RNA Sequencing and Bioinformatics Analysis of CircRNAs in Asphyxial Newborns with Acute Kidney Injury

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Research Article

Keywords: Neonate, Acute kidney injury, CircRNA, High throughput sequencing, Bioinformatics

Posted Date: April 6th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1517853/v1

Abstract

Objective: As one kind of novel non-coding RNA with high stability and conservatism, circular RNAs (circRNAs) are involved in different biological processes. Although growing evidence has supported the important role of circRNAs in renal diseases, the specific mechanism still remains unknown in neonatal acute kidney injury (AKI).

Methods: High throughput sequencing analysis was applied to investigate the expression patterns of circRNAs in the comparison between hypoxia induced AKI neonates and controls. Then, bioinformatics analysis was conducted to predict the function of differentially expressed circRNAs and construct the network of circRNA-miRNA interaction. Finally, the differentially expressed circRNAs were further screened and determined by quantitative real-time PCR.

Results: (1) We identified 296 differentially expressed circRNAs (circRNAs with Fold change > 2 and *P* value < 0.05). Of them, 184 circRNAs were markedly up-regulated and 112 were significantly down-regulated in the AKI group. (2) Bioinformatics analysis indicated that the parental genes of the differentially expressed circRNAs were predominantly implicated in the cell and cell part, cellular process and cancer pathways. (3) Top five up-regulated and down-regulated circRNAs with higher fold change were selected for qPCR validation. hsa_circ_0008898, hsa_circ_0005519 were found significantly up-regulated and hsa_circ_0132279, hsa_circ_0017647, hsa_circ_0112327 were down-regulated in neonate with asphyxia-induced AKI group compared to control group.

Conclusion: This study could contribute to future research of circRNAs in the pathogenesis of neonatal AKI and facilitate the identification of novel therapeutic targets of circRNAs.

Introduction

Acute kidney injury (AKI) is a severe complication in neonate intensive care unit (NICU), which is defined as significantly elevated level of serum creatinine (SCr) or/and decreased urine output according to the modification of acute kidney injury network (AKIN) staging system and kidney disease improving global outcomes (KIDGO) classification [1–3]. The risk factors of neonatal AKI include sepsis, perinatal asphyxia, necrotizing enterocolitis, nephrotoxic drugs and *etc*. The incidence of AKI could reach up to 70% in asphyxial newborns, which leading to increased mortality and prolonged hospitalization [4–5]. Newborns who survive AKI are prone to develop chronic kidney disease (CKD) and hypertension [6]. Early diagnosis of AKI in newborns is difficult because many of them show non-oliguric renal failure, and SCr is not a sensitive marker for neonates. Although growing progress has been made in treating neonatal AKI, there is still a lack of sufficient researches on the mechanism. Therefore, it is of great significance to fully understand the etiology and pathogenesis of AKI after neonatal asphyxia, which will provide a new insight for the prevention and treatment of AKI.

Circular RNAs (circRNAs) are short RNA molecules with co-valently bound structures, which could play an important role in regulating gene expression by functioning as competitive endogenous RNAs (ceRNAs)

to sponge microRNAs (miRNAs) [7–9]. Most circRNAs are originated in 'back-splicing' reaction. Different from the linear RNA molecular, circRNAs show higher stability in *vivo* [10]. Moreover, previous studies have showed that circRNAs are involved in the pathogenesis of various diseases [11–12]. Such as circular RNA YAP1 has been found act as a sponge of microRNA-21-5p to secure HK-2 cells from ischaemia/reperfusion-induced injury [13]. Additionally, circ_0023404 has been identified to play important role in HK-2 cells injury triggered by hypoxia/reoxygenation [14]. However, the roles of circRNAs in neonatal AKI, especially after asphyxia, are far from being fully elucidated.

In the present study, we used high-throughput sequencing to detect differential expression of circRNAs in peripheral blood between AKI neonates and controls. Then, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were performed to evaluate the potential role of differentially expressed circRNAs in AKI. Finally, we validated the differentially expressed circRNAs by qPCR.

Materials And Methods

Subjects and study design

All AKI samples were obtained from the patients in NICU of the First Affiliated Hospital of Nanjing Medical University from October 2020 to November 2021. Blood samples of controls were drawn from mild to moderate indirect hyperbilirubinemia at Obstetrics at the third day after birth. Firstly, to perform the circRNA profile, 2mL peripheral venous blood were drawn from five infants with asphyxia induced AKI and five controls. The time point of blood drawing is when the AKI diagnosis was established. Subsequently, for qPCR validation, peripheral blood samples were collected from 20 asphyxial AKI newborns and 16 controls. Samples were mixed with TRIzol and then frozen in liquid nitrogen until they were tested for circRNA sequencing or quantification. The clinical characteristics of AKI infants and controls are showed in Table1.

The indicators of acute perinatal asphyxia were as follows: a) history of fetal distress (bradycardia, late decelerations, absence of heart rate variability); b) the presence of sentinel hypoxia event immediately before or during delivery; c) metabolic acidosis in the arterial umbilical cord (base deficit ≧ -16 mmol/l), and PH≦7.0; d) clinical indications of hypoxic-ischemic encephalopathy and multiple organ involvement [15].

The definition of AKI was based on the neonatal modified KDIGO criteria [16], as persistently increased level of serum creatinine (>1.5mg/dl) for at least 24hs or decreased level of urine output (<1.0ml/kg·h).

High-throughput RNA sequencing

Five samples from AKI group and five samples from control group were pooled into two independent tubes for high throughput sequencing. Total RNA was isolated from the above two tubes and purified by

Trizol reagent (Invitrogen, Carlsbad, CA, US) following the manufacturer's instruction. The RNA amount and purity of samples was quantified through NanoDrop ND-1000 (NanoDrop, Wilmington, DE, US). The RNA integrity was assessed by Agilent 2100 with RIN number >7.0. 5 ug of total RNA was used to deplete ribosomal RNA according to the instruction of the Ribo-Zero™ rRNA Removal Kit (Illumina, SanDiego, US). Then left RNAs were treated with Rnase R (Epicentrelnc, Madison, WI, US) to remove linear RNAs and to enrich circRNAs. After removing linear RNAs and ribosomal, the enriched circRNAs were fragmented into small pieces by divalent cations. Then, the cleaved RNA fragments were reverse-transcribed to create the cDNA, which were further used to synthesize U-labeled second-stranded DNAs with E.coli DNA polymerase I, dUTP and RNase H. An A-base was added to the blunt ends of each strand, preparing them for ligation to the indexed adapters. Each one contains a T-base overhang for ligating the adapter to the A-tailed fragmented DNA. Single-ordual-index adapters are ligated to the fragments, and size selection was performed with AMPureXP beads. After the heat-labile UDG enzyme treatment of the U-labeled second-stranded DNAs, the ligated products were amplified with PCR by the following conditions: initial denaturation at 95℃ for 3 min; 8 cycles of denaturation at 98℃ for 15 sec, annealing at 60℃ for 15 sec, and extension at 72℃ for 30 sec; and final extension at 72℃ for 5min. The average insert size for the final cDNA library was 300bp (±50bp). Finally, we performed the paired-end sequencing on an Illumina Hiseq 4000 (LCBio, China) following the vendor's recommended protocol.

Firstly, cutadapt was used to remove the reads that contained adaptor contamination, low quality bases and undetermined bases. Then sequence quality was verified using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). We used Bowtie2 and Tophat2 to map reads to the genome of species. Remaining reads (unmapped reads) were still mapped to genome using tophat-fusion. CIRCExplorer2 and CIR was used to denovo assemble the mapped reads to circular RNAs at first. Then, back splicing reads were identified in unmapped reads by tophat-fusion. All samples were generated unique circular RNAs. CircRNA expressions from different samples or groups were calculated by scripts in house. Only the comparisons with ‘P value’ less than 0.05 were considered as differential expression by R package. This part was implemented by the company named Obio Technology, Shanghai, China.

Bioinformatics analysis

Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/) and Gene Ontology (GO; http://www.geneontology.org/) were used to analyze the parent genes to predict circRNA function. GO functional analysis is divided into three parts: molecular function, biological processes, and cellular components. GO analysis was used to annotate gene function based on the GO database to obtain all functions of the gene parameters.

RT-qPCR validation for candidate circRNAs

The extraction and quantification of total RNA were processed as previously described. The RNA quantity control was detected using a NanoDrop2000 Spectrophotometer (Thermo Fisher Scientific, Inc. US). Then, 1μg of total RNA was isolated as previous report and converted into cDNA by the HiScript® II Q Select RT SuperMix for qPCR (R232-01; Vazyme; China) according to the manufacturer's protocol [4μl 5X HiScript II Select qRTSuperMix, 1μl Random hexamers (50ng/μl) and 1μg RNA]. The RT reaction was conducted at 37˚C for 15min and 85˚C for 2min. Next, the RT-PCR reaction was performed using AceQ® qPCR (Q131-01; Vazyme; China). For RT-PCR, 1μl cDNA was added to 9μl master mix, including 5μl SYBR®Green Master Mix (Low Rox Premixed; Q131-01; Vazyme; China), 0.2μl reverse and forward primers, and 3.6μl diethypyrocarbonate water. Then, the PCR was performed under the reaction conditions included an initial step at 95˚C for 5min, and 40 cycles at 90˚C for 15sec and at 60˚C for 15sec, 72˚C for 1min and final extension at 72˚C for 10min. The primer sequences are presented in Table2. PCR was performed in each plate using GAPDH as an endogenous control. All primer sequences were designed and synthesized by Guangzhou RiboBio Co. Ltd. The expression level of circRNAs were calculated through the $2^{-\Delta\Delta Ct}$ method.

Statistical analysis

All data are presented as Mean±SEM. All experiments were repeated at least three times. The data were analyzed by SPSS 17.0 (SPSS, Inc. US) and GraphPad Prism 8.0 (GraphPad Software, Inc. US) statistical packages. For quantitative data, Student's *t*-test was applied to analyze statistical significance between two groups. For qualitative data, Fisher's exact test was used to compare between two groups. $P < 0.05$ was considered as a statistically significant difference.

Results

Differential expression of circRNAs between AKI and control groups

The differentially expressed circRNAs between the AKI and control groups were screened by fold change and *P*-values statistical criteria. We first analyzed the differences in expression levels using volcano plot and bar chart (Figure 1a and 1b). Moreover, hierarchical clustering and heatmap visualization of circRNA profile showed distinguishable expression patterns between controls and AKI patients (Figure 2a). We identified 296 differentially expressed circRNAs (Fold change > 2 and *P* value < 0.05). Of them, 186 circRNAs were markedly up-regulated and 112 were down-regulated in the AKI group. Subsequently, a box plot was used for visualizing the intensities of expression values after normalization, which showed a similar distribution (Figure 2b). Among all identified circRNAs, top 10 up-regulated and down-regulated circRNAs with higher fold change were selected for qPCR validation. Summary of selected circRNAs is shown in Table3.

Bioinformatics analysis of differentially expressed circRNAs

To explore the potential molecular function of the circRNAs, GO and KEGG enrichment analysis were performed to determine the function of the parental gene of the differentially expressed circRNA. GO analysis showed the above circRNAs are involved in protein monoubiquitination, ubiquitin–dependent protein catabolic process and *etc* (Figure 3 A and B). The pathway analysis found ubiquitin mediated proteolysis, renal cell carcinoma, Jak-STAT signaling pathway, HIF-1 signaling pathway are participated in AKI. The figure below shows the 20 most significant enrichment pathways (Figure 3 C).

qPCR validation of circRNA expression

Top five up-regulated and five down-regulated circRNAs with higher fold change were further selected (Table3). Candidate circRNAs expression were validated by qPCR based on the blood samples from 20 AKI newborns and 16 controls. Of all these selected circRNAs, hsa_circ_0008898 and hsa_circ_0005519 were found significantly up-regulated, and hsa_circ_0132279, hsa_circ_0017647, hsa_circ_0112327 were significantly down-regulated as the same as RNA sequencing results (Figure 4).

Discussion

Accumulating evidence have showed that neonatal AKI affects 18%-70% of newborns in NICU and is associated with higher mortality and higher risk of CKD [4–6]. Newborns who develop AKI in the first week after birth are at nearly three-times-higher adjusted odds of death than those who did not [17]. Identify the mechanism of pathogenesis kidney damage would be useful for early diagnosis and therapy.

In recent years, circRNAs have been demonstrated as important regulators of various renal diseases, such as AKI, renal cancer, chronic nephritis and diabetic glomerular injury [18–20]. Compared to linear RNA, circRNA is resistant to exonucleases and fairly stable. CircRNAs could regulate the expression of target gene by interacting with RNA binding proteins. However, there is still a lack of studies to discuss the relationship between circRNA and neonatal AKI.

In this article, we used high throughput RNA sequencing to investigate the role of circRNA in neonatal AKI. Among all identified circRNAs, a total of 296 differentially expressed circRNAs were found, including 184 up-regulated and 112 down-regulated circRNAs between AKI neonate and controls. Their function was further predicted by bioinformatics analysis. During the GO analysis, the top 20 GO enriched terms are focused on various biological process, like protein monoubiquitination, DNA repair, protein sumoylation and protein phosphatase binding. We noticed that HIF-1 signaling pathway is enrichened in KEGG analysis, HIF-1 is a heterodimer composed of an O2-regulated HIF-1α subunit and a constitutively expressed HIF-1β subunit [21], which is a hallmark change in ischemia/reperfusion injury [22], which could be a potential target in asphyxia induced AKI.

Based on the RNA sequencing results, we selected top 5 up-regulated and down-regulated circRNAs for further validation. Finally, qPCR showed that the expression level of hsa_circ_0008898 and hsa_circ_0005519 were significantly increased in AKI group. Meanwhile, the expression level of hsa_circ_0132279, hsa_circ_0017647 and hsa_circ_0112327 were significantly decreased, which was consistent with sequencing results.

The pathological mechanism of AKI is complicated. Multiple factors, such as inflammation, mitochondrial damage and oxidative stress, have been shown to be involved [23–24]. Inflammation and oxidative stress have been described as key mediators of the initial phase of AKI. Among these circRNAs, hsa_circ_0005519 is located in SNX13 gene, which is involved in the modulation of T-cell differentiation, apoptosis and secretion of cytokines [25–26]. Hsa_circ_0005519 was up-regulated in $CD4^+$ T cells of asthmatic patients and was likely to target hsa-let-7a-5p to promote the expression of IL-13 and IL-6 in $CD4^+$ T cells [25–26]. This result may help to prove that has_circ_0005519 could participate in the inflammation response in AKI development.

In summary, we identified 5 circRNAs (hsa_circ_0008898, hsa_circ_0005519, hsa_circ_0132279, hsa_circ_0017647 and hsa_circ_0112327) are associated with asphyxia induced AKI. Among these candidate circRNAs, has_circ_0005519 could participate in the inflammation response in AKI development. The present study also had some limitations. Firstly, the sample size was relatively small which limited the accuracy of results. Secondly, the potential function of circRNAs in the pathogenesis of AKI need further research and verification.

Declarations

Ethics approval and consent to participate

This study was approved by the Committee on Human Rights Related to Research Involving Human Subjects, Faculty of Affiliated Children Hospital, Nanjing Medical University (Approved Number: 202107071-1).

Consent for publication

Informed consent has been obtained from the parents of included newborns.

Availability of data and materials

The datasets used and analyzed during the current study are available from the link 10.6084/m9.figshare.18665768.

Competing interests

The authors declare that they have no competing interests.

Funding

This work was supported by grant from the Young Talents Startup Fund of Jiangsu Women and Children Health Hospital, China (FYRC202012 Q,C.).

Authors' contributions

JJP and YY collected and analyzed the patient data of microarray profile regarding AKI. XQC was a major contributor in writing and revising the manuscript. JS and MZW helped to perform the experiments of qPCR and bioinformatics analysis. MLT and XGZ designed this research and revised this manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors would like to thank the parents of the patients for their understanding, patience and endurance.

References

1. Mehta RL, Kellum JA, Shah SV, et al. Acute kidney injury network: report of an initiative to improve outcomes in acute kidney injury. *Crit Care.* 2007;11 (2): R31.
2. Ricci Z, Cruz D, Ronco C. The RIFLE criteria and mortality in acute kidney injury: a systematic review. *Kidney Int.* 2008;73 (5):538-546.
3. Uchino S. Outcome prediction for patients with acute kidney injury. *Nephron Clin Pract.* 2008;109 (4):c217-c223.
4. Mok TYD, Tseng M-H, Lee J-C, et al. A retrospective study on the incidence of acute kidney injury and its early prediction using troponin-I in cooled asphyxiated neonates. *Sci Rep*. 2020; 10 (1):15682.
5. Aslam M, Arya S, Chellani H, Kaur C. Incidence and predictors of acute kidney injury in birth asphyxia in a tertiary care hospital. *J Clin Neonatology*. 2017; 6 (4):240.
6. Jetton JG, Boohaker LJ, Sethi SK, et al. Incidence and outcomes of neonatal acute kidney injury (AWAKEN): a multicentre, multinational, observational cohort study. *Lancet Child Adolesc Health*. 2017; 1 (3):184-194.
7. Jeck WR, Sharpless NE. Detecting and characterizing circular RNAs. *Nat Biotechnol*. 2014; 32 (5):453-461.
8. Haddad G, Lorenzen JM. Biogenesis and function of circular RNAs in health and in disease. *Front Pharmacol*. 2019; 10:428.
9. Szabo L, Salzman J. Detecting circular RNAs: bioinformatic and experimental challenges. *Nat Rev Genet*. 2016; 17 (11):679-692.
10. Li Y, Zheng Q, Bao C, et al. Circular RNA is enriched and stable in exosomes: a promising biomarker for cancer diagnosis. *Cell Res.* 2015; 25 (8):981-4.

11. Wang S, Zhang X, Li Z, et al. Circular RNA profile identifies circOSBPL10 as an oncogenic factor and prognostic marker in gastric cancer. *Oncogene.* 2019; 38 (44):6985-7001.
12. Yang J, Cong X, Ren M, et al. Circular RNA hsa_circRNA_0007334 is Predicted to Promote MMP7 and COL1A1 Expression by Functioning as a miRNA Sponge in Pancreatic Ductal Adenocarcinoma. *J Oncol.* 2019; 2019: 7630894.
13. Huang T, Cao Y, Wang H, Wang Q, Ji J, Sun X, Dong Z. Circular RNA YAP1 acts as the sponge of microRNA-21-5p to secure HK-2 cells from ischaemia/reperfusion-induced injury. J Cell Mol Med. 2020 Apr;24(8):4707-4715.
14. Xu Y, Li X, Li H, Zhong L, Lin Y, Xie J, Zheng D. Circ_0023404 sponges miR-136 to induce HK-2 cells injury triggered by hypoxia/reoxygenation via up-regulating IL-6R. J Cell Mol Med. 2021 Jun;25(11):4912-4921.
15. Bona E, Hagberg H, Loberg EM, Bågenholm R, Thoresen M. Protective effects of moderate hypothermia after neonatal hypoxia-ischemia: Short- and long-term outcome. *Pediatr Res*. 1998; 43 (6):738-745.
16. Selewski DT, Charlton JR, Jetton JG, et al. Neonatal Acute Kidney Injury. *Pediatrics.* 2015; 136 (2):e463-73.
17. Charlton JR, Boohaker L, Askenazi D, et al. Incidence and Risk Factors of Early Onset Neonatal AKI. *Clin J Am Soc Nephrol*. 2019; 14 (2):184-195.
18. Hao JF, Ren KM, Bai JX, et al. Identification of potential biomarkers for clear cell renal cell carcinoma based on microRNA-mRNA path way relationships. *J Cancer Res Ther*. 2014;10 (Suppl):C167-C177.
19. Brandenburger T, Salgado Somoza A, Devaux Y, Lorenzen JM. Noncoding RNAs in acute kidney injury. *Kidney Int*. 2018; 94 (5):870-881.
20. Kolling M, Haddad G, Wegmann U, et al. Circular RNAs in urine of kidney transplant patients with acute T cell-mediated allograft rejection. *Clin Chem*. 2019; 65 (10):1287-1294.
21. Zhang Z, Haimovich B, Kwon YS, Lu T, Fyfe-Kirschner B, Olweny EO. Unilateral Partial Nephrectomy with Warm Ischemia Results in Acute Hypoxia Inducible Factor 1-Alpha (HIF-1α) and Toll-Like Receptor 4 (TLR4) Overexpression in a Porcine Model. *PLoS One*. 2016; 11 (5):e0154708.
22. Fu ZJ, Wang ZY, Xu L, et al. HIF-1α-BNIP3-mediated mitophagy in tubular cells protects against renal ischemia/reperfusion injury. *Redox Biol*. 2020; 36:101671.
23. Gu Y, Huang F, Wang Y, et al. Connexin32 plays a crucial role in ROS-mediated endoplasmic reticulum stress apoptosis signaling pathway in ischemia reperfusion-induced acute kidney injury. J Transl Med. 2018; 16 (1):117.
24. Andres-Hernando A, Li N, Cicerchi C, et al. Protective role of fructokinase blockade in the pathogenesis of acute kidney injury in mice. *Nat Commun*. 2017; 8:14181.
25. Taheri M, Barth DA, Kargl J, Rezaei O, Ghafouri-Fard S, Pichler M. Emerging Role of Non-Coding RNAs in Regulation of T-Lymphocyte Function. *Front Immunol*. 2021; 12:756042.

26. Huang Z, Cao Y, Zhou M, et al. Hsa_circ_0005519 increases IL-13/IL-6 by regulating hsa-let-7a-5p in CD4+ T cells to affect asthma. *Clin Exp Allergy*. 2019; 49 (8):1116-1127.

Tables

Table 1

The clinical characteristics of AKI patients and controls

	AKI (n = 20)	Non-AKI (n = 16)	*P*-value
	Mean ± SEM or n (%)	Mean ± SEM or n (%)	
Infants' characteristics			
Male	16（80%）	12（75%）	1.0000
Birthweight (g)	3458±452	3324±450	0.5405
Gestational age (weeks)	39.2±1.25	38.8 ±0.81	0.4477
Apgar 1 min	5.2±1.38	9.8±0.32	0.0007
Apgar 5 min	6.4±1.58	9.9±0.13	0.0004
Delivery			
Vaginal	12 (60%)	6(37.5%)	0.315
Cesarean	8(40%)	10(62.5%)	0.315
AKI by creatinine	5(35%)	0	0.053
AKI by urine output	15 (65%)	0	0.0003
Cord blood pH<7	12（60%）	0	0.0002

Table 2

Primers for RT-qPCR

circRNA ID	Primers
hsa_circ_0008898	F:TATGAATACAGGAGTGGAGGCTG
	R:CAAGTAGAAAAACCACAGATCTGCA
hsa_circ_0005519	F:TTGTCAGGGAAATCCTTGCA
	R:CCAGTCTATTTCTTTTGACCATCCA
hsa_circ_0132279	F:AGTGACACACCACAAAAACACA
	R:GGAGCCCAAGAAGACCACAT
hsa_circ_0017647	F:CCGACTTCTGGTGTGACGTA
	R:TCTCCCCAGTTAAAGCCAGTT
hsa_circ_0112327	F:CTCAGCCCATTTCTCAGCAT
	R:GCTAGAGAAGCAGCCCACAG
GAPDH	F:GAACGGGAAGCTCACTGG
	R:GCCTGCTTCACCACCTTCT

F, forward; R, reverse

Table 3

Biological information of the selected circRNAs.

ID	Fold Change	Regulation	Chromosome	Gene symbol
hsa_circ_0008898	5.48	up	chr10	OAT
hsa_circ_0093681	5.24	up	chr10	ZFAND4
hsa_circ_0001355	4.79	up	chr3	RSRC1
hsa_circ_0120331	4.65	up	chr2	PSME4
hsa_circ_0005519	4.65	up	chr7	SNX13
hsa_circ_0021781	-4.56	down	chr11	TTC17
hsa_circ_0132279	-4.47	down	chr6	SLC17A5
hsa_circ_0017647	-4.26	down	chr13	SFMBT2
hsa_circ_0031071	-4.26	down	chr10	RASA3
hsa_circ_0112327	-4.15	down	chr17	NUP133

Figures

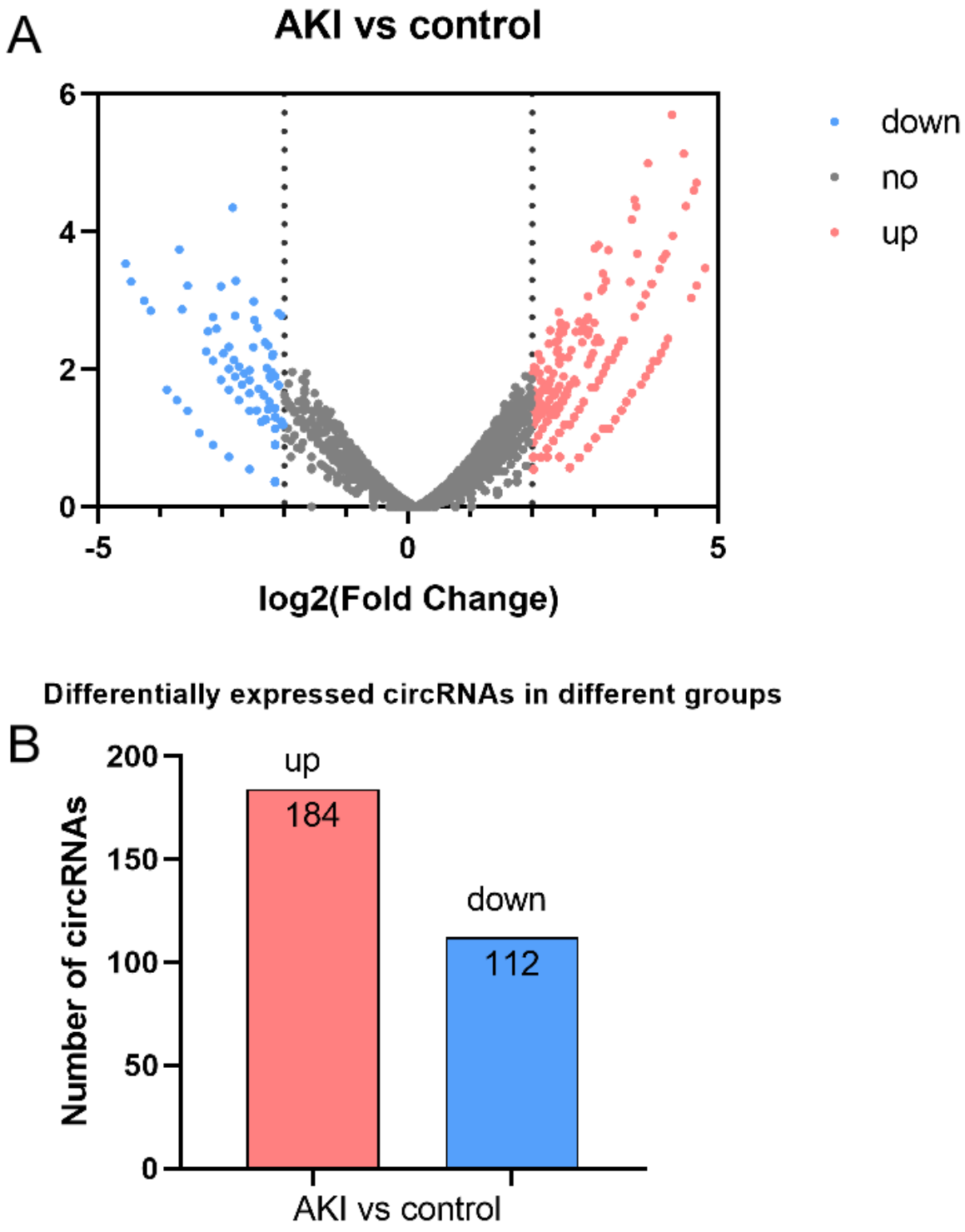

Figure 1

CircRNA profile between AKI patients and controls. **(A)** Volcano plots show the expression pattern of circRNAs. The red dots are the down-regulated circRNAs with statistical significance [log2 (Fold change) >2 and a *P*-value < 0.05]. The blue dots are the differentially up-regulated circRNAs with statistical significance [log2 (Fold change) >2 and a *P*-value < 0.05]. **(B)** In total, 112 significantly down-regulated

circRNAs and 184 up-regulated circRNAs were observed. Red represents high-expressed circRNAs. Blue represents down-expressed ones.

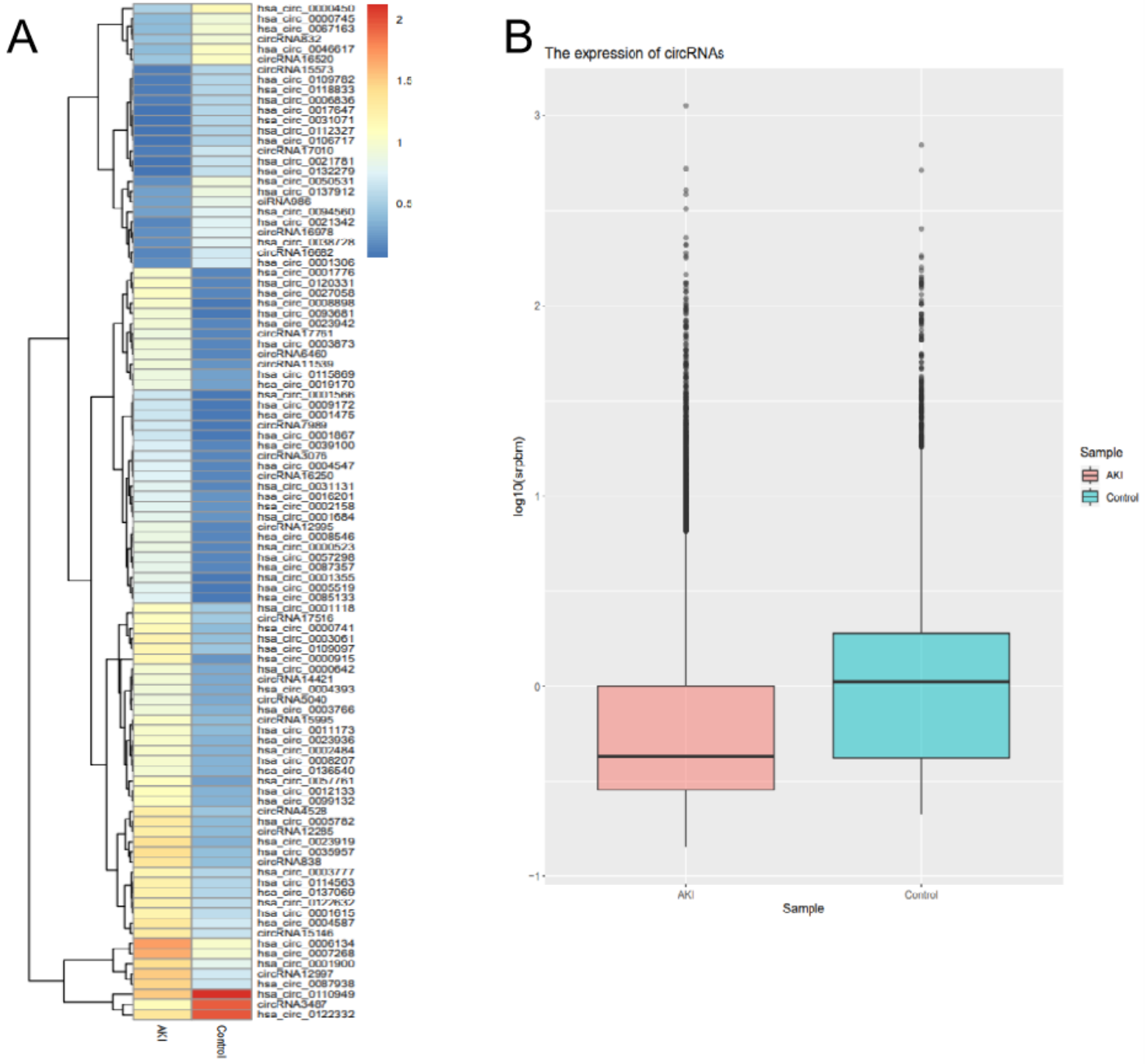

Figure 2

Differentially expressed circRNAs between AKI patients and controls. **(A)** Heatmap and hierarchical clustering analysis of circRNAs. **(B)** Boxplot, a method of describing data in terms of minimum, first quartile (25%), median (50%), third quartile (75%), and maximum. The abscissa is the sample name, and the ordinate is the value of the signal value of the probe after log2.

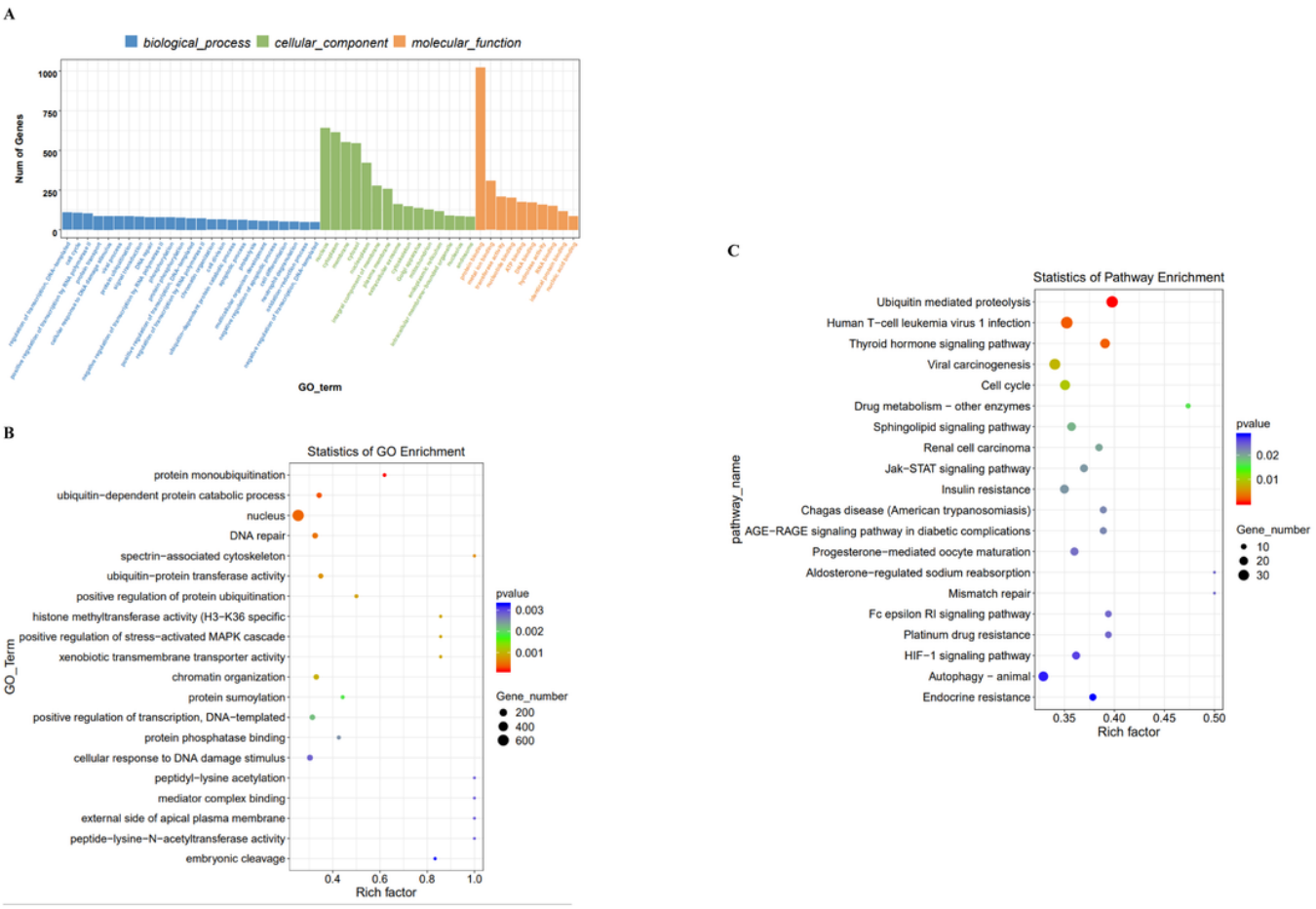

Figure 3

The analysis of the gene function of differentially expressed circRNAs by GO and KEGG. **(A** and **B)** Top 20 enriched GO terms according to the negative logarithm of the p-value of enriched target genes. **(C)** Top 20 KEGG pathways of target genes were described according to the enrich factors of enriched target genes.

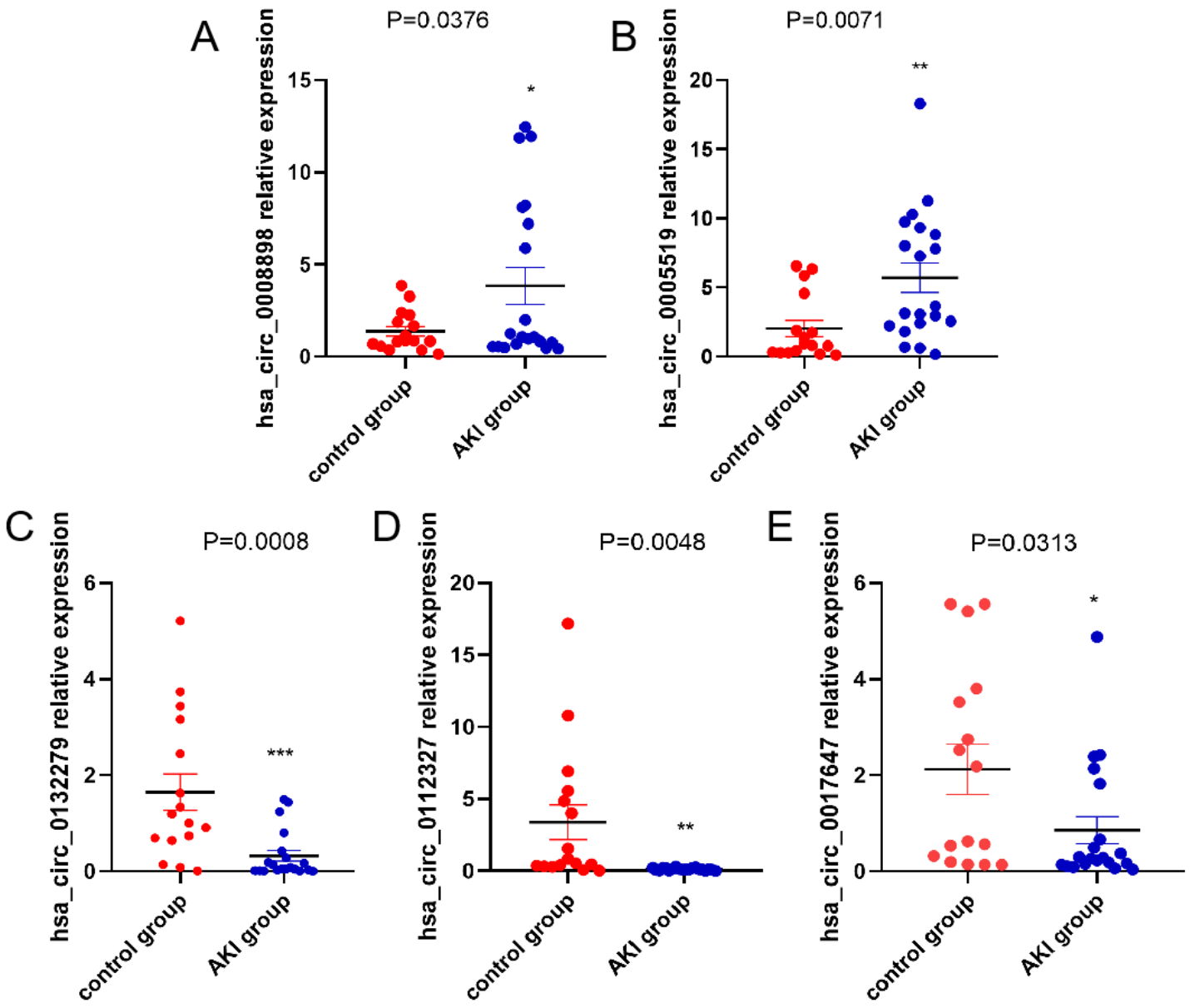

Figure 4

RT-qPCR validation of top 5 up-regulated and down-regulated circRNAs [including **(A)** hsa_circ_0008898, **(B)** hsa_circ_0005519, **(C)** hsa_circ_0132279, **(D)** hsa_circ_0112327 and **(E)** hsa_circ_0017647]. *$P < 0.05$.