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Microarray expression profile of circular RNAs in chronic thromboembolic pulmonary hypertension

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Abstract

Background: Chronic thromboembolic pulmonary hypertension (CTEPH) is a rare but debilitating and life-threatening complication of acute pulmonary embolism. Circular RNAs (circRNAs), presenting as covalently closed continuous loops, are RNA molecules with covalently joined 3′- and 5′-ends formed by back-splicing events. circRNAs may be significant biological molecules to understand disease mechanisms and to identify biomarkers for disease diagnosis and therapy. The aim of this study was to investigate the potential roles of circRNAs in CTEPH.

Methods: Ten human blood samples (5 each from CTEPH and control groups) were included in the Agilent circRNA chip. The differentially expressed circRNAs were evaluated using *t* test, with significance set at a *P* value of $< .05$. A functional enrichment analysis for differentially expressed circRNAs was performed using DAVID online tools, and a Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis for target genes of miRNAs was performed using the R package clusterProfiler. Furthermore, miRNAs that interacted with differentially expressed circRNAs were predicted using the miRanda package. mRNAs that had clear biological functions and were regulated by miRNAs were predicted using miRWalk2.0 and then combined into a circRNA–miRNA–mRNA network.

Results: In total, 351 differentially expressed circRNAs (122 upregulated and 229 downregulated) between CTEPH and control groups were obtained; among these circRNAs, hsa_circ_0002062 and hsa_circ_0022342 might be important because they can regulate 761 (e.g., hsa-miR-942–5p) and 453 (e.g., hsa-miR-940) miRNAs, respectively. Target genes (e.g., cyclin-dependent kinase 6) of hsa-miR-942–5p were mainly enriched in cancer-related pathways, whereas target genes (e.g., CRK-Like Proto-Oncogene, Adaptor Protein) of hsa-miR-940 were enriched in the ErbB signaling pathway. Therefore, these pathways are potentially important in CTEPH.

Conclusions: Our findings suggested that hsa_circ_0002062 and hsa_circ_0022342 may be key circRNAs for CTEPH development and that their targeted regulation may be an effective approach for treating CTEPH.

Abbreviations: CircRNAs = Circular RNAs, CTEPH = Chronic thromboembolic pulmonary hypertension, GO = Gene Ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

Keywords: chronic thromboembolic pulmonary hypertension, circRNA–miRNA–mRNA network, circular RNAs, functional enrichment

1. Introduction

Chronic thromboembolic pulmonary hypertension (CTEPH), a rare but debilitating and life-threatening complication of acute pulmonary embolism, is caused by persistent obstruction of pulmonary arteries and progressive vascular remodeling.[1] The estimated prevalence of CTEPH is 0.1% to 9.1% at 2 years after

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The authors identified 351 differentially expressed circRNAs in CTEPH compared with those in the control group.

Ten miRNAs were regulated by circRNAs, which then regulated target genes in the network.

CircRNAs may have potential roles in the diagnosis and treatment of CTEPH.

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acute pulmonary embolism.[2,3] The risk factors for CTEPH include some clinical disorders, such as inflammatory bowel disease, splenectomy, and myeloproliferative disorders.[4–7] Patients with CTEPH exhibit a poor prognosis unless they receive treatment at an early stage.[8] Thus, early diagnosis and treatment of this disease are important.

Circular RNAs (circRNAs), presenting as covalently closed continuous loops, are RNA molecules with covalently joined 3′- and 5′-ends formed by back-splicing events.[9] Recently, circRNAs have increasingly been recognized as an abundant class of regulatory transcripts primarily derived from protein-coding exons.[10,11] CircRNAs are characterized by scrambled exons, and until 20 years ago, they were mostly misinterpreted as resulting from splicing errors.[12] Only recently, circRNAs have been rediscovered from RNA-seq data.[10,11,13] Further, circRNAs functioning as sponges of miRNAs are believed to regulate the expression of miRNA targets, thus contributing to the competing endogenous RNA network.[14] Some studies have demonstrated that CDR1as functions as a sponge for miR-7, whereas circRNA generated from the Sry gene (circSry) functions as a sponge for miR-138.[15,16] In addition, circRNAs play key roles in diseases, especially in cancer, and thus constitute new potential diagnostic and therapeutic targets for diseases.[17,18] Lukiw [19] have suggested that deficits in other circRNA-mediated "miRNA sponging systems" and ambient upregulation of specific inducible miRNAs help in explaining downregulated gene expression in the brains of patients with sporadic Alzheimer disease. Some other studies have indicated that circRNAs are involved in the development of nervous system disorders and atherosclerosis.[20,21] Therefore, circRNAs may be significant biological molecules to understand disease mechanisms and to identify biomarkers for disease diagnosis and therapy.[14]

To investigate the potential roles of circRNAs in CTEPH, we performed a microarray analysis to identify differentially expressed circRNAs in a CTEPH group compared with those in a control group. These circRNAs were then subjected to functional enrichment analyses, including Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. Furthermore, miRNAs regulated by differentially expressed circRNAs were predicted, and enrichment analyses for the target genes of these miRNAs were also performed.

2. Methods

2.1. CircRNA expression profile data

Peripheral blood samples from 5 CTEPH patients at Beijing Chao-Yang Hospital, Capital Medical University, and 5 healthy controls who had undergone a routine physical examination at a physical examination center from March 2016 to April 2016 were collected. The criterion for inclusion in the CTEPH group was a previous diagnosed of CTEPH by right heart cardiac catheterization, ventilation-perfusion lung scans, or computed tomography (CT) pulmonary angiography (CTPA). The criterion for exclusion from the CTEPH group was the presence of a disease of the circulatory system, such as malignant tumor, hypertension, diabetes mellitus, coronary heart disease, or cerebrovascular disease. The control group comprised healthy controls with age and sex matched to those in the CTEPH group and normal routine blood test/routine urine test/biochemical test/carcinoembryonic antigen (CEA)/alpha-fetoprotein (AFP)/erythrocyte sedimentation rate (ESR)/chest X-ray. This study was approved by the Ethics Committee of Beijing Chao-Yang Hospital, Capital Medical University. This study was approved by the ethics review board of our hospital, and the requirement to obtain informed written consent was waived. Demographic information on the patients is summarized in Table 1. No significant difference was observed in risk factors for CTEPH between the CTEPH and control groups ($P > .1$).

Total RNAs were extracted from the samples using RNAprep Pure Blood Kit (Tiangen Biotech Co., Ltd., Beijing, China) according to the manufacturer's protocol. First-strand cDNA was synthesized using first-strand enzyme mix with random primers containing the T7 RNA polymerase promoter sequence. RNA in the DNA–RNA hybrid was transcribed to second-strand cDNA, and double-stranded DNA was synthesized. Subsequently, using second-strand cDNA as a template, cRNA was synthesized using T7 enzyme mix. Next, cRNA was purified and subjected to quantification and quality control. Reverse transcription of cRNA was then performed using random primers and CbcScript II reverse transcriptase. The obtained cDNA was purified and quantified. Subsequently, using cDNA, random primers, and DNA polymerase (Klenow fragment), the other chain of cDNA was synthesized and fluorescently labeled with dNTP (Cy3-dCTP). Lastly, the products were purified and quantified. This fluorescently labeled DNA was used subsequently for microarray hybridization.

2.2. Screening of differentially expressed circRNAs

CircRNA gene expression profiles (Agilent circRNA chip) were preprocessed using the Feature Extraction package, and subsequently, the data were normalized using the GeneSpring GX package. The CTEPH and control group samples could be clearly identified in the expression profile matrix file. Subsequently, the differentially expressed circRNAs between the 2 groups were evaluated using *t* test, with statistical significance set at a P value of $<.05$.

2.3. miRNA prediction of differentially expressed circRNAs

CircRNAs can regulate mRNA translation indirectly via combining with targeted miRNAs. The miRNA prediction of differentially expressed circRNAs was performed using the miRanda package (http://www.microrna.org/microrna/home.do).[22] The top 10 key target miRNAs based on the number of circRNAs associated with them were selected for analysis. On the basis of these findings, the miRNA–circRNA network was constructed using Cytoscape software.[23]

2.4. Construction of the circRNA–miRNA–mRNA network

mRNAs that had clear biological functions and were regulated by miRNAs were predicted using miRWalk2.0 (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/).[24,25] The results were also combined with the former predicted miRNA–circRNA results into a circRNA–miRNA–mRNA network.

2.5. Functional enrichment analysis for differentially expressed circRNAs and target genes of miRNAs

GO is a tool used for gene annotation by collecting defined, structured, controlled vocabulary.[26] KEGG is a database used to categorize associated gene sets into appropriate pathways.[27] DAVID is an integrated data-mining environment to analyze gene lists.[28]

Table 1

The baseline characteristics of the 10 samples.

	Sex	Collection date	Age, y	Clinical description	BMI, kg/m^2	Family history of blood clots	Smoking	Long periods of inactivity	Other CTEPH risk factors
CTEPH group									
160039E&H-1	Male	March 2016	41	CTEPH	25.99	No	15 y, quit smoking for 3 y	No	No
160039E&H-3	Female	March 2016	53	CTEPH	27.99	No	No	No	Unilateral lower extremity edema, a year before onset
160039E&H-4	Female	March 2016	71	CTEPH	18.96	No	No	No	Varicosity
160039E-2	Male	March 2016	67	CTEPH	23.66	No	40 y, quit smoking for 4 y	No	No
160039G-1	Male	April 2016	55	CTEPH	17.58	No	30 y, not quit	No	Deep venous thrombosis of lower extremities in 20 y
Control group									
160039F-1	Male	April 2016	50	No abnormal for routine urine and blood tests, biochemistry test, CEA, AFP, ESR, chest radiography	—	No	—	No	No
160039F-2	Male	April 2016	56	No abnormal for routine urine and blood tests, biochemistry test, CEA, AFP, ESR, chest radiography	—	No	—	No	No
160039F-3	Female	April 2016	71	No abnormal for routine urine and blood tests, biochemistry test, CEA, AFP, ESR, chest radiography	—	No	—	No	No
160039F-4	Female	April 2016	64	No abnormal for routine urine and blood tests, biochemistry test, saccharification test, CEA, AFP, ESR, chest radiography	—	No	—	No	No
160039G-2	Male	April 2016	50	No abnormal for routine urine and blood tests, biochemistry test, CEA, AFP, ESR, chest radiography	—	No	—	No	No
Fisher (*P*)	1.000		.908		—	—	—	1.000	.167

For smoking, we did not investigate this information for control group, but there was no correction between smoking and CTEPH according to previous studies. For BMI, we did not investigate this information for control group. 160039E&H-1, 160039E&H-3, 160039E&H-4, 160039E-2, 160039G-1, 160039F-1, 160039F-2, 160039F-3, 160039F-4, and 160039G-2 were chip number.

GO and KEGG pathway enrichment analyses were performed using DAVID online tools (Version 6.8, https://david-d.ncifcrf.gov/), with the classification stringency set to "medium." *P* value of <.05 and a count of >2 were regarded as the cut-off values.

KEGG pathway enrichment analysis for target genes of miRNAs was performed using the R package clusterProfiler.[29] A *P* value of <.01 was set as the threshold value.

3. Results

3.1. Screening of differentially expressed circRNAs

In the CTEPH group, 351 differentially expressed circRNAs (122 upregulated and 229 downregulated) compared with those in the control group were identified. The heat map is shown in Fig. 1, and information on these 351 differentially expressed circRNAs is summarized in Supplemental Table 1, http://links.lww.com/MD/B772.

3.2. miRNA prediction of differentially expressed circRNAs

The results for predicting miRNAs associated with differentially expressed circRNAs (limited to circRNAs with >100 predicted associated miRNAs) are summarized in Table 2. The circRNAs hsa_circ_0002062 and hsa_circ_0022342 were associated with most miRNAs (761 and 453, respectively).

The miRNA–circRNA network is shown in Fig. 2. The 23 circRNAs shown in Fig. 2 are the differentially expressed circRNAs associated with >100 miRNAs provided in Table 2. From their status, we inferred that these 23 circRNAs were the key circRNAs. These 23 key circRNAs and the miRNAs regulated by them are summarized in Table 3. hsa_circ_0011232, hsa_circ_0017198, hsa_circ_0047515, hsa_circ_0051769, hsa_circ_0064391, hsa_circ_0076020, hsa_circ_0026738, hsa_circ_0047474, hsa_circ_0053212, and hsa_circ_0058098 regulated hsa-miR-939–5p; hsa_circ_0016070, hsa_circ_0002062, and hsa_circ_0084265 regulated hsa-miR-

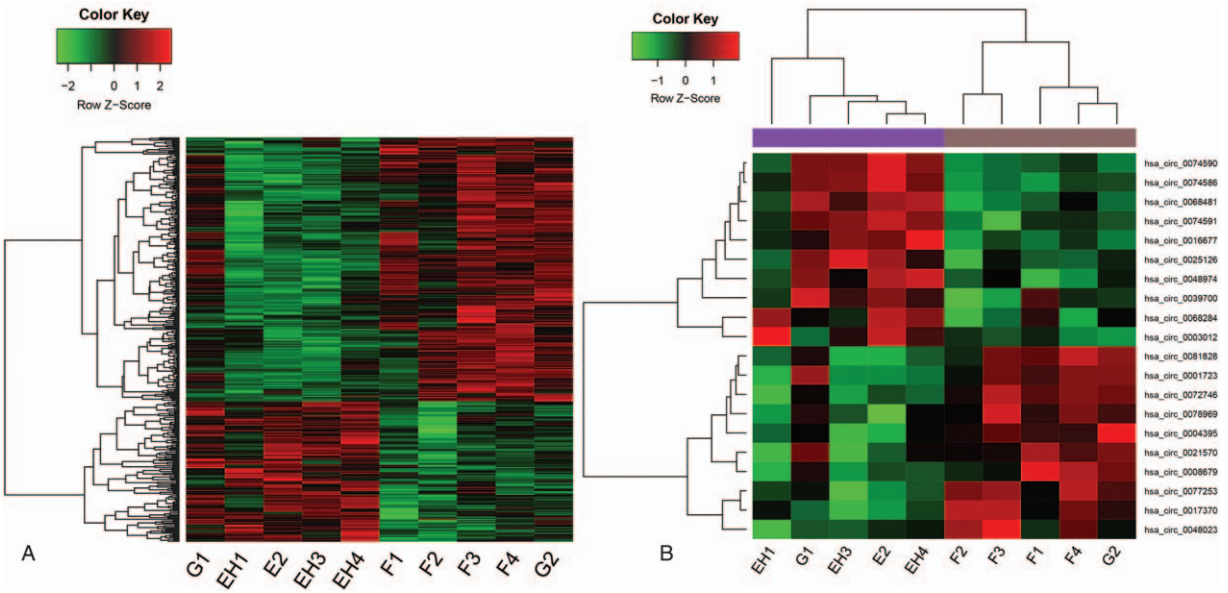

Figure 1. (A) A heat map of 351 differentially expressed circRNAs. (B) A heat map of the top 10 upregulated and top 10 downregulated differentially expressed circRNAs. Green represents a low expression level, whereas red represents a high expression level.

942–5p; hsa_circ_0049875, hsa_circ_0017373, hsa_circ_0022342, and hsa_circ_0042590 regulated hsa-miR-940; hsa_circ_0007311, hsa_circ_0051439, hsa_circ_0065157, and hsa_circ_0065158 regulated hsa-miR-92a-2–5p, and hsa_circ_0057797 and hsa_circ_0074900 regulated hsa-miR-939–3p. These circRNAs were related to cell movement and cell migration, as revealed by the enrichment analysis of the circRNA sequence annotation results.

3.3. Construction of the circRNA–miRNA–mRNA network

The target genes of miRNAs were predicted, and the circRNA–miRNA–mRNA network is shown in Fig. 3. It shows that each of the 10 miRNAs (hsa-miR-939–5p, hsa-miR-939–3p, hsa-miR-942–5p, hsa-miR-940, hsa-miR-92a-2–5p, hsa-miR-7107–3p, hsa-miR-7161–3p, hsa-miR-6831–5p, hsa-miR-7974, and hsa-miR-8089) were regulated by circRNAs, which then regulated the target genes of miRNAs.

3.4. Functional enrichment analysis for differentially expressed circRNAs and target genes of miRNAs

The results of GO and KEGG pathway enrichment analyses for differentially expressed circRNAs are summarized in Table 4. Upregulated circRNAs were mainly enriched in the purine ribonucleotide biosynthetic process and ribonucleotide biosynthetic process. Downregulated circRNAs were mainly enriched in cellular response to stress, post-transcriptional regulation of gene expression, response to DNA damage stimulus, DNA repair, and mRNA metabolic processes.

Furthermore, target genes of six miRNAs (hsa-miR-942–5p, hsa-miR-940, hsa-miR-939–3p, hsa-miR-92a-2–5p, hsa-miR-

Table 2

The results for miRNA prediction of differentially expressed circRNA with the number of miRNA greater than 100.

Name	Regulation	No. of miRNA targets	Name	Regulation	No. of miRNA targets
hsa_circ_0002062	down	761	hsa_circ_0047515	up	152
hsa_circ_0007400	down	735	hsa_circ_0086140	down	152
hsa_circ_0050087	down	595	hsa_circ_0008679	down	151
hsa_circ_0022342	down	453	hsa_circ_0018142	down	148
hsa_circ_0047474	down	420	hsa_circ_0017198	up	145
hsa_circ_0084265	down	344	hsa_circ_0064391	up	144
hsa_circ_0040799	down	343	hsa_circ_0065158	down	134
hsa_circ_0042590	down	280	hsa_circ_0049875	up	132
hsa_circ_0051439	down	274	hsa_circ_0074900	down	126
hsa_circ_0026738	down	267	hsa_circ_0065157	down	123
hsa_circ_0078982	up	233	hsa_circ_0080991	up	122
hsa_circ_0058098	down	210	hsa_circ_0053212	down	114
hsa_circ_0016070	up	190	hsa_circ_0076020	up	112
hsa_circ_0057797	down	175	hsa_circ_0087742	down	108
hsa_circ_0017373	down	172	hsa_circ_0011232	up	106
hsa_circ_0027194	up	168	hsa_circ_0007311	down	106
hsa_circ_0051769	up	157			

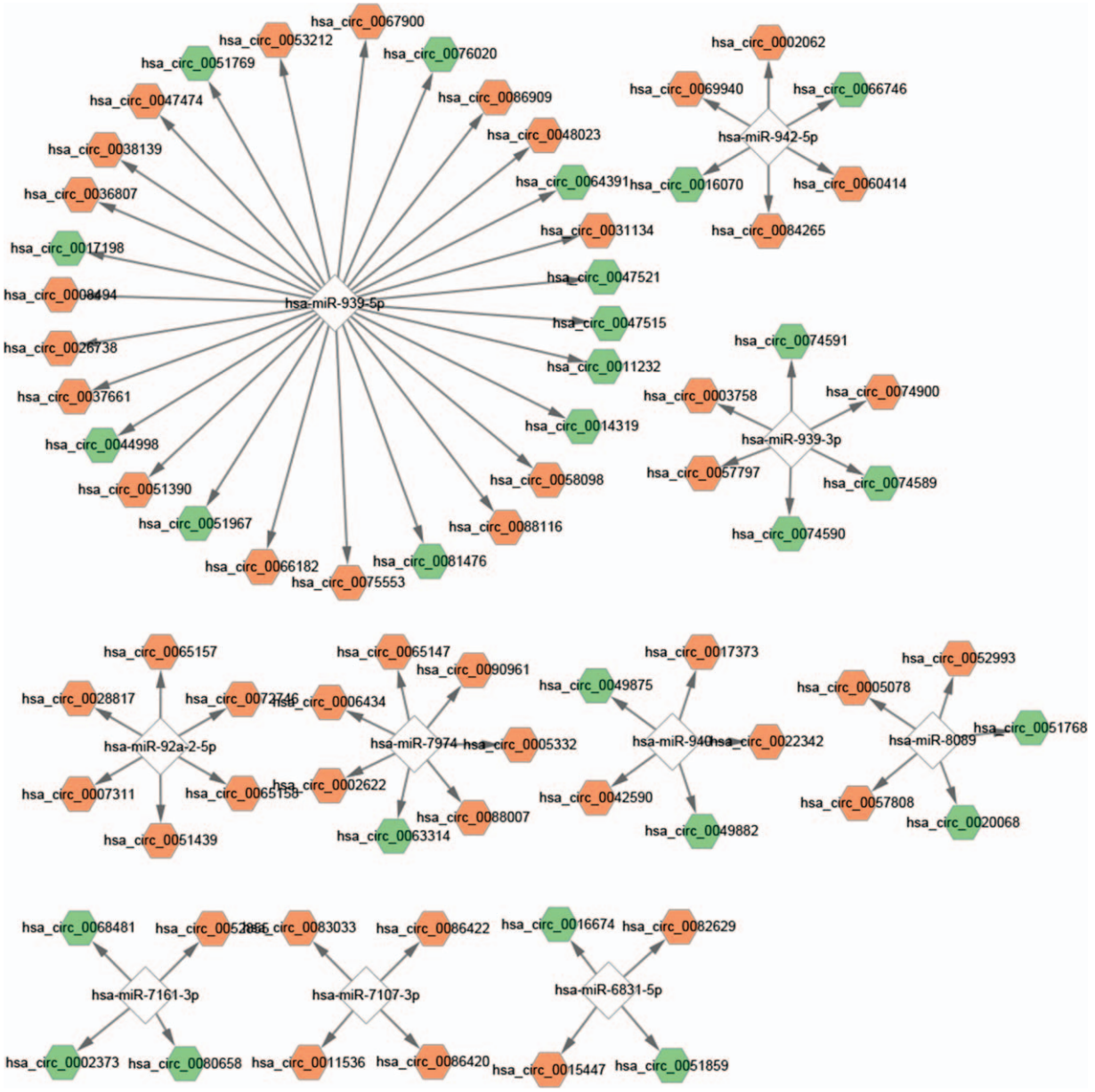

Figure 2. The miRNA–circRNA network. Green hexagon: upregulated differentially expressed circRNA; orange hexagon: downregulated differentially expressed circRNA; white diamond: miRNA.

8089, and hsa-miR-6831–5p) were significantly enriched into function terms (Fig. 4). For example, the target genes [e.g., cyclin-dependent kinase 6 (CDK6)] of hsa-miR-942–5p were mainly enriched in cancer-related pathways, whereas the target genes (e.g., CRK-Like Proto-Oncogene, Adaptor Protein) of hsa-miR-940 were enriched in the ErbB signaling pathway, among others (Supplemental Table 2, http://links.lww.com/MD/B773). Thus, these pathways were considered as potentially important in CTEPH.

4. Discussion

CircRNAs represent a new special class of endogenous noncoding RNAs. Some studies have shown that circRNAs can function as miRNA sponges or regulate parent gene expression to influence disease initiation and progression.[18,30] In the present study, we analyzed differentially expressed circRNAs in the CTEPH group compared with those in the control group. In total, 351 differentially expressed circRNAs (122 upregulated and 229 downregulated) were identified, which suggested that circRNAs play a role in CTEPH.

Furthermore, in the present study, upregulated circRNAs were found to be mainly enriched in the purine ribonucleotide biosynthetic process and ribonucleotide biosynthetic process. Downregulated circRNAs were mainly enriched in cellular response to stress, post-transcriptional regulation of gene expression, response to DNA damage stimulus, DNA repair, and mRNA metabolic process. Therefore, we inferred that upregulated circRNAs may function in CTEPH mainly by affecting the ribonucleotide biosynthetic process, whereas downregulated circRNAs may function mainly by regulating cellular response to stress, response to DNA damage stimulus, and gene

Table 3

The 23 key circRNAs and miRNAs regulated by these circRNAs.

miRNA	circRNAs	Gene_symbol	Regulate
hsa-miR-939–5p	hsa_circ_0011232	PUM1	Up
hsa-miR-942–5p	hsa_circ_0016070	ATP2B4	Up
hsa-miR-939–5p	hsa_circ_0017198	TCONS_l2_00002816	Up
hsa-miR-939–5p	hsa_circ_0047515	EPG5	Up
hsa-miR-940	hsa_circ_0049875	AP1M1	Up
hsa-miR-939–5p	hsa_circ_0051769	MAMSTR	Up
hsa-miR-939–5p	hsa_circ_0064391	NUP210	Up
hsa-miR-939–5p	hsa_circ_0076020	ITPR3	Up
hsa-miR-942–5p	hsa_circ_0002062	PTK2	Down
hsa-miR-92a-2–5p	hsa_circ_0007311	LRRC8A	Down
hsa-miR-940	hsa_circ_0017373	DIP2C	Down
hsa-miR-940	hsa_circ_0022342	SYT7	Down
hsa-miR-939–5p	hsa_circ_0026738	ITGA5	Down
hsa-miR-940	hsa_circ_0042590	KIAA0100	Down
hsa-miR-939–5p	hsa_circ_0047474	KIAA1328	Down
hsa-miR-92a-2–5p	hsa_circ_0051439	PPP1R37	Down
hsa-miR-939–5p	hsa_circ_0053212	CAD	Down
hsa-miR-939–3p	hsa_circ_0057797	BMPR2	Down
hsa-miR-939–5p	hsa_circ_0058098	FN1	Down
hsa-miR-92a-2–5p	hsa_circ_0065157	SETD2	Down
hsa-miR-92a-2–5p	hsa_circ_0065158	SETD2	Down
hsa-miR-939–3p	hsa_circ_0074900	SLIT3	Down
hsa-miR-942–5p	hsa_circ_0084265	PRKDC	Down

We made annotations for the obtained circRNAs, and the circRNAs were located on the chromosome. "Gene symbol" represents the genes name targeted by the position of circRNAs. "Regulate" represents the expression change of circRNAs. logFC>0 was defined as upregulation, and logFC<0 was defined as downregulation.

expression. However, additional studies to confirm these findings are warranted.

The circRNA–miRNA–mRNA network (Fig. 3) revealed that each miRNA (hsa-miR-939–5p, hsa-miR-939–3p, hsa-miR-942–5p, hsa-miR-940, hsa-miR-92a-2–5p, hsa-miR-7107–3p, hsa-miR-7161–3p, hsa-miR-6831–5p, hsa-miR-7974, and hsa-miR-8089) in this network is regulated by circRNAs, which in turn regulates the target genes of miRNAs. Furthermore, hsa_circ_0002062 and hsa_circ_0022342 targeted more miRNAs (761 and 453, respectively). To our knowledge, few studies have focused on these miRNAs, but miR-940 has been studied in several diseases, such as pancreatic ductal adenocarcinoma, hepatocellular carcinoma, and prostate cancer.[31–33] Furthermore, 1 study has reported that HIV-1 Vpr can induce miR-942–5p expression, activate NF-κB signaling, and inhibit Kaposi sarcoma associated herpesvirus lytic replication.[34] Rothman et al[35] highlighted the potential of using miRNA signatures as diagnostic and prognostic tools in pulmonary hypertension. Hansen et al[17] have suggested that miR-7 is closely coupled to ciRS-7 and that the fine-tuning of the miR-7/miR-671/ciRS-7 axis probably plays profound roles in diseases such as cancer. However, the functions of these potential miRNAs and circRNAs in CTEPH were unclear. Owing to our limited understanding of miRNAs and circRNAs, more studies on the interactions between

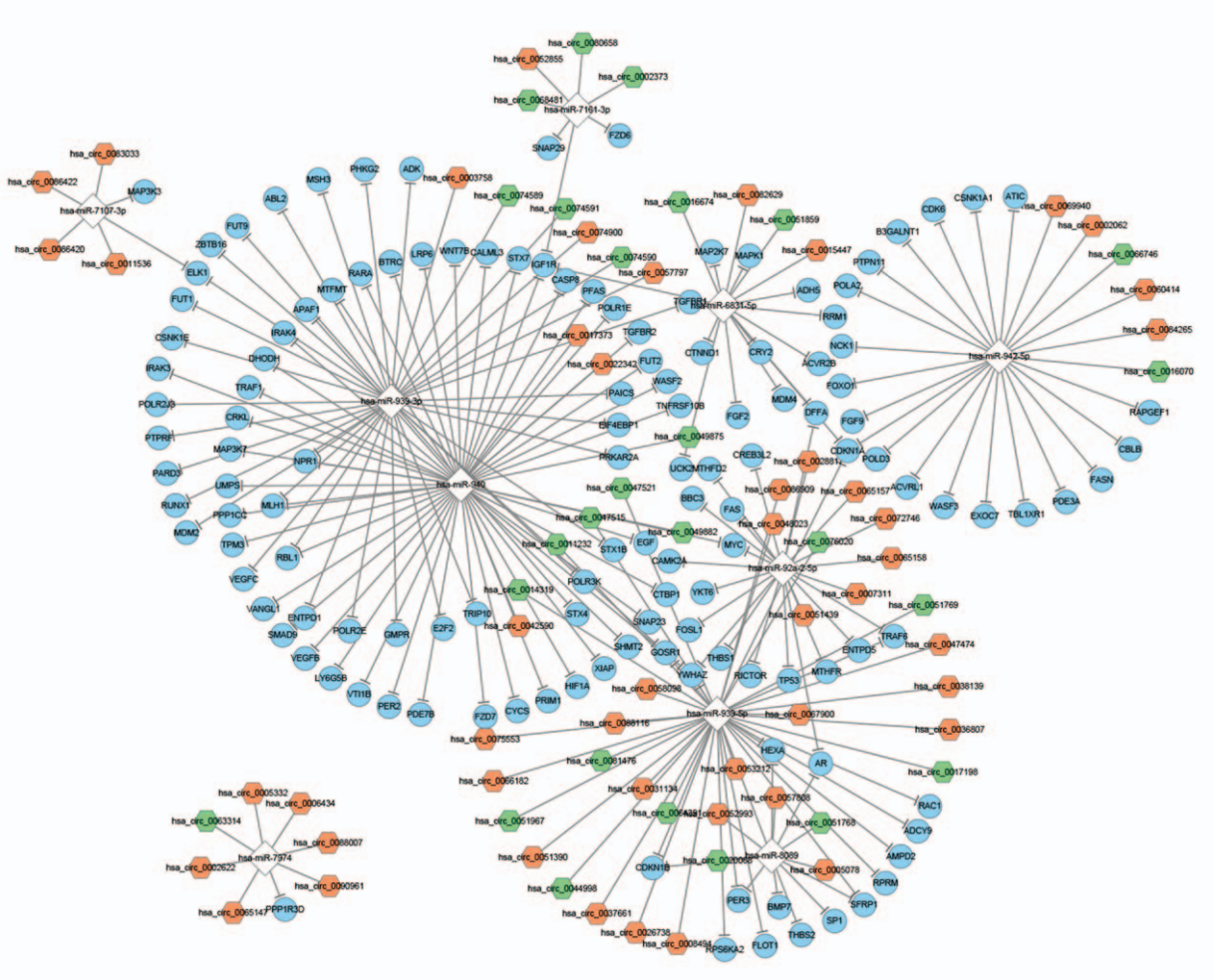

Figure 3. The circRNA–miRNA–mRNA network. Green hexagon: upregulated differentially expressed circRNA; orange hexagon: downregulated differentially expressed circRNA; white diamond: miRNA; blue circle: mRNA regulated by miRNA.

Table 4

GO and KEGG pathway enrichment analysis for differentially expressed circRNA.

Term	Description	Count	*P*
Upregulated			
GO_BP: 0009152	Purine ribonucleotide biosynthetic process	6	7.13E-4
GO_BP: 0009260	Ribonucleotide biosynthetic process	6	9.27E-4
GO_BP: 0009150	Purine ribonucleotide metabolic process	6	1.50E-03
GO_BP: 0006816	Calcium ion transport	6	1.70E-03
GO_BP: 0006812	Cation transport	11	1.80E-03
KEGG: hsa05010	Alzheimer disease	5	2.70E-02
KEGG: hsa04142	Lysosome	4	4.89E-02
Downregulated			
GO_BP:0033554	Cellular response to stress	20	1.45E-5
GO_BP:0010608	Posttranscriptional regulation of gene expression	12	2.16E-5
GO_BP:0006974	Response to DNA damage stimulus	15	6.41E-5
GO_BP:0006281	DNA repair	13	7.14E-5
GO_BP:0016071	mRNA metabolic process	13	8.05E-4
KEGG: hsa04662	B cell receptor signaling pathway	5	1.35E-02
KEGG: hsa04370	VEGF signaling pathway	5	1.35E-02
KEGG: hsa05222	Small cell lung cancer	5	1.97E-02
KEGG: hsa05215	Prostate cancer	5	2.38E-02
KEGG: hsa04062	Chemokine signaling pathway	7	2.72E-02

BP represents biological process; Term represents the identification number of GO and KEGG term; Description represents the names of GO and KEGG term; Counts represent the number of genes enriched in GO and KEGG terms.
GO = gene ontology, KEGG = Kyoto Encyclopedia of Genes and Genomes.

them are needed. Improving our understanding of the disease-related mechanisms of circRNAs should help improve the diagnosis and prevention of such diseases.[14]

In the present study, target genes of hsa-miR-942–5p were mainly enriched in cancer-related pathways. Furthermore, *CDK6*, a target gene of hsa-miR-942–5p, was enriched in cancer-related pathways (Supplemental Table 2, http://links.lww.com/MD/B773). Auger et al[36] have reported that the presence of an organized thrombus in major pulmonary arteries is typically associated with diseases, such as lung cancer. Although no previous studies have reported the direct associations between cancer-related pathways and CTEPH, we inferred that such pathways and axon guidance might be related to CTEPH, based on this previous study by Auger et al.[36] We searched the relevant literature and found that CDK6 was involved in the cell cycle and proliferation in pulmonary hypertension.[37] Combining these findings with the results of our present study showing that hsa_circ_0002062 regulated hsa-miR-942–5p, we suggested that circRNAs function in CTEPH via hsa_circ_0002062–hsa-miR-942–5p–CDK6–pathways involved in cancer.

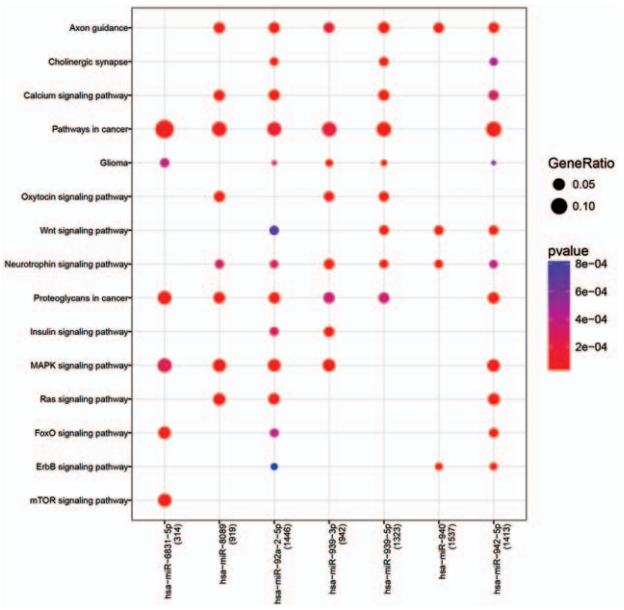

Figure 4. KEGG pathway enrichment analyses for target genes of miRNAs. Node size: gene ratio; node color: *P* value.

In addition, target genes of hsa-miR-940 were enriched in the ErbB signaling pathway. *CRKL*, one target gene of hsa-miR-940, was enriched in the ErbB signaling pathway in the present study (Supplemental Table 2, http://links.lww.com/MD/B773). Grant et al[38] have indicated that modulation of the ErbB signaling pathway can lead to increased apoptosis and loss of clonogenic survival, and cell proliferation has also been shown to be related to pulmonary hypertension.[39,40] Although no previous studies have reported the direct associations between the ErbB signaling pathway and CTEPH, we inferred that the ErbB signaling pathway might be related to CTEPH. From a literature search, we identified several articles reporting that CRKL overexpression can promote cell proliferation in many diseases.[41,42] Combined with the results of our present study showing that hsa_circ_002234 regulated hsa-miR-940, we suggested that circRNAs play potential roles in CTEPH via the hsa_circ_0022342–hsa-miR-940–CRKL–ErbB signaling pathway.

In conclusion, we inferred that hsa_circ_0002062–hsa-miR-942–5p-CDK6 pathways in cancer and the hsa_circ_0022342–-hsa-miR-940–CRKL–ErbB signaling pathway may be key mechanisms in CTEPH development. The identification of novel differentially expressed circRNAs is a key step to obtaining a better understanding of CTEPH. However, because of our limited knowledge of circRNAs, more studies are needed for verifying our results. A limitation of the present study is the small sample size. Thus, we plan to collect more samples to support the conclusions of the present study.

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