Original Paper

The Landscape of Circular RNA Expression Profiles in Papillary Thyroid Carcinoma Based on RNA Sequencing

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Key Words

Circular RNA • MicroRNA • Papillary thyroid carcinoma • RNA sequencing

Abstract

Background/Aims: Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer. However, the molecular mechanisms responsible for its tumorigenesis and progression remain largely unknown. Circular RNA (circRNA) is a novel type of noncoding RNA that can serve as an ideal biomarker due to its stability. Recent evidence suggests that circRNAs play important roles in tumorigenesis. This study aims to investigate circRNA expression profiles and their potential biological functions in PTC. **Methods:** High-throughput RNA sequencing was used to assess circRNA expression profiles in PTC, and quantitative real-time polymerase chain reaction (qRT-PCR) was used to validate dysregulated circRNAs. Receiver operating characteristic (ROC) curves were generated to evaluate the diagnostic value of circRNAs for PTC. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were employed to determine the biological functions of differentially expressed circRNAs. Bioinformatic analyses were applied to predict interactions between circRNAs and microRNAs (miRNAs), and a circRNA-miRNA-mRNA network was constructed using Cytoscape software. **Results:** We identified a number of differentially expressed circRNAs in PTC tissues compared with paired normal thyroid tissues, with chr5:160757890-160763776-, chr12:40696591-40697936+, chr7:22330794-22357656-, and chr21:16386665-16415895- being upregulated, and chr7:91924203-91957214+, chr2:179514891-179516047-, chr9:16435553-16437522-, and chr22:36006931-36007153- being downregulated. These findings were confirmed by qRT-PCR, and ROC curves indicated that they can serve as potential biomarkers for PTC. GO and KEGG pathway analyses showed that some of these circRNAs are related to cancers. Additionally, bioinformatic analyses revealed a potential competing-endogenous-RNA-regulating network among circRNAs, miRNAs, and mRNAs. **Conclusions:**

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Our study results depict the landscape of circRNA expression profiles in PTC and also provide potential biomarkers for PTC. Further functional and mechanistic studies of these circRNAs may improve our understanding of PTC tumorigenesis.

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Introduction

The incidence of thyroid cancer has increased rapidly (>5% per year in both men and women) over the past several decades [1]. Accounting for more than 80% of all cases, papillary thyroid carcinoma (PTC) represents the major histopathologic type of thyroid cancer [2]. Although PTC is usually curable, with a 5-year survival of over 95%, PTCs with more aggressive phenotypes, such as extrathyroidal extension, multifocal tumors, and lymph node and distant metastases, can dedifferentiate into more lethal thyroid cancers. These more aggressive PTCs may not be efficiently managed by traditional treatments, such as surgery, thyroid hormone therapy, or radioactive iodine therapy [3]. Therefore, it is necessary to identify individuals with a genetic predisposition toward PTC and those with potentially more aggressive types of PTC who may need individual treatment strategies or novel therapeutic approaches.

Some genetic alterations associated with PTC pathogenesis (e.g., *BRAF*, *RAS*, and *RET/PTC* mutations) have been evaluated as diagnostic or prognostic markers [2, 3], though the molecular markers reported thus far are mainly protein-coding genes. However, it is estimated that less than 2% of the human genome encodes proteins, and the majority of the human transcriptome consists of noncoding RNAs. Accumulating evidence demonstrates that noncoding RNAs, such as microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), play an important regulatory role in many physiological and pathophysiological processes. More recently, a novel class of noncoding RNA termed circular RNA (circRNA) has become a hot topic in RNA research. In contrast to linear RNAs that are terminated with 5' caps and 3' tails, circRNAs are characterized by covalently closed continuous loops with neither 5'–3' polarity nor a polyadenylated tail [4]. circRNAs are commonly found in mammalian cells and can regulate gene expression at the transcriptional or post-transcriptional level by acting as miRNA sponges or by interacting with other molecules [5]. Although circRNAs were discovered decades ago, studies about their functions have emerged only recently. Moreover, circRNAs appear to be expressed in tissue- and developmental-stage-specific manners, which indicates that they function in many physiological and pathophysiological processes [6, 7]. Similar to miRNAs and lncRNAs, researchers have also found that circRNAs are related to many diseases, such as atherosclerosis, nervous system disorders, and a variety of cancers [8–11]. circRNAs are stable in cells because they are resistant to exonucleases; thus, circRNAs may constitute ideal biomarkers for cancer diagnosis [12]. Regardless, identifying dysregulated circRNAs in different cancers and elucidating their functions remains an ongoing process in cancer research.

To date, little is known about the associations between circRNAs and PTC. Therefore, in the current study, we screened the circRNA profiles of PTC patients using high-throughput RNA sequencing (RNA-Seq) and validated dysregulated circRNAs in PTC tissues by quantitative real-time polymerase chain reaction (qRT-PCR). Bioinformatic analysis was then performed to predict their potential roles in PTC and lay a foundation for future studies of circRNAs that are related to PTC.

Materials and Methods

Patient samples

A total of 90 pairs of PTC and adjacent normal thyroid tissues were collected from patients at the Department of Head and Neck Surgery of the Zhejiang Cancer Hospital between September 2016 and August 2017 (Tables 1 and 2). The diagnosis of PTC and the adjacent normal tissues was independently

Table 1. Clinicopathologic characteristics of patients from whom samples were obtained for circRNA sequencing

Patient No.	Sex	Age	TNM (AJCC, 7th, ed. 2010)
1	female	51	T4aN1bM0, IVa
2	female	55	T3N1aM0, III
3	female	57	T4aN1aM0, IVa

confirmed by two pathologists (Fig. 1). The patients did not receive any chemotherapy or radiotherapy prior to surgical resection. All tissue samples were snap-frozen and stored in liquid nitrogen until further experiments were performed. Written informed consent was obtained from all patients, and this study was approved by the ethics committee of the Zhejiang Cancer Hospital, Hangzhou, China.

RNA extraction and quality control

Total RNA was isolated from each tissue sample using TRIzol reagent (Life Technologies, Carlsbad, CA, USA) following the manufacturer's instructions. The quality of the RNA samples was determined by OD 260/280 assessment using a NanoDrop ND-2000 instrument (Thermo Fisher Scientific, Waltham, MA, USA). RNA integrity was determined by denaturing agarose gel electrophoresis.

circRNA sequencing and bioinformatic analysis

Total RNA from each sample was used to prepare circRNA sequencing libraries according to a previously described protocol [13]. Paired-end reads were harvested from an Illumina HiSeq 4000 sequencer, and quality control was performed with a Q30. After 3' adapter trimming and low-quality read removal with cutadapt software (v1.9.3), high-quality reads were aligned to the reference genome/transcriptome with STAR software [14]. circRNAs were detected and annotated with DCC software [15] using two public circRNA databases: circBase [16] and circ2Traits [17]. The junction read counts were normalized, and differentially expressed circRNAs were determined using the edgeR package of R software [18]. circRNAs that exhibited fold changes (FCs) ≥ 2.0 with P values < 0.05 were considered to be significantly differentially expressed. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed for the host genes of the differentially expressed circRNAs. circRNA-miRNA interactions and miRNA targets were predicted with popular target prediction software [19, 20], and a network was constructed with Cytoscape software [21].

Table 2. Clinicopathologic characteristics of patients from whom samples were obtained for qRT-PCR

Characteristics	Discovery cohort (n)	Validation cohort (n)	P value
Total	44	43	
Sex			
Male	18	10	0.108
Female	26	33	
Age (years)			
< 45	25	20	0.394
≥ 45	19	23	
Extrathyroidal extension			
Yes	19	13	0.268
No	25	30	
Primary tumor			
T ₁₋₂	24	30	0.186
T ₃₋₄	20	13	
Lymph node metastasis			
Yes	34	31	0.628
No	10	12	
TNM stage			
I-II	29	26	0.660
III-IV	15	17	
Multicentricity			
Yes	25	19	0.286
No	19	24	
Microcarcinoma			
Yes	6	8	0.572
No	38	35

Fig. 1. Representative hematoxylin-eosin-stained images of PTC (C1–C3) and adjacent normal tissues (N1–N3).

qRT-PCR validation

Qualified total RNA was reverse transcribed to synthesize cDNA using PrimeScript RT Master Mix (Perfect Real Time, TaKaRa, Shiga, Japan) and then analyzed by qRT-PCR with SYBR Premix Ex Taq II (Tli RaseH Plus, TaKaRa) and a LightCycler 480 system (Roche, Basel, Switzerland). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as the internal control. The primers used for qRT-PCR analysis were as follows: chr5:160757890-160763776– (F 5'-TGGGGTGGCTTTGTCTTCGTT-3', R 5'-TCAGTTGTGTATCCATCTTGTGAC-3'), chr12:40696591-40697936+ (F 5'-ACTGAAAGAGCTGCTATGCCT-3', R 5'-AAGACCGCAAGTGTGGAAGA-3'), chr7:22330794-22357656– (F 5'-TGCCTCTCATTCCTGCCAGA-3', R 5'-TCTTGATAGAGTCGAGATGTTAGA-3'), chr21:16386665-16415895– (F 5'-TCTGAAGACTCCGGATGACA-3', R 5'-GTGTGCATCTTCTGGCTGTG-3'), chr7:91924203-91957214+ (F 5'-TCGAGCTCATGAATGTGAAAGATG-3', R 5'-GTGCTTTACGGAATTTGGTGGT-3'), chr2:179514891-179516047– (F 5'-AAGCCAGTTCTGTTCAGA-3', R 5'-TTTGCTGGTGGGACTTCTGG-3'), chr9:16435553-16437522– (F 5'-CGAAGCCGAGACAGGATGC-3', R 5'-GCCTGCCCTTCTTCTCCTG-3'), chr22:36006931-36007153– (F 5'-TACCTGGAGGCTCTTAAGGGT-3', R 5'-CAGACGCCTTCATCTCGTCC-3') and GAPDH (F 5'-CAGGAGGCATTGCTGATGAT-3', R 5'-GAAGGCTGGGGCTCATTT-3'). The relative expression level of each circRNA was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical analyses

Statistical analyses were performed using Prism 6 (GraphPad Software, La Jolla, CA) and SPSS 19.0 (IBM, Chicago, IL, USA). $P < 0.05$ was considered statistically significant. Differences between groups were assessed using Student's *t* test or Wilcoxon's rank-sum test, as appropriate. Receiver operating characteristic (ROC) curves were established to evaluate the diagnostic value of circRNAs for PTC.

Results

Overview of circRNA profiles

A total of 9,103 circRNAs were identified by RNA-Seq in three pairs of PTC and normal thyroid tissue samples. Statistically significant differentially expressed circRNAs between the two groups are displayed through FC filtering (Fig. 2A, B). The heat map

Fig. 2. Overview of circRNA profiles (CA: PTC tissues; N: normal thyroid tissues; chr: chromosome). (A) Volcano plot of differentially expressed circRNAs in PTC. The vertical green lines correspond to two-fold increased and decreased expression, and the horizontal green line represents $P = 0.05$. The red points represent circRNAs that were differentially expressed with statistical significance. (B) The scatter plot presents the circRNA expression variations between the PTC and paired adjacent normal thyroid tissues. The values of the x and y axes are the normalized signal values of two groups of samples (log2 scaled). The circRNAs above the top green line and below the bottom green line indicate greater than 2-fold change between the two groups of samples. (C) Hierarchical cluster analysis of differentially expressed circRNAs. The red and green colors indicate high and low expression, respectively. (D) Chromosomal distributions of differentially expressed circRNAs. (E) Classification of differentially expressed circRNAs based on genomic origin.

shown in Fig. 2C illustrates the circRNA expression profiles that were distinguishable between the cancer and normal tissues. Consequently, 87 circRNAs were detected as differentially expressed by $FC \geq 2.0$ and $P < 0.05$, of which 41 were upregulated and 46 downregulated; the numbers of upregulated circRNAs and downregulated circRNAs were approximately equal. As presented in Fig. 2D, the transcription of differentially regulated circRNAs was found to be widely distributed among all chromosomes except chr13 and chr20, with chr2, chr7, chr12, chr1, and chr8 being the top five. Among these differentially expressed circRNAs, 70 are exonic and seven intronic; one belongs to the group of intergenic circRNAs, and nine are transcribed from overlapping areas (Fig. 2E). Moreover, 33 were identified as new circRNAs that have not been annotated in the circBase or circ2Traits database. The top 20 up- and downregulated circRNAs are listed in Table 3 according to FC.

Validation of circRNA expression

Eight circRNAs (four were the most differentially expressed, and four were randomly chosen) were selected to verify the RNA-Seq in another 44 pairs of samples by qRT-PCR. The results revealed that expression of chr5:160757890-160763776-, chr12:40696591-40697936+, chr7:22330794-22357656-, and chr21:16386665-16415895- was upregulated, whereas that of chr7:91924203-91957214+, chr2:179514891-179516047-, chr9:16435553-16437522-, and chr22:36006931-36007153- was downregulated (Fig. 3A). The qRT-PCR data were strongly consistent with the circRNA sequencing data (Fig. 3B), indicating the reliability of the RNA-Seq results.

Diagnostic value of qRT-PCR-validated circRNAs

To assess whether the identified circRNAs can serve as potential diagnostic biomarkers for PTC, we built ROC curves for qRT-PCR-validated circRNAs using both a discovery cohort ($n = 44$, Fig. 4A) and a validation cohort ($n = 43$, Fig. 4B). The results revealed that these

Table 3. Top 20 differentially expressed circRNAs in PTC (FC, fold change)

circRNA ID	logFC	P Value	regulation	chrom	circBase ID	Gene Name	Catalog	length
chr5:160757890-160763776-	7.0594638	4.455E-06	up	chr5		GABRB2	exonic	536
chr12:40696591-40697936+	6.9757106	1.224E-05	up	chr12	hsa_circ_0025887	LRRK2	exonic	281
chr12:94562929-94580249+	6.1494217	1.873E-06	up	chr12		PLXNC1	exonic	377
chr7:22347958-22357656-	6.1289577	0.0002622	up	chr7		RAPGEF5	exonic	449
chr7:22330794-22357656-	5.3544779	0.0045872	up	chr7	hsa_circ_0001681	RAPGEF5	exonic	516
chr15:94899366-94928754+	5.2425776	2.232E-05	up	chr15		MCTP2	exonic	783
chr19:57088081-57089646+	5.2420838	0.0064241	up	chr19		ZNF470	intronic	1566
chr15:94910834-94928754+	4.9201652	0.0178311	up	chr15		MCTP2	exonic	487
chr22:23243156-23260373+	4.9161654	0.0162592	up	chr22			intergenic	17218
chr11:89165952-89185063-	4.8185828	0.0196645	up	chr11	hsa_circ_0023990	NOX4	exonic	395
chr2:203817282-203826149+	4.741227	0.0235486	up	chr2	hsa_circ_0003747	CARF	exonic	526
chr5:170305101-170323119+	4.6828141	0.0278566	up	chr5	hsa_circ_0001554	RANBP17	exonic	471
chr4:174153603-174169591+	4.6395841	0.0308816	up	chr4		GALTNT7	sense overlapping	15989
chr18:20570900-20581692+	4.5742248	0.0328106	up	chr18	hsa_circ_0047136	RBBP8	exonic	1480
chr16:29845054-29845387+	4.5645496	0.0410921	up	chr16	hsa_circ_0000688	MVP	exonic	256
chr5:123974826-124036962-	4.5342373	0.0294251	up	chr5	hsa_circ_0073716	ZNF608	exonic	3626
chr8:18622959-18662408-	4.5014113	0.000377	up	chr8	hsa_circ_0002111	PSD3	exonic	538
chr2:9028148-9098771-	4.4839511	0.0346522	up	chr2	hsa_circ_0008847	MBOAT2	exonic	376
chr2:196544756-196545774+	4.4623798	0.0410323	up	chr2	hsa_circ_0057550	SLC39A10	exonic	1019
chr6:32623627-32729488-	4.4523293	0.0376792	up	chr6		HLA-DQB1	sense overlapping	96862
chr22:36006931-36007153-	-7.833294	0.0002446	down	chr22	hsa_circ_0063050	MB	exonic	223
chr9:16435553-16437522-	-7.610093	0.0001563	down	chr9	hsa_circ_0086414	BNC2	exonic	1970
chr2:179517185-179517463-	-7.2153	0.000688	down	chr2		TTN	exonic	168
chr2:179534319-179535022-	-6.211052	0.0041329	down	chr2		TTN	exonic	162
chr2:179545806-179546177-	-6.211052	0.0041329	down	chr2		TTN	exonic	168
chr2:179514891-179516047-	-6.169081	0.0044674	down	chr2		TTN	exonic	270
chr2:179594015-179594293-	-5.889572	0.007312	down	chr2		TTN	exonic	279
chr2:179545806-179546141-	-5.889572	0.007312	down	chr2		TTN	intronic	336
chr8:62531537-62566219-	-5.779114	0.0026795	down	chr8	hsa_circ_0084606	ASPH	exonic	654
chr8:41518948-41519459-	-5.722471	0.0056978	down	chr8	hsa_circ_0001792	ANK1	exonic	201
chr5:137219073-137219280+	-5.479514	0.0140916	down	chr5		MYOT	exonic	208
chr14:23873505-23900690-	-5.205989	0.0040941	down	chr14		MYH7	sense overlapping	27186
chr2:179559556-179560107-	-5.167508	0.0229073	down	chr2		TTN	intronic	552
chr1:200816768-200822623+	-5.167508	0.0229073	down	chr1		CAMSAP2	exonic	2631
chr19:4511523-4511918-	-5.079222	0.0259603	down	chr19		PLIN4	sense overlapping	396
chr2:179560592-179560759-	-5.079222	0.0259603	down	chr2		TTN	intronic	168
chr6:170846322-170858201-	-4.984135	0.0296471	down	chr6	hsa_circ_0078784	PSMB1	exonic	427
chr18:6301902-6312055-	-4.975557	0.0131084	down	chr18		L3MBTL4	exonic	217
chr2:168101672-168101821+	-4.877317	0.0345266	down	chr2		XIRP2	intronic	150
chr16:24979672-24981901-	-4.813434	0.0231379	down	chr16	hsa_circ_0004260	ARHGAP17	exonic	263

Table 4. Area under the curve (AUC) of qRT-PCR-validated circRNAs in the discovery and validation cohorts

circRNA	Discovery cohort (n = 44)			Validation cohort (n = 43)		
	AUC	95%CI	P	AUC	95%CI	P
chr5:160757890-160763776-	0.9566	0.9088–1.004	< 0.0001	0.9502	0.8995–1.001	< 0.0001
chr12:40696591-40697936+	0.9476	0.9041–0.9911	< 0.0001	0.9448	0.8966–0.9931	< 0.0001
chr7:22330794-22357656-	0.9099	0.8433–0.9764	< 0.0001	0.9186	0.8607–0.9765	< 0.0001
chr21:16386665-16415895-	0.7200	0.6128–0.8272	0.0003813	0.7309	0.6212–0.8407	0.0002284
chr7:91924203-91957214+	0.8763	0.8045–0.9481	< 0.0001	0.8661	0.7922–0.9401	< 0.0001
chr2:179514891-179516047-	0.8270	0.7343–0.9196	< 0.0001	0.8253	0.7318–0.9189	< 0.0001
chr9:16435553-16437522-	0.8192	0.7306–0.9078	< 0.0001	0.8213	0.7319–0.9106	< 0.0001
chr22:36006931-36007153-	0.9305	0.8796–0.9814	< 0.0001	0.9348	0.8794–0.9902	< 0.0001

circRNAs are promising biomarkers for identifying PTC, with consistent areas under the curve in both the validation and discovery cohorts (Table 4).

GO analysis and pathway analysis of circRNA genes

GO annotation revealed the host genes of the identified differentially expressed circRNAs, as presented in Fig. 5. The most significantly enriched GO terms in the biological process, cellular component, and molecular function categories were muscle system process (GO: 0003012, $P = 1.42E-05$), Z disc (GO: 0030018, $P = 1.33E-07$), and alpha-actinin binding (GO: 0051393, $P = 1.11E-06$), respectively. The results of KEGG pathway analysis of the circRNA genes are presented in Fig. 6. The differentially expressed circRNA genes are mainly associated with autoimmune thyroid disease, viral myocarditis, allograft rejection, graft-versus-host disease, type I diabetes mellitus, epithelial cell signaling in *Helicobacter pylori* infection, PPAR signaling pathway, adherens junction, axon guidance, antigen processing and presentation, and herpes simplex infection.

miRNA prediction and circRNA-miRNA-mRNA network construction

Because circRNAs contain corresponding miRNA binding sites and can function as miRNA sponges, to evaluate their potential functions in PTC, we investigated miRNAs potentially associated with the identified differentially expressed circRNAs using miRNA target prediction software. The first five predicted miRNAs for the top three up- and downregulated circRNAs are listed in Table 5. For example, circRNA chr5:160757890-160763776- is predicted to harbor hsa-miR-3154, hsa-miR-2392, hsa-miR-6833-5p, hsa-miR-7114-5p, and hsa-miR-5004-5p, with different seed sequence matching types (i.e., 7mer-m8, 8mer, 6mer, or imperfect; Fig. 7). Additionally, a circRNA-miRNA-

Fig. 3. qRT-PCR validation of eight differentially expressed circRNAs. (A) The relative expression levels of circRNAs in 44 PTC tissues and paired normal thyroid tissues (N: normal thyroid tissues; C: PTC tissues; $P < 0.05$). (B) Comparison of log2 fold changes (FCs) in circRNAs between RNA-Seq and qRT-PCR results.

Fig. 4. Receiver operating characteristic (ROC) curves of eight qRT-PCR-validated circRNAs. (A) Discovery cohort ($n = 44$). (B) Validation cohort ($n = 43$).

Table 5. Predicted miRNAs for the top three up- or downregulated circRNAs in PTC

circRNA	miRNA	miRNA	miRNA	miRNA	miRNA
chr5:160757890-160763776-	hsa-miR-3154	hsa-miR-2392	hsa-miR-6833-5p	hsa-miR-7114-5p	hsa-miR-5004-5p
chr12:40696591-40697936+	hsa-miR-648	hsa-miR-6833-5p	hsa-miR-6505-5p	hsa-miR-5683	hsa-miR-767-3p
chr12:94562929-94580249+	hsa-miR-6507-5p	hsa-miR-5571-5p	hsa-miR-3927-3p	hsa-miR-3190-5p	hsa-miR-4529-5p
chr2:179517185-179517463-	hsa-miR-128-1-5p	hsa-miR-6779-5p	hsa-miR-939-5p	hsa-miR-4266	hsa-miR-765
chr9:16435553-16437522-	hsa-miR-378g	hsa-miR-6779-5p	hsa-miR-4674	hsa-miR-149-5p	hsa-miR-92a-1-5p
chr22:36006931-36007153-	hsa-miR-103a-2-5p	hsa-miR-764	hsa-miR-636	hsa-miR-4659b-3p	hsa-miR-767-5p

Fig. 5. Gene Ontology (GO) terms for differentially expressed circRNA genes. The bar plot presents the enrichment scores ($-\log_{10}$ [P value]) of the top 10 significantly enriched GO terms: biological process (A), cellular component (B), and molecular function (C).

Fig. 6. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of differentially expressed circRNA genes. The top 10 significantly enriched pathways and their scores ($-\log_{10}$ [P value]) are listed as the y axis and the x axis, respectively.

targeted mRNA network was also constructed (Fig. 8), in which each circRNA has five associated miRNAs and every miRNA has 10 predicted mRNA targets. The network indicates the potential competing endogenous RNA (ceRNA) relationships among circRNAs, miRNAs, and mRNAs in PTC.

Fig. 7. Detailed annotation of circRNA-miRNA interactions between chr5:160757890-160763776- and its target miRNAs.

Discussion

circRNAs were originally thought to be by-products of mRNA splicing errors [22]. However, recent studies have indicated that circRNAs are a stable, diverse, and conserved class of RNA molecules [7]. Compared with mRNAs, miRNAs, and lncRNAs, circRNAs are promising diagnostic and prognostic biomarkers because of their stability [23]. Owing to the advent of high-throughput RNA-Seq, numerous circRNAs have been identified as being dysregulated in certain cancers, such as gastric cancer [24], breast cancer [25], hepatocellular carcinoma [6, 26], pancreatic carcinoma [27], and esophageal carcinoma [28]; however, their expression statuses and roles in PTC remain to be determined. In this study, we used circRNA sequencing to obtain circRNA expression profiles for PTC tissues and paired adjacent normal tissues. Consequently, we identified 87 significantly differentially expressed circRNAs, among which chr5:160757890-160763776- was the most upregulated, and chr22:36006931-36007153- the most downregulated in PTC. Among these differentially expressed circRNAs, we chose eight for qRT-PCR validation, the results of which were strongly consistent with those of circRNA sequencing, confirming the reliability of RNA-Seq. ROC curves also suggested that the validated circRNAs can serve as biomarkers for diagnosing PTC. Additionally, we identified 33 novel circRNAs from the RNA-Seq data. Although much work is needed, these novel circRNAs are specifically interesting for further study because of the lack of information about them in circRNA databases.

As circRNAs have been shown to regulate the function of their host mRNAs or parent genes [29], we attempted to explore the potential functions of circRNAs differentially expressed in PTC by analyzing their parent genes. GO and KEGG pathway analyses revealed that the parent genes are involved in certain pathways associated with cancer, such as the PPAR signaling pathway (related circRNAs and genes: chr19:4511523-4511918- and PLIN4; chr10:97154758-97170534- and SORBS1) and adherens junction (related circRNAs and genes: chr7:116339125-116340338+ and MET; chr10:97154758-97170534- and SORBS1). Previous studies have suggested that the PPAR signaling pathway contributes to thyroid cancer [30-32], and MET is a well-known proto-oncogene associated with multiple

Fig. 8. Predicted circRNA-miRNA-mRNA network for the top three up- and downregulated circRNAs in PTC. Yellow represents circRNAs, green represents miRNAs, and pink represents miRNA target mRNAs.

human cancers [33-35]. Therefore, circRNA chr19:4511523-4511918-, chr10:97154758-97170534-, and chr7:116339125-116340338+ may participate in the tumorigenesis of PTC by regulating their parent genes.

circRNAs are closed continuous loops of RNA with multiple miRNA binding sites [4, 36], which enables them to sequester miRNAs and act as sponges of miRNA that regulate expression of target miRNAs [37, 38]. For example, the circRNA CiRS-7 contains more than 70 binding sites for miR-7 and can function as a sponge of this miRNA [5, 39]. Due to their abundance and stability, circRNAs are more effective in sponging miRNA than are other types of RNAs [40-44]. We also predicted the potential miRNA targets of circRNAs and constructed a circRNA-miRNA-mRNA network to explore the function of dysregulated circRNAs in PTC. The network illustrates the potential ceRNA relationships among circRNAs, miRNAs, and mRNAs. For example, chr5:160757890-160763776-, chr12:40696591-40697936+, and chr12:94562929-94580249+, as well as chr22:36006931-36007153-, chr9:16435553-16437522-, and chr2:179517185-179517463-, are the top three up- and downregulated circRNAs and possibly regulate expression of certain mRNAs by binding to their target miRNAs. Dysregulated circRNAs may participate in the pathogenesis of PTC by regulating miRNAs and their target mRNAs. Future studies should focus on these circRNAs and their related miRNAs and mRNAs.

Several limitations should be acknowledged in the present study. First, only three pairs of samples were chosen for circRNA sequencing. The sample size for RNA-Seq is relatively small and thus some selection bias may exist. Second, the prognostic value of the circRNAs was not investigated in the present study due to the relatively short-term follow-up. Therefore, future studies with large sample sizes and long-term follow-up are still needed to verify our findings.

In conclusion, our study revealed a landscape of circRNA expression profiles in PTC. Numerous circRNAs were found to be dysregulated in PTC tissues compared with adjacent normal tissues. Our RNA-Seq and qRT-PCR results were strongly consistent. Bioinformatic analysis predicted the potential functions related to these circRNAs as well as several potential circRNA-miRNA-mRNA ceRNA-regulating networks. Additionally, the findings provide potential biomarkers for PTC. Further functional and mechanistic studies of these circRNAs will improve our understanding of tumorigenesis in PTC.

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Disclosure Statement

The authors declare to have no conflict of interests.

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