

Expression profile of circular RNAs in the peripheral blood of neonates with hypoxic-ischemic encephalopathy

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Abstract. Circular RNAs (circRNAs) are a class of non-coding RNAs that participate in various biological processes. However, the function of circRNAs in neonatal hypoxic-ischemic encephalopathy (HIE) is not fully understood. In the present study, the differentially expressed circRNAs in the peripheral blood of neonates with HIE and control samples were characterized by a microarray assay. A total of 456 circRNAs were significantly differentially expressed in the peripheral blood of neonates with HIE, with 250 upregulated and 206 downregulated circRNAs in HIE compared with the control samples. Reverse transcription-quantitative PCR was used to investigate specific circRNAs. Gene Ontology, and Kyoto Encyclopedia of Genes and Genomes pathway analyses were used to determine the function of the parent genes of the dysregulated circRNAs. In addition, microRNAs that may be associated with specific circRNAs were predicted using miRanda. Collectively, the present results indicated the potential importance of circRNAs in the peripheral blood of neonates with HIE.

Introduction

Neonatal hypoxic-ischemic encephalopathy (HIE) is mainly triggered by perinatal asphyxia, leading to neural tissue damage caused by deprivation of oxygen and glucose (1,2). The

pathophysiology of HIE is complex and may be related to complications in the neonate, mother or placenta (3). Cerebral hypoxic ischemia causes a strong neuroinflammatory response, which results in delayed cell death. Neonatal HIE can cause mortality or major disability, as well as cerebral palsy, neuromotor and cognitive delays, growth restriction and epilepsy (4-8).

Hypothermic therapy is an effective treatment for moderate and severe HIE by improving the neurological function of survivors (8). Effective hypothermia therapy can enhance neural repair, which is thought to improve neurological outcomes (9). However, current examinations of neonatal HIE have limitations and different levels of effectiveness, as diagnosis largely depends on clinical judgement and instrumental examination (10). Therefore, effective methods to define the degree of insult of HIE are required urgently.

Circular RNAs (circRNAs) are a class of non-coding RNAs that are produced from precursor mRNA back-splicing of exons (11). The downstream 5' splice site and upstream 3' splice site of circRNA are ligated by a 3'-5' phosphodiester bond at the junction site (11,12). Previous studies have shown that circRNAs play essential roles in neuronal function, cell proliferation and immune responses (13-15). Furthermore, circRNAs are usually stable both in extracellular and intracellular environments (16,17). However, the expression profiles and functions of circRNAs in neonatal HIE have not been previously reported.

In the present study, the expression profile of circRNAs in three neonatal HIE samples and control samples were detected by microarray analysis. Additionally, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses results suggested that several processes were enriched in the HIE group compared with the control group, and are related to the cellular processes, immune system, signal transduction and cellular community. Thus, the present results may provide a novel insight into the progress of neonatal HIE.

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Materials and methods

Sample collection. Samples were collected from Jingjiang People's Hospital from Aug. 2017 to May, 2018. Peripheral

blood samples (6 ml) from ten neonates with HIE and ten control infants without HIE were collected. The clinical parameters of neonatal patients and controls are listed in Table I. A diagnosis of HIE was confirmed by routine examination; An Apgar score of 2-6 for 1 min and <8 after first 5 min after birth indicates HIE (18) (Table I). PAXgene Blood RNA tubes (Qiagen GmbH) with 4 ml RNA protect reagent were used for collection of peripheral blood samples. All HIE samples were collected prior to hypothermia therapy. The present study was approved by the Medical Ethics Committee of Jingjiang People's Hospital in China [approval no. (2017)25].

Total RNA extraction and purification. Total RNA was extracted and purified from the whole blood sample using the PAXgene Blood RNA kit (Qiagen, Inc.) following the manufacturer's instructions. Total RNA was then checked for RNA integrity using an Agilent Bioanalyzer 2100 (Agilent Technologies, Inc.).

Microarray analysis. Microarray was performed using an Agilent microarray platform (Shanghai Biotechnology Co., Ltd.), according to the manufacturer's protocols. Total RNA was amplified and labeled using a Low Input Quick Amp Labeling kit, One-Color (Agilent Technologies, Inc.), following the manufacturer's instructions. Then, the labeled cRNA were purified using a RNeasy mini kit (Qiagen, Inc.). Each slide was hybridized with 1.65 μ g Cy3-labeled cRNA using a Gene Expression Hybridization kit (Agilent Technologies, Inc.) in a hybridization oven at 65°C for 17 h (Agilent Technologies, Inc.), according to the manufacturer's protocol. After 17 h of hybridization, the slides were washed in staining dishes (Thermo Shandon; Thermo Fisher Scientific, Inc.) with Gene Expression Wash Buffer kit (Agilent Technologies, Inc.), following the manufacturer's instructions. Then, slides were scanned by an Agilent Microarray scanner (Agilent Technologies, Inc.) with the following default settings: Dye channel, green; Scan resolution=3 μ m; photomultiplier tube 100%, 20 bit. Data were extracted using Feature Extraction software 10.7 (Agilent Technologies, Inc.). Raw data were normalized using a Quantile algorithm, according to the manufacturer's instructions provided by the microarray.

RNA-sequence analysis of HIF-1 α . The microarray data discussed in the present study, with regards to the expression of HIF-1 α , have been deposited in the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database. The GEO accession number is GSE121178.

GO and KEGG pathway analysis. The GO and KEGG pathway was predicted using Amigo 2 (<http://amigo.geneontology.org/>; V2.5.12) and GenomeNet website (<http://www.genome.ad.jp/kegg/>).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted as described above. RT-qPCR was performed using HiScript II Q RT SuperMix for qPCR kit (Vazyme Biotech Co., Ltd.) and SYBR Green (Roche Diagnostics) method following the manufacturer's protocol. To investigate the results of the microarray analysis, the primers of

Table I. Population demographics.

Characteristic	HIE (n=10)	Control (n=10)
Age, day	1 (1-2)	5 (2-10)
Sex, M/F	6/4	4/6
1 min Apgar	3 (2-6)	10 (9-10)
5 min Apgar	7 (6-8)	10 (10-10)
Cord pH	7.34 (7.245-7.442)	-

Data in the brackets represent the minimum and maximum of the medical index. M, male; F, female; HIE, Hypoxic Ischemic Encephalopathy. HIE group included six moderate HIE and four severe HIE cases.

circRNA were designed by CircPrimer software (<http://www.bioinf.com.cn>; V1.2), and the sequences of primers used are listed in Table II. RT-qPCR was performed using an Applied Biosystems ViiA 7 Dx system (Thermo Fisher Scientific, Inc.) with SYBR Green (Roche Diagnostics). The qPCR conditions were as follows: Initial denaturation at 50°C for 2 min, 95°C for 10 min, followed by 40 cycle at 95°C for 15 sec and 60°C for 30 sec. The expression of circRNA was normalized to the 18S ribosomal RNA, using the 2^{- $\Delta\Delta$ C_q} method (19).

circRNA and microRNA (miRNA) interaction prediction. The circRNA and microRNA interaction was predicted using miRanda (<http://www.microrna.org/>; V21.0). The network map of circRNA and microRNA was drawn using Cytoscape (<https://cytoscape.org/>; V3.7.2).

Statistical analysis. Data were analyzed using the SPSS 20.0 software package (SPSS, Inc.) with an independent-sample t-test for comparisons between the HIE and control group. All experiments were repeated three times. Data are presented as the mean \pm SD. P<0.05 was considered to indicate a statistically significant difference.

Results

circRNA microarray profiling. Peripheral blood samples were collected from ten neonates with HIE and ten infants without HIE. The 10 HIE samples included samples from six infants with moderate HIE and four with severe HIE. Demographic data of the HIE group and non-HIE groups are shown in Table I. To detect the differentially expressed circRNAs in HIE, microarray profiling was performed on three randomly selected HIE samples and controls. A total of 88,750 circRNAs were detected, with 456 circRNAs found to be differentially expressed between the HIE samples and control samples. Hierarchical clustering showed the total number of differentially expressed circRNAs among the samples (Fig. 1A). A scatter plot was used to visualize the circRNAs with a fold change of >2.0. The red and green lines are the fold change lines for the differentially expressed circRNAs (Fig. 1B). Furthermore, a volcano plot was used to visualize the differentially expressed circRNAs with fold changes, >2.0 and with statistical significance (P<0.05;

Table II. Primer sequences used for reverse transcription-quantitative PCR of circular RNA expression.

CircID	Forward primer (5'→3')	Reverse primer (5'→3')
has_circ_0005537	GGAGAAGACCAGGCAGAAGA	TGGTCATGATTCATCCCAGCT
hsa_circ_0050345	CCTGAGACCAAACCTTACAGCC	ACGTGGCAAGGTAGACAGAT
hsa_circ_0050705	GCCACCACTTTGAGACACTG	TGTAGTCCATCCGAACCCTG
hsa_circ_0069578	TGGCTACTTTGGTTTCTGTCTG	CATCATGGGCTGCCTGTATG
hsa_circ_0070733	TGTGATGATGGCTGGACTGA	CCACTGTGCCTTCAAACCTCA

CircID, circular RNAs identity.

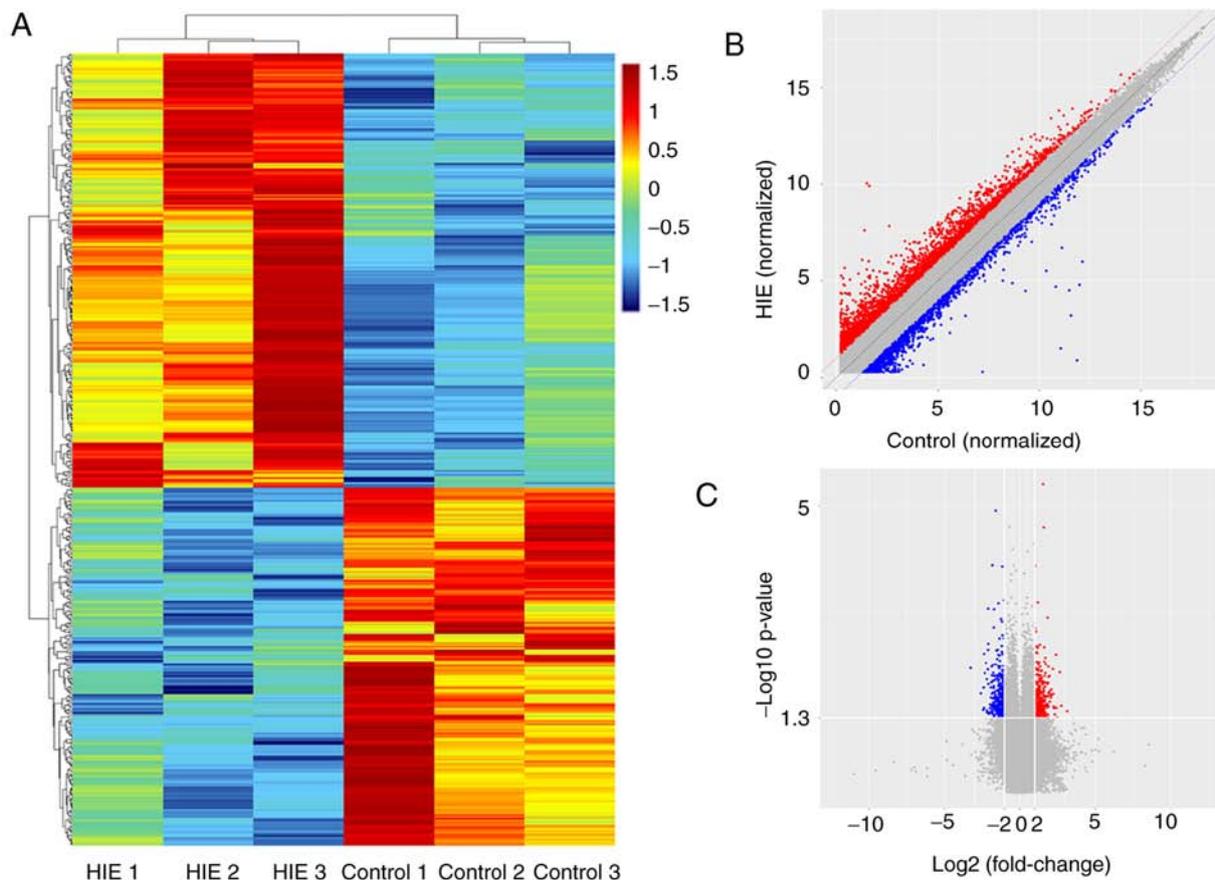


Figure 1. Analysis of differentially expressed circRNAs in HIE and control peripheral blood samples. (A) Heat map of differentially expressed circRNAs in peripheral blood from the HIE group compared with peripheral blood from the controls. (B) Scatter plots used to detect circRNA expression variation between the HIE and control groups. The circRNAs above the top red line and below the bottom green line indicated a >2.0-fold change of circRNAs between the HIE and control samples. (C) Volcano plots of differentially expressed circRNAs between the two samples. The blue and red points in the plot represents the down-regulated and up-regulated expressed circRNAs with statistical significance, respectively. circRNA, circular RNA; HIE, hypoxic-ischemic encephalopathy.

Fig. 1C). The present results suggested that 456 circRNAs, including 250 upregulated and 206 downregulated circRNAs, were differentially expressed in the HIE samples compared with the non-HIE samples. Tables III and IV demonstrated the characteristics of the top 20 upregulated circRNAs and the top 20 downregulated circRNAs, respectively (fold change, ≥ 2 ; $P < 0.05$).

RT-qPCR validation. To further investigate the microarray analysis results, the present study randomly selected five differentially expressed circRNAs in the Tables III and IV,

including three upregulated circRNAs (circRNA_0005537; circRNA_0050345; circRNA_0050705) and two downregulated circRNAs (circRNA_0069578; circRNA_0070733). RT-qPCR analysis was performed on additional HIE samples and non-HIE samples. The present results suggested that the mRNA expression levels of the selected circRNAs were significantly different in the HIE samples compared with the control samples (Fig. 2). Compared with the control samples, the expression levels of circRNA_0005537, circRNA_0050705 and circRNA_0050345 in the HIE samples was 5.21-, 2.32- and 2.59-fold higher, respectively. By contrast, the expression

Table III. Top 20 upregulated circRNAs in the hypoxic ischemic encephalopathy peripheral blood samples compared with normal blood samples.

circRNA	P-value	FC	circRNA_length (bp)	Gene symbol
hsa_circ_0015493	0.007985563	6.32061115	341	<i>QSOX1</i>
hsa_circ_0084605	0.003891836	5.277928402	1661	<i>ASPH</i>
hsa_circ_0080208	0.032305489	4.829873143	6577	<i>GRB10</i>
hsa_circ_0017745	0.031672451	4.771273975	313	<i>DHTKD1</i>
hsa_circ_0085851	0.019112436	4.676079992	1269	<i>TSTA3</i>
hsa_circ_0050345	0.007019217	4.596592582	617	<i>DPY19L3</i>
hsa_circ_0005537	0.019407829	4.111283582	326	<i>CDC25C</i>
hsa_circ_0034212	0.009278198	3.938135751	2362	<i>HERC2</i>
hsa_circ_0014223	0.009610085	3.769487918	523	<i>S100A8</i>
hsa_circ_0088696	0.006352905	3.743285501	718	<i>STXBP1</i>
hsa_circ_0050705	0.049517311	3.618324961	1452	<i>WDR62</i>
hsa_circ_0043217	0.032711809	3.572406618	4544	<i>ACACA</i>
hsa_circ_0080599	0.02837951	3.530978904	522	<i>GTF2IRD1</i>
hsa_circ_0083857	0.012124746	3.307009337	708	<i>GSR</i>
hsa_circ_0033633	0.040119053	3.253147202	255	<i>C14orf80</i>
hsa_circ_0064377	0.014170302	3.212198165	3786	<i>CAND2</i>
hsa_circ_0045827	0.043441186	3.178805121	1976	<i>MXRA7</i>
hsa_circ_0085853	0.024352592	3.178538435	65	<i>TSTA3</i>
hsa_circ_0010211	0.025240307	3.165611241	1788	<i>ARHGEF10L</i>
hsa_circ_0067605	0.009250664	3.16140903	378	<i>GK5</i>

circRNA, circular RNA; FC, Fold change.

Table IV. Top 20 downregulated circRNAs in the hypoxic ischemic encephalopathy peripheral blood samples compared with normal blood samples.

circRNA	P-value	FC	circRNA_length (bp)	Gene symbol
hsa_circ_0051858	0.006718587	9.691469839	1856	<i>TRPM4</i>
hsa_circ_0077755	0.018710732	6.059898228	2896	<i>GJA1</i>
hsa_circ_0012164	0.036270185	5.413530234	662	<i>KIF2C</i>
hsa_circ_0061590	0.014796495	5.306206578	4364	<i>SETD4</i>
hsa_circ_0076770	0.008277684	5.021701979	2765	<i>LOC730101</i>
hsa_circ_0075624	0.010552438	4.49619749	271	<i>SYCP2L</i>
hsa_circ_0069578	0.040281981	4.492267813	617	<i>UCHL1</i>
hsa_circ_0035951	0.003210402	4.217652612	729	<i>DENND4A</i>
hsa_circ_0062272	0.002515151	4.197587878	2975	<i>SEPT5-GP1BB</i>
hsa_circ_0070733	0.042574192	4.072228574	790	<i>PRSS12</i>
hsa_circ_0080184	0.039725995	3.946056497	11042	<i>TNS3</i>
hsa_circ_0056518	0.033688712	3.852150499	126	<i>NCKAP5</i>
hsa_circ_0073280	0.040374199	3.717489627	2346	<i>GPR98</i>
hsa_circ_0009506	0.022064444	3.708353404	117	<i>ACOT7</i>
hsa_circ_0008091	0.009072897	3.543094344	405	<i>NDUFS1</i>
hsa_circ_0025111	0.019091997	3.526641269	241	<i>VWF</i>
hsa_circ_0034711	0.042187121	3.456252673	4006	<i>RPAP1</i>
hsa_circ_0071519	0.038634183	3.451687321	2419	<i>STOX2</i>
hsa_circ_0077888	0.031798526	3.429293915	1469	<i>EYA4</i>
hsa_circ_0062751	0.005900349	3.389322219	2051	<i>GAS2L1</i>

circRNA, circular RNA; FC, Fold change.

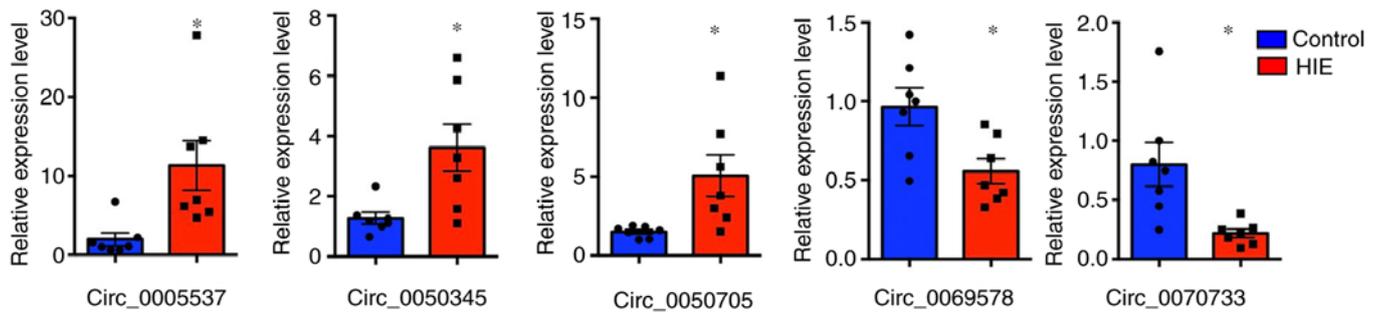


Figure 2. Reverse transcription-quantitative PCR validation. Differential expression of circRNAs in the blood samples between neonatal hypoxic-ischemic encephalopathy and controls. * $P < 0.05$. circRNA, circular RNA.

levels of circRNA_0070733 and circRNA_0069578 in the HIE samples was 3.58- and 1.84-fold lower, respectively, compared with the control samples.

GO and KEGG pathway analysis of host genes of the circRNAs in HIE. Previous studies have shown that circRNAs function by regulating the expression levels of their parent genes at the transcriptional level (20,21). To further identify the function of host genes of the differentially expressed circRNAs in HIE, GO and KEGG pathway analysis of the host genes was performed (Figs. 3 and 4). The GO results demonstrated that the host genes of the differentially expressed circRNAs are primarily involved in cellular processes (22), cell, cell part and binding (Fig. 3A), specifically in regulation of phospholipase C activity, hindbrain morphogenesis and fatty-acyl-CoA biosynthetic process (Fig. 3B). KEGG analysis results indicated that the immune system, signal transduction and cellular community (Fig. 4A), and in particular in the regulation of actin cytoskeleton, focal adhesion and ECM-receptor interaction (Fig. 4B), were related to the host genes of the differentially expressed circRNAs.

Interactions between circRNAs and miRNAs. Previous studies have shown that circRNAs can bind to miRNAs via miRNA response elements (MREs) and function as miRNA sponges (13,23). To investigate the potential miRNAs that may bind to circRNAs, miRanda was used to screen the MREs in the five validated circRNAs. The present results indicated that several miRNAs were associated with specific circRNAs (Table V). A total of 92 miRNAs were identified to potentially bind to circRNA_0050705. It was also found that 16 miRNAs could potentially bind to circRNA_0005537, nine miRNAs could potentially bind to circRNA_0069578, eight miRNAs could potentially bind to circRNA_0070733 and six miRNAs could potentially bind to circRNA_0050345 (Fig. 5).

Discussion

Neonatal HIE is one of the common causes of death or permanent disability, particularly in developing countries (24,25). The crucial role of miRNAs (miRs) and long non-coding RNAs in HIE pathologic processes have been previously demonstrated (19,26). The expression level of miR-374a is significantly downregulated in the umbilical cord blood of

neonatal HIE (26). Furthermore, miR-204 is reported to participate in the pathogenesis of HIE via the regulation of *KLLN* (27).

circRNAs are a novel type of non-coding RNA that stably exist in peripheral blood (28) and play a crucial role in the regulation of numerous pathological or biological processes (29,30). The expression profiles of circular RNAs in neonatal rats following hypoxic-ischemic brain damage (HIBD) has been previously reported (31). Jiang *et al* (31) identified that 66 circRNAs are differentially expressed in the early stages of HIBD. However, to the best of our knowledge, circRNA profiles determined by microarray analysis in the neonatal HIE have not been previously reported. Therefore, the present study investigated differentially expressed circRNAs in the peripheral blood of neonates with HIE and healthy controls using microarray analysis. The microarray expression profiles suggested that 250 upregulated circRNAs and 206 downregulated circRNAs were significantly differentially expressed in patients with HIE. Therefore, the distinct expression profile of circRNAs may participate in the pathogenesis of HIE and provide new biomarkers for HIE diagnosis. To further investigate the microarray data and identify potential clinically applicable biomarkers, the present study assessed five significant differentially expressed circRNAs in neonatal HIE. It was found that circRNA_0005537, circRNA_0050345 and circRNA_0050705 were significantly upregulated in patients with HIE compared with healthy controls, while circRNA_0069578 and circRNA_0070733 were downregulated.

Hypoxia-inducible factor 1 α (HIF-1 α) is a sensitive molecule regulated by oxygen tension and mediates the adaptive response of the cell to a hypoxic environment (32). Zhu *et al* (33) reported that HIF-1 α can regulate BCL2 Interacting Protein 3 (*BNIP3*) by binding to the *BNIP3* promoter in hypoxia-induced neurons, and participates in the process of HIE. The present results suggested that the expression level of *HIF-1 α* was upregulated in HIE group by RNA-seq (fold change, >2; data submitted to GEO; accession no. GSE121178). Previous studies have demonstrated that circRNAs may bind to miRNAs and regulate mRNAs expression (34). The present results indicated that miR-433 may potentially bind to circ_0005537, and that miR-338-3p could potentially bind to circ_0070733. However, microRNA.org was used to predict the miRNAs that could potentially bind to *HIF-1 α* , and found that *HIF-1 α* has miR-433 and miR-338-3p specific binding

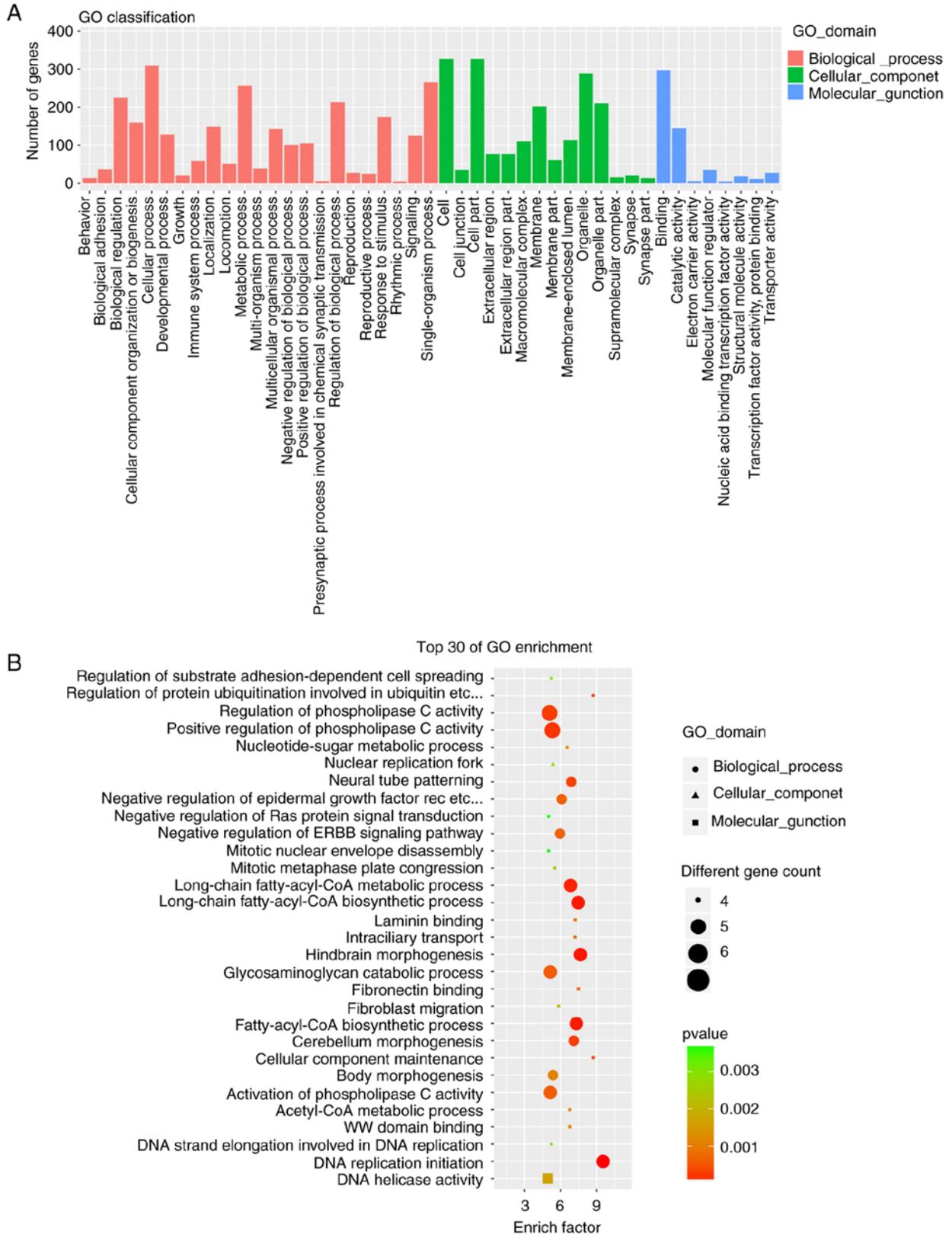


Figure 3. GO terms for host genes of dysregulated circRNAs between neonatal hypoxic-ischemic encephalopathy and control blood samples. (A) GO classification of differentially expressed circRNAs. (B) Top 30 circRNAs of GO enrichment. GO, Gene Ontology; circRNA, circular RNA.

sites (data not shown). Therefore, the present results suggested that dysregulated circ_0005537 and circ_0070733 in patients with HIE may regulate *HIF-1α* expression, and participate in the pathological process of HIE.

circRNA lack crucial elements for cap-dependent translation, however, few endogenous circRNAs have been shown to act as protein templated (35,36). According to the open reading frame prediction and functional internal ribosome

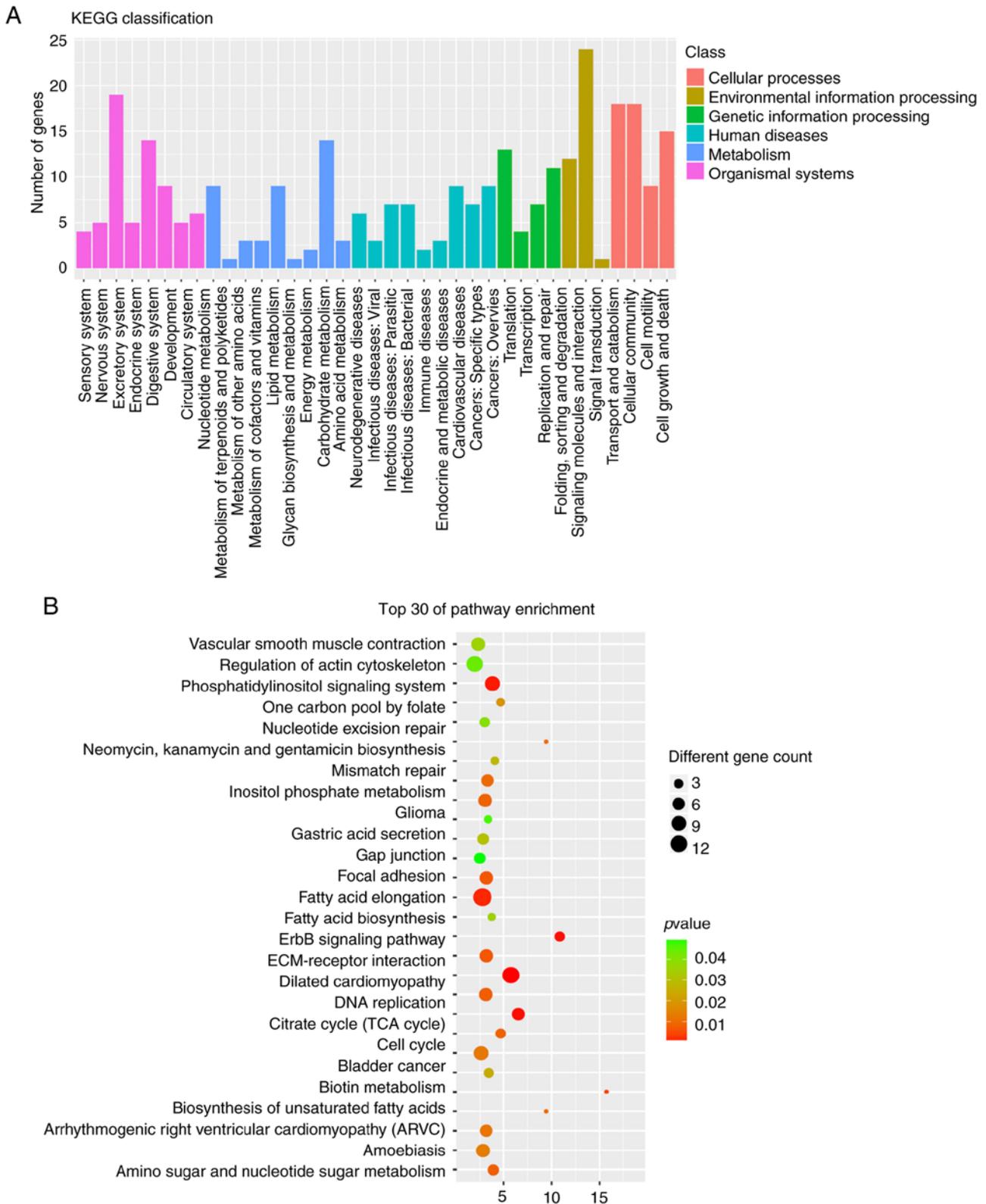


Figure 4. KEGG pathway analysis for host genes of dysregulated circRNAs between neonatal hypoxic-ischemic encephalopathy and control blood samples. (A) KEGG classification of differentially expressed circRNAs. (B) Top 30 circRNAs of pathway enrichment. KEGG, Kyoto Encyclopedia of Genes and Genomes; circRNA, circular RNA.

entry site element prediction of the circRNA_0005537, circRNA_0050345, circRNA_0050705, circRNA_0069578 and circRNA_0070733; it was suggested that these five circRNAs have the possibility to translate into short proteins

or peptides (data not shown). However, the functional relevance of these five circRNA requires further study.

Brain ischemia leads to a lack of ATP and initiates a cascade of biochemical events, including cell death and the induction

Table V. Interaction of circRNA and miRNAs.

circRNA	miRNA	miRNA number
circ_0005537	miR-1200, miR-1208, miR-1236, miR-1283, miR-1288, miR-1290, miR-136, miR-433, miR-526b, miR-579, miR-587, miR-221-3p, miR-222-3p, miR-6131, miR-3199, miR-8052	16
circ_0050345	miR-4717-5p, miR-518a-5p, miR-527, miR-505-5p, miR-4680-3p, miR-7974	6
circ_0050705	miR-8089, miR-4700-5p, miR-6738-3p, miR-637, miR-608, miR-6743-5p, miR-3187-5p, miR-612, miR-6721-5p, miR-486-3p, miR-4731-5p, miR-370-3p, miR-1207-5p, miR-6819-5p, miR-4739, miR-4690-5p, miR-6878-3p, miR-6734-5p, miR-3692-5p, miR-6774-5p, miR-4632-5p, miR-1199-3p, miR-1908-5p, miR-3162-5p, miR-6744-3p, miR-5088-5p, miR-149-5p, miR-6835-5p, miR-6738-5p, miR-6876-5p, miR-8069, miR-615-5p, miR-6773-3p, miR-6796-5p, miR-4640-5p, miR-3132, miR-6737-5p, miR-3907, miR-6742-5p, miR-6763-5p, miR-6860, miR-4726-3p, miR-6823-5p, miR-4778-3p, miR-6842-3p, miR-3192-5p, miR-661, miR-4667-5p, miR-4459, miR-6726-5p, miR-4749-5p, miR-6127, miR-3194-3p, miR-146b-3p, miR-6805-5p, miR-5189-5p, miR-4476, miR-760, miR-4498, miR-611, miR-3180-3p, miR-345-5p, miR-6821-5p, miR-7155-5p, miR-1229-5p, miR-1301-3p, miR-6791-5p, miR-5787, miR-6893-3p, miR-6848-3p, miR-4664-5p, miR-1587, miR-6816-5p, miR-4763-3p, miR-6865-5p, miR-1972, miR-6851-5p, miR-423-5p, miR-3922-3p, miR-6879-5p, miR-6775-5p, miR-1251-3p, miR-1914-3p, miR-484, miR-6756-5p, miR-3184-5p, miR-3620-5p, miR-3147, miR-885-3p, miR-3925-3p, miR-766-3p, miR-6852-5p	92
circ_0069578	miR-3158-5p, miR-3617-3p, miR-922, miR-4291, miR-761, miR-4725-3p, miR-6734-5p, miR-6780b-5p, miR-6825-5p	9
circ_0070733	miR-2355-5p, miR-6882-3p, miR-338-3p, miR-4286, miR-6762-3p, miR-24-3p, miR-6716-5p, miR-4252	8

circRNA, circular RNA; miRNA/miR, microRNA.

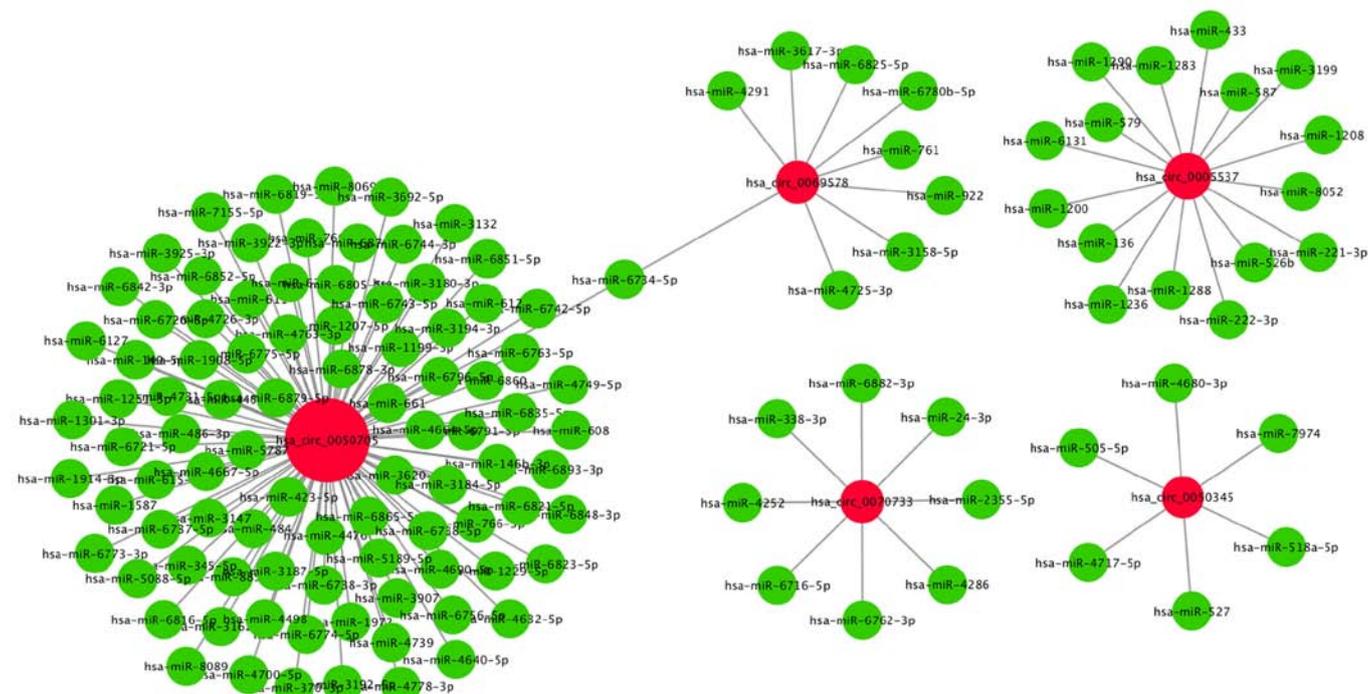


Figure 5. Network map of potential interaction between circRNAs and miRNAs. circ_0050705 had potential interactions with 92 miRNAs, circ_0005537 had potential interactions with 16 miRNAs, circ_0069578 had potential interactions with nine miRNAs, circ_0070733 had potential interactions with eight miRNAs and circ_0050345 had potential interactions with six miRNAs. circRNA, circular RNA; miRNA, microRNA.

of secondary brain injury in neonates (37). In addition, glucose and oxygen play important roles in the pathogenesis of brain injury (1). Moreover, acetyl-CoA is essential in generating ATP from ADP in the mitochondrion (1,38). In the present study, GO and KEGG pathway analyses results demonstrated that the host genes of dysregulated circRNAs participate in hindbrain morphogenesis and fatty-acyl-CoA biosynthetic processes. Therefore, the present results suggested that the dysregulated circRNAs may be involved in the pathogenesis of neonatal HIE.

In conclusion, microarray analysis was used to detect differentially expressed circRNAs between neonates with HIE and controls. To the best of our knowledge, the present study is the first to examine the potential relationship between circRNAs and HIE in neonates. In addition, the potential functions of the host genes of the dysregulated circRNAs were predicted by GO and KEGG pathway analyses. The GO and KEGG pathway analyses results indicated that the abnormally expressed circRNAs may be involved in the pathogenesis of HIE. However, whether the severity of HIE and the effect of hypothermia therapy are related to differentially expressed circRNAs in the progression of HIE requires further investigation.

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Availability of data and materials

The microarray data that included the expression profile of circular RNAs and the expression profile of long non-coding RNAs (19) have been deposited in the National Center for Biotechnology Information GEO database (accession no. GSE121178). The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

YZ designed the project and edited the manuscript. LY analyzed the data and wrote the manuscript. XD designed the experiment and performed the bioinformatics analysis. SZ performed the bioinformatics analysis. YH and XY performed sample collection and statistical analysis. YF performed the data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This work was approved by the Medical Ethics Committee of Jingjiang People's Hospital in China [approval no. (2017)25]. Informed consent was obtained from the guardian of subjects for participation in this study.

Patient consent for publication

Informed consent was obtained from the guardian of subjects for participation in this study.

Competing interests

The authors declare that they have no competing interests.

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