea that multiple pathways contribute to the etiology of PE [31].

Though we were unable to identify all pathways that may contribute to sPE development, we generated a circRNA-miRNA-mRNA interaction network that outlined the ceRNA mechanism underlying the upregulation of hsa_circ_0001438, hsa_circ_0001326, and hsa_circ_32340. Within the proposed network, several miRNAs and mRNAs were involved in certain pathophysiological processes when separately analyzed. In the network, the miR-145-5p subset was the largest, containing 13 mRNAs, including ROCK1, MMP12, and JAG1, which are involved in oxidative stress in trophoblasts, placental development, and endovascular remodeling [32–34] during PE. Especially, evidence shows that miR-145-5p is involved in the regulation of trophoblast invasion via down-regulation of Cyr61 [35]. In the present study, miR-145-5p and Cyr61 were included the circRNA-miRNA-mRNA interaction network. Hence, it is reasonable to suggest that these three circRNAs are involved in PE pathogenesis.

Conclusion

In summary, we characterized the global profiling of circRNAs in sPE patients by RNA-seq analysis. Furthermore, we identified three circRNAs that were upregulated in sPE and predicted a regulatory network involving trophoblast infiltration, placental development, and endovascular remodeling. It will be of great importance to validate the function of circRNAs in PE in the future. The upregulated circRNAs could function as biomarkers for the diagnosis of sPE.

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Conflicts of interest

The authors report no conflict of interest.

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References

[1] Mol BWJ, Roberts CT, Thangaratnam S, Magee LA, de Groot CJM, Hofmeyr GJ. Pre-eclampsia. *Lancet* (Lond, Engl) 2016;387(10022):999–1011.

[2] Ahmed R, Dunford J, Mehran R, Robson S, Kunadian V. Pre-eclampsia and future cardiovascular risk among women: a review. *J Am Coll Cardiol* 2014;63(18):1815–22.

[3] Pridjian G. Severe preeclampsia. *Curr Wom Health Rev* 2011;7(2):112–24.

[4] Brkic J, Dunk C, O'Brien J, Fu G, Nadeem L, Wang YL, et al. MicroRNA-218-5p promotes endovascular trophoblast differentiation and spiral artery remodeling. *Mol Ther J Am Soc Gene Ther* 2018;26(9):2189–205.

[5] Bell MJ. A historical overview of preeclampsia-eclampsia. *J Obstet Gynecol Neonatal Nurs JGNN* 2010;39(5):510–8.

[6] Zhao ZJ, Shen J. Circular RNA participates in the carcinogenesis and the malignant behavior of cancer. *RNA Biol* 2017;14(5):514–21.

[7] Xuan L, Qu L, Zhou H, Wang P, Yu H, Wu T, et al. Circular RNA: a novel biomarker for progressive laryngeal cancer. *Am J Trans Res* 2016;8(2):932–9.

[8] Zheng Q, Bao C, Guo W, Li S, Chen J, Chen B, et al. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat Commun* 2016;7:11215.

[9] Salzman J, Gawad C, Wang PL, Lacayo N, Brown PO. Circular RNAs are the predominant transcript isoform from hundreds of human genes in diverse cell types. *PLoS One* 2012;7(2):e30733.

[10] Li Y, Zheng Q, Bao C, Li S, Guo W, Zhao J, et al. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. *Cell Res* 2015;25(8):981–4.

[11] Qian Y, Lu Y, Rui C, Qian Y, Cai M, Jia R. Potential significance of circular RNA in human placental tissue for patients with preeclampsia. *Cell Physiol Biochem – Int J Exp Cell Physiol Biochem Pharmacol* 2016;39(4):1380–90.

[12] Zhang YG, Yang HL, Long Y, Li WL. Circular RNA in blood corpuscles combined with plasma protein factor for early prediction of pre-eclampsia. *BJOG An Int J Obstet Gynaecol* 2016;123(13):2113–8.

[13] Hu X, Ao J, Li X, Zhang H, Wu J, Cheng W. Competing endogenous RNA expression profiling in pre-eclampsia identifies hsa_circ_0036877 as a potential novel blood biomarker for early pre-eclampsia. *Clin Epigenet* 2018;10:48.

[14] Bai Y, Rao H, Chen W, Luo X, Tong C, Qi H. Profiles of circular RNAs in human placenta and their potential roles related to preeclampsia. *Biol Reprod* 2018;98(5):705–12.

[15] Brown MA, Magee LA, Kenny LC, Karumanchi SA, McCarthy FP, Saito S, et al. The hypertensive disorders of pregnancy: ISSHP classification, diagnosis & management recommendations for international practice. *Pregn Hypertens* 2018;13:291–310.

[16] Ye L, Lin ST, Mi YS, Liu Y, Ma Y, Sun HM, et al. Overexpression of LARP1 predicts poor prognosis of colorectal cancer and is expected to be a potential therapeutic target. *Tum Biol J Int Soc Oncodevelopment Biol Med* 2016;37(11):14585–94.

[17] Sabri A, Lai D, D'Silva A, Seeho S, Kaur J, Ng C, et al. Differential placental gene expression in term pregnancies affected by fetal growth restriction and macrosomia. *Fetal Diagn Ther* 2014;36(2):173–80.

[18] Workalemahu T, Enquobahrie DA, Moore A, Sanchez SE, Ananth CV, Pacora PN, et al. Genome-wide and candidate gene association studies of placental abruption. *Int J Molec Epidemiol Genet* 2013;4(3):128–39.

[19] Green BB, Kappil M, Lambertini L, Armstrong DA, Guerin DJ, Sharp AJ, et al. Expression of imprinted genes in placenta is associated with infant neuro-behavioral development. *Epigenetics* 2015;10(9):834–41.

[20] Yao Z, Luo J, Hu K, Lin J, Huang H, Wang Q, et al. ZKSCAN1 gene and its related circular RNA (circZKSCAN1) both inhibit hepatocellular carcinoma cell growth, migration, and invasion but through different signaling pathways. *Molec Oncol* 2017;11(4):422–37.

[21] Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, et al. Natural RNA circles function as efficient microRNA sponges. *Nature* 2013;495(7441):384–8.

[22] Xu H, Guo S, Li W, Yu P. The circular RNA Cdr1as, via miR-7 and its targets, regulates insulin transcription and secretion in islet cells. *Sci Rep* 2015;5:12453.

[23] Xu L, Zhang M, Zheng X, Yi P, Lan C, Xu M. The circular RNA ciRS-7 (Cdr1as) acts as a risk factor of hepatic microvascular invasion in hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2017;143(1):17–27.

[24] Yu L, Gong X, Sun L, Zhou Q, Lu B, Zhu L. The circular RNA Cdr1as act as an oncogene in hepatocellular carcinoma through targeting miR-7 expression. *PLoS One* 2016;11(7):e0158347.

[25] Granados-Riveron JT, Aquino-Jarquín G. The complexity of the translation ability of circRNAs. *Biochim Biophys Acta* 2016;1859(10):1245–51.

[26] Massimiani M, Salvi S, Piccirilli D, Vecchione L, Moresi S, Ferrazzani S, et al. A4. EGFL7 in placenta trophoblast and endothelial cells: implications in the pathogenesis of pre-eclampsia. *J Matern Fetal Med* 2016;29(Suppl. 2):4–4.

[27] Liu X, Deng Q, Luo X, Chen Y, Shan N, Qi H. Oxidative stress-induced Gadd45alpha inhibits trophoblast invasion and increases sFlt1/sEng secretions via p38 MAPK involving in the pathology of pre-eclampsia. *J Matern Fetal Neonatal Med – Offic J Eur Assoc Perinat Med Federat Asia Ocean Perinat Soc Int Soc Perinat Obstet* 2016;29(23):3776–85.

[28] Ding GC, Chen M, Wang YX, Rui C, Xu W, Ding HJ, et al. MicroRNA-128a-induced apoptosis in HTR-8/SVneo trophoblast cells contributes to pre-eclampsia. *Biomed Pharmacother Biomed Pharmacother* 2016;81:63–70.

[29] Fu J, Zhao L, Wang L, Zhu X. Expression of markers of endoplasmic reticulum stress-induced apoptosis in the placenta of women with early and late onset severe pre-eclampsia. *Taiwan J Obstet Gynecol* 2015;54(1):19–23.

[30] Macklin PS, McAuliffe J, Pugh CW, Yamamoto A. Hypoxia and HIF pathway in cancer and the placenta. *Placenta* 2017;56:8–13.

- [31] Brew O, Sullivan MH, Woodman A. Comparison of normal and pre-eclamptic placental gene expression: a systematic review with meta-analysis. *PLoS One* 2016;11(8):e0161504.
- [32] Xu J, Jia X, Gu Y, Lewis DF, Gu X, Wang Y. Vitamin D reduces oxidative stress-induced procaspase-3/ROCK1 activation and MP release by placental trophoblasts. *J Clin Endocrinol Metab* 2017;102(6):2100–10.
- [33] Han J, Yang BP, Li YL, Li HM, Zheng XH, Yu LL, et al. RhoB/ROCK mediates oxygen-glucose deprivation-stimulated syncytiotrophoblast microparticle shedding in preeclampsia. *Cell Tissue Res* 2016;366(2):411–25.
- [34] Fragkiadaki P, Soulitzis N, Sifakis S, Koutroulakis D, Gourvas V, Vrachnis N, et al. Downregulation of notch signaling pathway in late preterm and term placentas from pregnancies complicated by preeclampsia. *PLoS One* 2015;10(5):e0126163.
- [35] Wen Z, Chen Y, Long Y, Yu J, Li M. Tumor necrosis factor-alpha suppresses the invasion of HTR-8/SVneo trophoblast cells through microRNA-145-5p-mediated downregulation of Cyr61. *Life Sci* 2018;209:132–9.