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RESEARCH ARTICLE

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Expression profile of circular RNAs in cystic echinococcosis pericystic tissue

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Abstract

Objective: The aim of the present study was to investigate the expression profiles of circular RNAs (circRNAs) in the pericystic tissue of patients with cystic echinococcosis (CE).

Patients and methods: CircRNA expression profiles were obtained by circRNA microarray of four matched pairs of pericystic tissues affected by CE and adjacent normal liver tissues. qRT-PCR was used to validate the differential expression of some circRNAs identified by the microarray analysis. The potential functions of the differentially expressed circRNAs in the CE pericystic tissues were predicted by bioinformatic analysis.

Results: Compared with the adjacent normal liver tissues, 177 circRNAs were upregulated and 166 circRNAs were downregulated in CE pericystic tissues based on a ≥2.0-fold change. The top 10 upregulated circRNAs were hsa_circRNA_001654,hsa_circRNA_103361,hsa_circRNA_001490,hsa_circRNA_104310,hsa_circRNA_100395,hsa_circRNA_102485,hsa_circRNA_001459,hsa_circRNA_104193,hsa_circRNA_400043, and hsa_circRNA_006773;The top 10 downregulated circRNAs were hsa_circRNA_400633,hsa_circRNA_404974,hsa_circRNA_068482 ,hsa_circRNA_100974,hsa_circRNA_049637,hsa_circRNA_404798,hsa_circRNA_400064,hsa_circRNA_004045,hsa_circRNA_101379, and hsa_circRNA_016771;The circRNA-seq results for 15 selected differentially expressed circRNAs were validated by qRT-PCR. The qRT-PCR analysis showed that hsa_circRNA_006773, hsa_circRNA_049637, hsa_circRNA_104349, and hsa_circRNA_406281 were differentially expressed in CE pericystic tissues when compared with their expression in the adjacent normal liver tissues. Interestingly, 319 miRNAs and 52 mRNAs were predicted to be adsorbed by these four differentially expressed circRNAs. Gene Ontology analysis revealed that these circRNAs may be involved in the response to organic cyclic compounds and endogenous stimuli and in cellular organismal processes.

Conclusion: Differential expression of circRNAs may be associated with the development and progression of CE, and these circRNAs might be useful as biomarkers for prognosis prediction and as treatment targets.

KEYWORDS
circular RNA, cystic echinococcosis, gene ontology analysis, microarray, pathways analysis

1 | INTRODUCTION

Cystic echinococcosis (CE), caused by *Echinococcus granulosus*, is a chronic zoonotic parasitic disease that endangers human health. Although the main endemic areas of the disease are regions in Oceania, Europe, China, and Central Asia, parts of Africa, and the Americas that have developed sheep and cattle industries,[1-3] CE can spread worldwide through globalization and immigration.[4] For example, Xinjiang (Northwest China) is one of the main areas affected by CE. The life cycle of *E. granulosus* requires two hosts: dogs as definitive hosts and livestock (mostly sheep) as intermediate hosts[5]. Humans can be accidental intermediate hosts. Although the liver and lungs are the most frequently affected organs (70% and 20% of total cases, respectively),[6,7] any organ or tissue can be infected with the parasite. In the liver, parasitic larvae, also called metacestodes, develop as fluid-filled cysts, which consist of an outer fibrous membrane, a middle laminated membrane, and an inner germinal layer. The cysts increase persistently, up to a diameter of 20 cm or more. The cyst is full of both hydatid cyst fluid (HCF),[8] which is derived from the secretion of soluble components by the germinal layer of the metacestode and may provide nutrients and other elements needed by the larval cyst and invaginated tissue layers called protoscoleces that are collected within the HCF. In definitive hosts, protoscoleces generate the adult worm.[9] The reaction of the host immune system to a transient granuloma results in the formation of a fibrotic pericyst. This extracellular matrix (ECM)-rich pericyst is a shell-like barrier, limiting the growth of the cyst and sometimes triggering spontaneous parasite degradation.[10,11] Some authors assumed that cysts may induce chronic parenchymal liver disease which could lead to the development of liver carcinoma, but there is not enough scientific evidence to confirm this thesis since there is a low number of relevant cases for analyzing the relation between these 2 focal liver lesions.[12] The host immune response to the fibrotic shell surrounding the metacestode may play a major role in limiting the growth of the cyst and determining its characteristics.[13,14] However, the pericystic wall also acts as a barrier for drugs. As a result, drugs have poor effects against the pericyst. Therefore, at present, treatment of hydatid disease is mainly limited to surgery. In summary, the hydatid cyst wall plays an important role in the occurrence and development of CE and possible treatment. However, very few studies on CE have addressed the specific role of the ECM-rich pericyst in counteracting CE. Therefore, we explored the mechanisms regulating the development of the fibrotic pericyst surrounding *E. granulosus* metacestodes by examining the fine-tuning of gene expression by small RNAs.[15,16]

According to previous reports, non-coding RNAs, including transfer RNAs (tRNAs), microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), participate in various biological and pathological processes.[17-19] However, compared with other non-coding RNAs, circRNAs have been poorly characterized. They are a new class of single-stranded, covalently closed RNA molecules without 5′ caps and 3′ tails that are widely expressed across species.[20] Recently, several studies have suggested that circRNAs can function as sponges for miRNAs, and thus act as competing endogenous RNAs (ceRNAs), and RNA-binding proteins, thereby regulating splicing or transcription. Interestingly, similar ceRNAs can compete for the same miRNA response elements to regulate each other's expression. Moreover, various reports revealed that dysregulation of circRNAs may be associated with the pathogenesis of a variety of diseases, including cancer,[21-23] cardiovascular diseases,[22-25] type 2 diabetes mellitus,[26] nervous system diseases,[27] and autoimmune diseases.[28] In addition, given that circRNAs alter miRNA levels to regulate their target genes, circRNA may be involved in the emergence of diseases correlated with miRNA dysregulation.

Previous studies revealed that circulating non-coding RNAs, including miRNAs and lncRNAs, can be detected in the blood of hosts infected with *E. granulosus* and *Echinococcus multilocularis*.[29] These stably circulating non-coding RNAs may play significant roles in the establishment and development of host-parasite interactions and could serve as diagnostic markers and therapeutic targets. However, the circRNA expression profiles in CE have been poorly explored.

To fill this knowledge gap, we first used human circRNAs microarrays to acquire the circRNAs expression profiles of CE pericystic tissues and adjacent normal liver tissues. We then performed quantitative reverse transcription-polymerase chain reaction (qRT-PCR) to validate the differentially expressed circRNAs in CE pericystic tissues. Subsequently, we performed a bioinformatic analysis to explore the potential of the differentially expressed circRNAs as ceRNAs.

2 | PATIENTS AND METHODS

2.1 | Sample collection

Four patients with CE who underwent cystectomy or lobectomy at the Department of Hepatobiliary Surgery, People's Hospital of Xinjiang, Uygur Autonomous Region between July 2018 and December 2019 were enrolled in the study. Paired CE pericystic tissues and adjacent non-CE liver tissues were extracted from the four patients, immediately frozen in liquid nitrogen, and stored at −80°C until use. This study was approved by the Ethics Committee of the People's Hospital of Xinjiang, Uygur Autonomous Region. Informed consent was obtained from all participants.

2.2 | Human circular RNA microarray

Human circRNA expression analysis was performed on four pairs of pericystic tissues of CE and adjacent non-CE liver tissue. Total RNA was extracted from each sample using a homogenizer and

TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc). The quantity and quality of purified RNA were assessed using a NanoDrop ND-1000 (NanoDrop Technologies; Thermo Fisher Scientific, Inc). RNA integrity was assessed by electrophoresis on a denaturing 1.5% agarose gel. Sample preparation and array hybridization were performed with the Super RNA Labeling Kit (Arraystar, Inc) according to the manufacturer's protocol. In brief, total RNA was digested with RNase R (Epicentre, Illumina Inc) to remove linear RNAs and obtain samples enriched in circRNAs, which were then amplified and transcribed to fluorescent cRNA using a random priming method (Super RNA Labeling Kit). The labeled cRNAs were purified using the RNeasy Mini Kit (Qiagen, Hilden, Germany). Labeled cRNAs were then hybridized to an Arraystar Human circRNA Array (8 × 15 K, Arraystar). The hybridized arrays were washed, fixed, and scanned using an Agilent G2505C Microarray Scanner System (Agilent).

The collected array images were analyzed using Feature Extraction software (Agilent). Quantile normalization and subsequent data processing were carried out with the limma package in R software. Independent samples were compared using a two-tailed Student's *t* test, and circRNA expression differences with a fold change ≥ 2.0 and $p < 0.05$ were considered statistically significant. Microarray analyses were performed by KangChen Bio-tech.

2.3 | Quantitative real-time reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA (1 μg) was used as a template for reverse transcription with the FastQuant RT kit (with gDNase) (Tiangen) according to the manufacturer's protocol. An aliquot (2 μl) of each cDNA sample was used for qRT-PCR in a total reaction volume of 20 μl with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems) in the presence of SYBR Green Realtime PCR Master Mix (Toyobo). Each sample was tested in triplicate. The PCR primers used to detect randomly chosen circRNAs to validate the microarray results are shown in Table 1.

2.4 | Bioinformatic analysis

The raw expression value of each circRNA obtained from the microarray experiment was normalized for further analysis. Then, a Volcano plot was generated to filter circRNAs displaying a ≥2.0-fold change in expression and $p < 0.05$, which were considered significantly differentially expressed between CE pericyst and adjacent non-CE liver tissues and were used for hierarchical clustering analysis with MeV 4.9 software. As circRNAs could adsorb specific miRNAs, we investigated the predicted circRNA-miRNA-mRNA interactions using the TargetScan and miRanda databases. A network connecting circRNAs and target miRNAs and mRNAs was constructed using Cytoscape v3.6.1.

Subsequently, the parent linear genes of the differentially expressed circRNAs were annotated using Gene Ontology (GO) (http://www.geneontology.org) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg) to predict the functions of these circRNAs.

TABLE 1 Primers sequences used in this study

circRNA	Primers (5′–3′)
hsa_circRNA_104349	F:5′CATTACCACCGTTACCTTTGC3′
	R:5′GGGATTTTCCAGACCGTCTAT3′
hsa_circRNA_049637	F:5′ATTAAATTTTGTGTCTCCGCG3′
	R:5′CCTTTAAAACGACCCTCCG3′
hsa_circRNA_006773	F:5′AGTTTTCATGGATGCCCCTGT3′
	R:5′AATCCAACTGGAGTCTTTGAAGC3′
hsa_circRNA_406281	F:5′AGGACTGGGAAAATGTAAACC3′
	R:5′CGTCTGGATTACAACCCTACTA3′

2.5 | Statistical analysis

Statistical analyses were carried out with SPSS 19.0 software, and a scatter diagram was drawn using GraphPad Prism (GraphPad Software, Inc). Data are expressed as mean ± standard deviation. Differences between two groups were analyzed using Student's *t* test. Differences with *p* values less than 0.05 were considered statistically significant.

3 | RESULTS

3.1 | Differentially expressed circRNAs in CE pericyst tissues

Paired samples from four patients with similar clinical characteristics were selected for microarray analysis. All patients were diagnosed with CE, male, and aged 27–51 years (average age: 38 years) (Table 2). The circRNA expression profiles in CE pericysts and adjacent normal liver tissues are displayed in Figure 1A. Based on the microarray analysis, there were a total of 343 differentially expressed circRNAs in the CE pericyst tissues; 177 were significantly upregulated, and 166 were significantly downregulated (Figure 1B). The top 10 abnormally expressed circRNAs were summarized in Tables 3 and 4. Moreover, hierarchical clustering revealed that all differentially expressed circRNAs could clearly identify the CE pericyst and adjacent normal liver tissues (Figure 1C).

3.2 | qRT-PCR validation of differentially expressed circRNAs

Based on the criteria of a fold change ≥ 2, normalized signal intensity > 7, and false discovery rate < 0.05, we selected 15 differentially expressed circRNAs and verified their expression in CE pericystic and adjacent normal liver tissues by qRT-PCR. We detected significant

TABLE 2 Clinical characteristics of all four patients

	Patient 1	Patient 2	Patient 3	Patient 4
Age (years)	38	51	27	42
Sex	Male	Female	Male	Male
Location	VI	VI VII	II III	VIII
Size (cm)	7 × 6	8 × 6	5 × 8	11 × 9
Cyst types	CE1	CE3	CE2	CE2
Complication (Clavien-Dindo classification)	Grade I	Grade I	Grade I	Grade I
Type of surgery	Cystectomy	Cystectomy	Hepatectomy	Cystectomy
Anthelmintic therapy	No	No	No	No

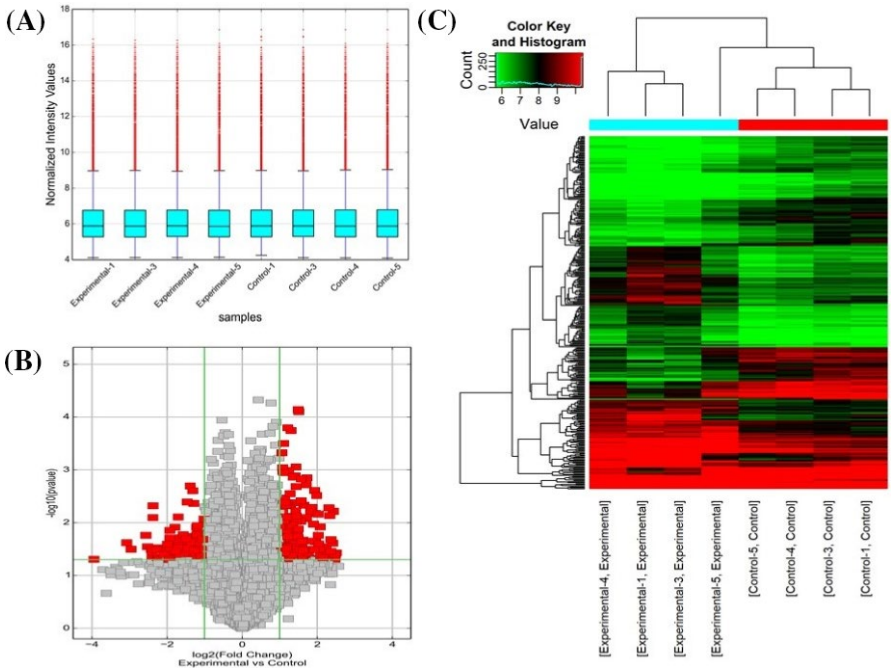

FIGURE 1 CircRNA microarray expression data between pericystic tissue of CE and adjacent normal liver tissue. (A) Normalized data were plotted as a Box plot to determine the overall characteristics of circRNA distribution. (B) Volcano plot analysis based on circRNA expression levels. The vertical blue lines represent 2.0-fold change, and the horizontal blue line represents $p < 0.05$. The red points represent upregulated and downregulated circRNAs in CE, respectively. (C) Hierarchical clustering of differentially expressed circRNAs between pericyst tissue of CE and adjacent normal liver tissue. The color bar indicates gene with expression intensity, high level of circRNA is shown in red, and low level is shown in green

differences in the expression levels of four circRNAs: hsa_circRNA_006773, hsa_circRNA_049637, hsa_circRNA_104349, and hsa_circRNA_406281 (Figure 2).

3.3 | Construction of a ceRNA network

To verify whether the detected differentially expressed circRNAs could function as ceRNAs, regulating the expression levels of target miRNAs and mRNAs, we performed a bioinformatic analysis to predict the miRNA response elements that they target. Notably, all four differentially expressed circRNAs were predicted to act as ceRNAs, affecting the expression of 319 miRNAs and 52 mRNAs (Figure 3).

3.4 | GO and KEGG pathway enrichment analysis of target genes of the differentially expressed circRNAs

Enrichment analysis of Gene Ontology (GO) categories and Kyoto Encyclopedia of Genes and Genomes (KEGG) biological pathways provided an overview of the potential functions of the target genes of differentially expressed circRNAs. The KEGG pathways

TABLE 3 Top 10 upregulated circRNAs in microarray and biological information

circRNA	chrom	circRNA type	Gene symbol	Fold change	*p* value
hsa_circRNA_001654	chr6	intronic	CNPY3	5.6753566	0.037355484
hsa_circRNA_103361	chr3	exonic	SMARCC1	5.5802604	0.040923063
hsa_circRNA_001490	chr5	exonic	KIF2A	5.541695	0.0493015
hsa_circRNA_104310	chr7	exonic	ZDHHC4	5.5193179	0.039805039
hsa_circRNA_100395	chr1	exonic	KLHL20	5.3812305	0.006083363
hsa_circRNA_102485	chr19	exonic	PGPEP1	5.3153808	0.024395294
hsa_circRNA_001459	chr11	intronic	SPI1	5.1365428	0.020894153
hsa_circRNA_104193	chr6	exonic	AHI1	5.1277631	0.034957804
hsa_circRNA_400043	chr19	intronic	CACTIN	5.0344456	0.007309864
hsa_circRNA_006773	chr7	exonic	HIBADH	4.9575465	0.005244304

TABLE 4 Top 10 downregulated circRNAs in microarray and biological information

circRNA	chrom	circRNA type	Gene symbol	Fold change	*p* value
hsa_circRNA_400633	chr10	exonic	SCD	15.4104085	0.049336666
hsa_circRNA_404974	chr12	exonic	SOX5	8.415951	0.023889853
hsa_circRNA_068482	chr3	exonic	ST6GAL1	7.7567368	0.03173318
hsa_circRNA_100974	chr11	exonic	ARHGEF12	5.9132985	0.017305558
hsa_circRNA_049637	chr19	exonic	CALR	5.7364489	0.028494207
hsa_circRNA_404798	chr10	exonic	DNMBP	5.3713019	0.032326286
hsa_circRNA_400064	chr21	intronic	PWP2	5.2920082	0.043770596
hsa_circRNA_004045	chr18	exonic	ATP5A1	5.2821791	0.048581929
hsa_circRNA_101379	chr14	exonic	SMOC1	5.2632059	0.036183956
hsa_circRNA_016771	chr1	exonic	OBSCN	5.2036355	0.007994328

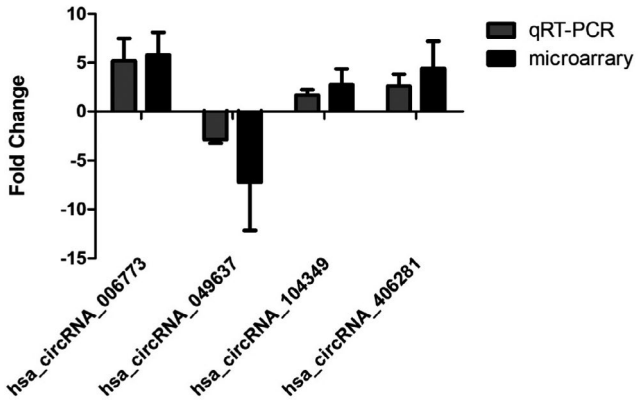

FIGURE 2 qRT-PCR validation of expression levels of circRNAs identified by microarray analysis. The error bar stands for standard deviations

for the enriched target genes were associated with several cellular processes, including inflammation, cell stress response, cell differentiation, cell division, cell proliferation, metabolism, motility, and apoptosis (via the MAPK, cAMP, PI3K-AKT, JAK-STAT, and cGMP-PKG signaling pathways), and immunologic processes (ie, Th1 and Th2 cell differentiation and Th17 cell differentiation), and other pathways, including the PD-L1 expression and PD-1 checkpoint pathway in cancer, focal adhesion, and ECM-receptor interaction. The significantly enriched KEGG pathways are displayed in Figure 4. GO analysis showed that these target genes were mainly associated with the response to organic cyclic compounds and endogenous stimuli as well as cellular organismal processes (Figure 5).

4 | DISCUSSION

CE pericystic tissues, which may be derived from fibrotic tissues surrounding the laminated layer of *E. granulosus* metacestodes, play an important role in the development, abortion, and degradation of the parasite.[30] As some miRNAs have been shown to be related to the development of CE, the aim of the current study was to examine the expression profile of circRNAs, a type of non-coding RNA that might regulate miRNA expression, in CE pericystic tissues.

Previous studies have shown that miRNA profiles change when intermediate hosts are infected with *E. granulosus*; in

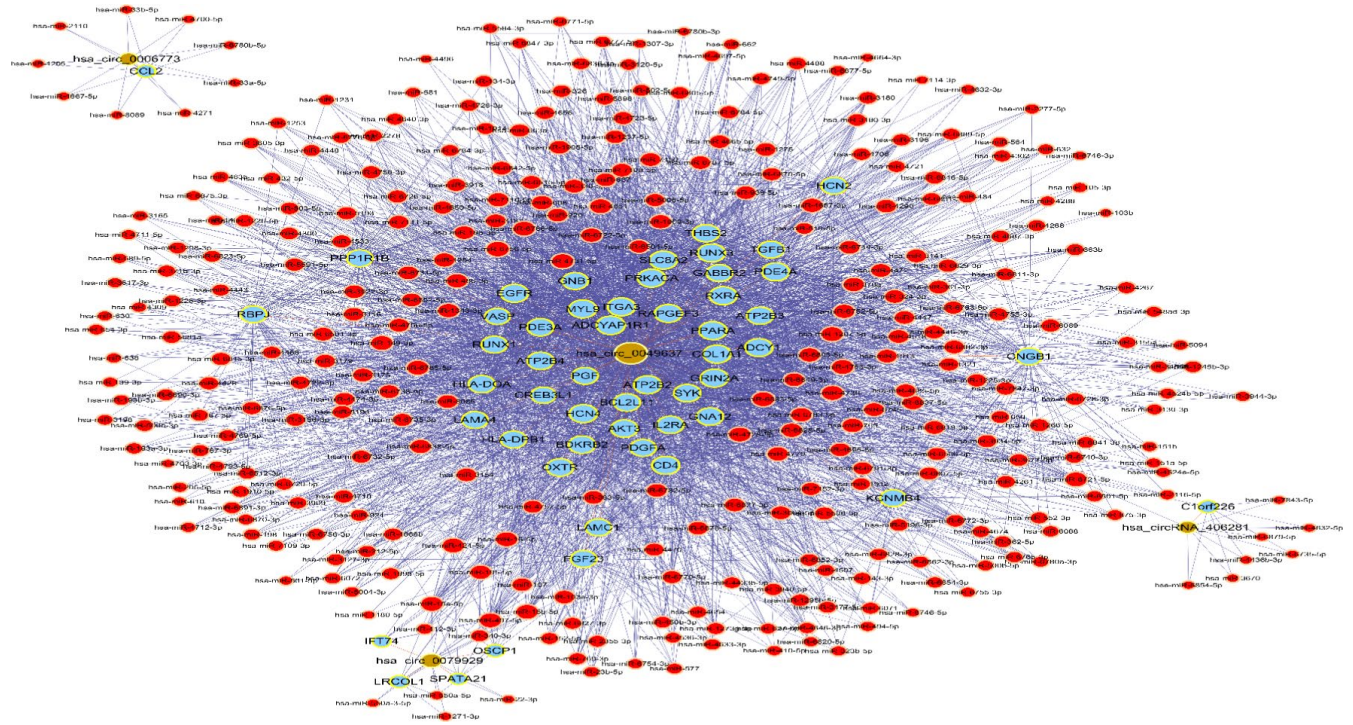

FIGURE 3 ceRNA network of the four circRNAs

particular, miR-27a, miR-542-5p, miR-134-5p, miR-21-3p, miR-26b, and miR-671, which are related to the inflammation process, are significantly more highly expressed in CE-resistant sheep than in non-CE-resistant sheep.[32] Moreover, reduced miR-19b levels promote pericystic tissue formation during CE by inducing fibrosis through increased expression of TβRII, activation of hepatic stellate cells, and production of ECM.[30] A previous report highlighted the differentially expressed lncRNAs and mRNAs between splenic monocytic myeloid-derived suppressor cells of *E. granulosus* protoscolex-infected mice and normal mice.[29] Further research on the functional mechanisms suggested that lncRNAs play a role in the immune response of the intermediate hosts, mice, against *E. granulosus* and might be useful as specific biomarkers for CE.[29]

Similarly, in this study, we detected 343 differentially expressed circRNAs in CE pericystic tissues; 177 were significantly upregulated, and 166 were significantly downregulated. Through qRT-PCR validation, the expression profiles of four key circRNAs were confirmed in another four paired tissue sets. Therefore, we propose that these circRNAs are derived from hub genes implicated in the pathogenesis of CE.

Because circRNAs can function as ceRNAs, regulating the expression of miRNA target genes by sequestering miRNAs,[31] we analyzed the sequences of the differentially expressed circRNAs to predict their miRNA binding sites. Bioinformatic analysis predicted a total of 139 miRNAs and 52 mRNAs as putative targets of the differentially expressed circRNAs, suggesting the involvement of complex circRNA-miRNA-mRNA networks in CE development. Among the identified differentially expressed circRNAs, hsa-miR-1912 may be an important candidate for further study.[30]

KEGG pathway enrichment analysis of the circRNA target genes showed an overrepresentation of genes involved several crucial pathways, including the MAPK signaling pathway, Th17 cell differentiation, and Th1 and Th2 cell differentiation; these pathways have already been reported to be associated with *Echinococcus* infection.[33,34] Other pathways identified by our analysis, such as the cAMP signaling pathway, PD-L1 expression and PD-1 checkpoint pathway in cancer, and cGMP-PKG signaling pathway, are potentially associated with CE.[35] In fact, secreted proteins that are highly expressed by *E. granulosus* or *Echinococcus lactis* in the adult stage and are delivered via vesicles can inhibit, hijack, and modify the host immune response by influencing host cytokine signaling and other immune signal transduction pathways or by inhibiting the activity of important enzymes, thus providing an anti-inflammatory environment for *E. granulosus* and facilitating its survival. Therefore, studying the signaling pathways related to *E. granulosus* infection is important for diagnosis, treatment, and prevention.[36]

In conclusion, the present study suggested that some circRNAs are differentially expressed in CE pericystic tissue. We showed their potential involvement in the development of CE in humans, thereby laying a foundation for future research on the potential roles of circRNAs in the pathogenesis of CE.

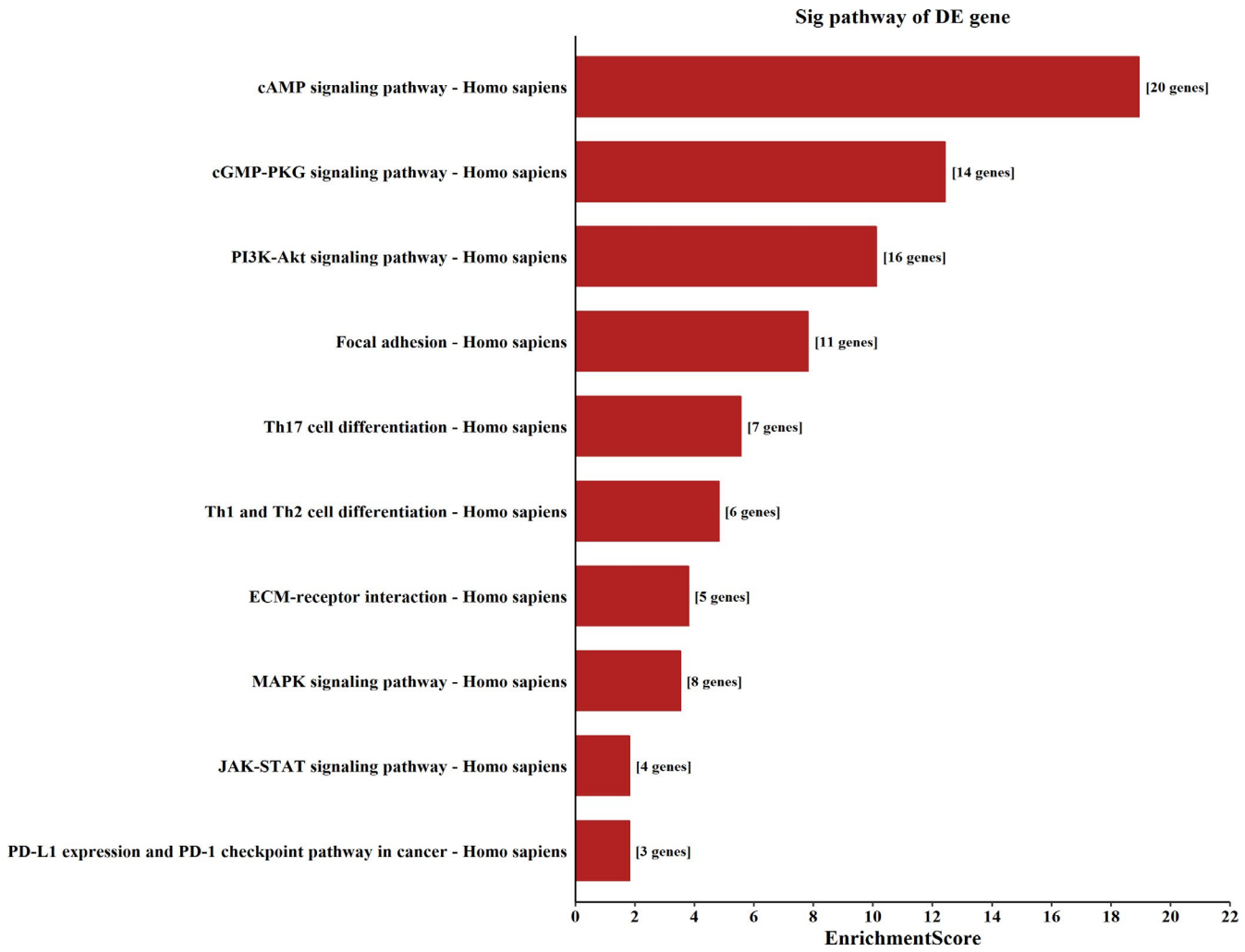

FIGURE 4 Top 10 significant KEGG pathway of the target genes of miRNAs interacted with differentially expressed circRNAs

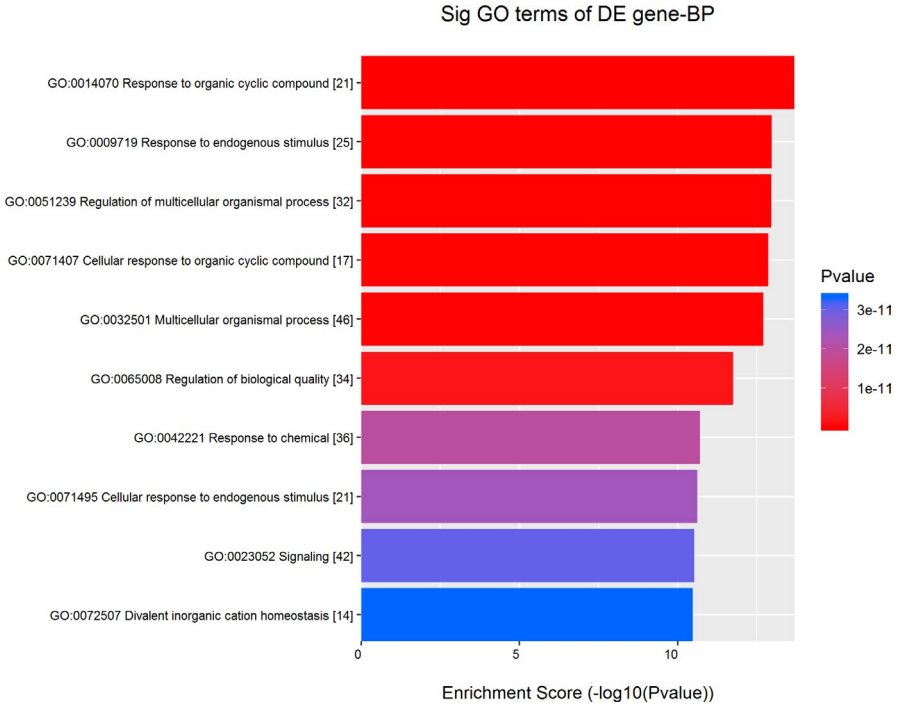

FIGURE 5 Top 10 significant GO biological processes of the target genes of miRNAs interacted with differentially expressed circRNAs

DATA AVAILABILITY STATEMENT

All data supporting the conclusions were shown in this article.

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REFERENCES

1. Deplazes P, Rinaldi L, Rojas CA, et al. Global distribution of alveolar and cystic echinococcosis. *Adv Parasitol*. 2017;95:315-493.
2. Zhang W, Wang S, Mcmanus DP. Echinococcus granulosus genomics:a new dawn for improved diagnosis, treatment, and control of echinococcosis. *Parasite-Journal De La Societe Francaise De Parasitologie*. 2014;21:66.
3. Craig PS, Mcmanus DP, Lightowlers MW, et al. Prevention and control of cystic echinococcosis. *Lancet Infect Dis*. 2007;7(6):385-394.
4. Budke CM, Carabin H, Ndimubanzi PC, et al. A systematic review of the literature on cystic echinococcosis frequency worldwide and its associated clinical manifestations. *AmJTrop Med Hyg*. 2013;88:1011-1027.
5. Gharbi HA, Hassine W, Brauner MW, et al. Ultrasound examination of the hydatid liver. *Radiology*. 1981;139(2):459–463.
6. McManus DP, Zhang W, Li J, et al. Echinococcosis. *Lancet*. 2003;362:1295-1304.
7. Mandal S, Mandal MD, Human cystic echinococcosis: epidemiologic, zoonotic, clinical, diagnostic and therapeutic aspects. *Asian Pac J Trop Med*. 2012;5:253-260.
8. Ahn CS, Han X, Bae YA, et al. Alteration of immune proteome profile of Echinococcus granulosus hydatid fluid with progression of cystic echinococcosis. *Parasit Vectors*. 2015;8:10.
9. Peng XY, Wu XW, Zhang SJ, et al. The new aspects in recognition of pathogenesis of the pericyst of human hepatic *Echinococcus granulosus*. *World Chin J Digestol*. 2005;13:276-279.
10. Golzari SE, Sokouti M. Pericyst: the outermost layer of hydatid cyst. *World J Gastroenterol*. 2014;20:1377-1378.
11. Peng X, Li J, Wu X, et al. Detection of Osteopontin in the pericyst of human hepatic *Echinococcus granulosus*. *Acta Trop*. 2006;100:163-171.
12. Romic B, Romic I, Petrovic I, Romic M, Romic R, Mance M, Pavlek G. A synchronous occurrence of hepatocellular carcinoma and echinoccocal liver cyst - can parasite promote carcinogenesis? Literature review and classification proposal. *Chirurgia (Bucur)*. 2016;111(4):297-303.
13. Vuitton DA. The ambiguous role of immunity in echinococcosis: protection of the host or of the parasite? *Acta Trop*. 2003;85:119-132.
14. Anthony B, Allen JT, Li YS, et al. Hepatic stellate cells and parasiteinduced liver fibrosis. *Parasit Vectors*. 2010;3:60.
15. Bala S, Marcos M, Szabo G. Emerging role of microRNAs in liver diseases. *World J Gastroenterol*. 2009;15:5633-5640.
16. Friedman SL. Evolving challenges in hepatic fibrosis. *Nat Rev Gastroenterol Hepatol*. 2010;7:425-436.
17. Xie Y, Yao L, Yu X, Ruan Y, Li Z, Guo J. Action mechanisms and research methods of tRNA-derived small RNAs. *Signal Transduct Target Ther*. 2020;5(1):109.
18. Mo X, Wu Y, Chen L, et al. Global expression profiling of Han J, Shen X. Long noncoding RNAs in osteosarcoma via various signaling pathways. *J Clin Lab Anal*. 2020;34(6):e23317.
19. Wang Y, Li Z, Xu S, Guo J. Novel potential tumor biomarkers: Circular RNAs and exosomal circular RNAs in gastrointestinal malignancies. *J Clin Lab Anal*. 2020;34(7):e23359.
20. Ruan Y, Li Z, Shen Y, Li T, Zhang H, Guo J. Functions of circular RNAs and their potential applications in gastric cancer. *Expert Rev Gastroenterol Hepatol*. 2020;14(2):85-92.
21. Zhang H, Shen Y, Li Z, et al. The biogenesis and biological functions of circular RNAs and their molecular diagnostic values in cancers. *J Clin Lab Anal*. 2020;34(1):e23049.
22. Nair AA, Niu N, Tang X, et al. Circular RNAs and their associations with breast cancer subtypes. *Oncotarget*. 2016;7:80967-80979.
23. Shang X, Li G, Liu H, et al. Comprehensive circular RNA profiling reveals that hsa_circ_0005075, a new circular RNA biomarker, is involved in hepatocellular crcinoma development. *Medicine (Baltimore)*. 2016;95(22):e3811.
24. Burd CE, Jeck WR, Liu Y, et al. Expression of linear and novel circular forms of an INK4/ARF-associated non-coding RNA correlates with atherosclerosis risk. *PLoS Genet*. 2010;6:e1001233.
25. Zhao Z, Li X, Gao C, et al. Peripheral blood circular RNA hsa_circ_0124644 can be used as a diagnostic biomarker of coronary artery disease. *Sci Rep*. 2017;7:39918.
26. Zhao Z, Li X, Jian D, et al. Hsa_circ_0054633 in peripheral blood can be used as a diagnostic biomarker of pre-diabetes and type 2 diabetes mellitus. *Acta Diabetol*. 2017;54:237-245.
27. Cui X, Niu W, Kong L, et al. Hsa_circRNA_103636: potential novel diagnostic and therapeutic biomarker in Major depressive disorder. *Biomark Med*. 2016;10:943-952.
28. Leire I, Maider MC, Iñigo P, et al. Circular RNA profiling reveals that circular RNAs from ANXA2 can be used as new biomarkers for multiple sclerosis. *Hum Mol Genet*. 2017;26:3564-3572.
29. He Z, Yan T, Yuan Y, et al. miRNAs and lncRNAs in Echinococcus and Echinococcosis. *Int J Mol Sci*. 2020;21(3):730.
30. Zhang C, Wang L, Ali T, et al. Hydatid cyst fluid promotes peri-cystic fibrosis in cystic echinococcosis by suppressing miR-19 expression. *Parasit Vectors*. 2016;9:278.
31. Hansen TB, Jensen TI, Clausen BH, et al. Natural RNA circles function as efficient microRNA sponges. *Nature*. 2013;495(7441):384-388.
32. Jiang S, Li X, Wang X, et al. MicroRNA profiling of the intestinal tissue of Kazakh sheep after experimental *Echinococcus granulosus* infection, using a high-throughput approach. *Parasite*. 2016;23:23.
33. Tsai IJ, Zarowieeki M, Holroyd N, et al. The genomes of four tapeworm species reveal adaptations to parasitism. *Nature*. 2013;496(7443):57-63.
34. Brehm K. The role of evolutionarily conserved signalling systems in *Echinococcus multilocularis* development and host parasite interaction. *Med Microbial Immunol*. 2010;199(3):247-259.
35. Zheng HJ, Zhang WB, Zhang L, et al. The genome of the hydatid tapeworm *Echinococcus granulosus*. *Nat Genet*. 2013;45(10):1168-1175.
36. Wen H, Vuitton L, Tuxun T, et al. Echinococcosis: Advances in the 21st century. *Clin Microbiol Rev*. 2019;32(2):e00075–18.

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