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Circular RNA profiling reveals circEXOC6B and circN4BP2L2 as novel prognostic biomarkers in epithelial ovarian cancer

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Abstract. Circular RNAs (circRNAs) are regarded as a novel class of widespread endogenous non-coding RNAs, which may play important roles in tumorigenesis by regulating gene expression. Nevertheless, the characterization of circRNAs in epithelial ovarian cancer (EOC) remains largely unknown. This study aimed to investigate circRNA expression profiles in EOC. A total of 54 EOC specimens and 54 normal ovarian tissues (controls) were collected. circRNA-sequencing based circRNA expression profiles were identified in 4 EOC specimens and compared with 4 normal ovarian tissues. circRNA-sequencing data were validated by reverse transcription-quantitative polymerase chain reaction (RT-qPCR) in the 54 EOC specimens and 54 normal ovarian tissues. The association between differentially expressed circRNAs and various clinicopathological features of EOC was determined using a non-parametric test. Univariate analysis was performed using the log-rank test. A total of 4,388 circRNAs (2,556 up- and 1,832 downregulated; fold change of ≥2 and P<0.05) were identified to be differentially expressed in the EOC specimens compared with the normal ovarian tissues. Of these, the levels of 6 circRNAs (circBNC2, circEXOC6B, circFAM13B, circN4BP2L2, circRHOBTB3 and circCELSR1) were confirmed by RT-qPCR. Our data further indicated that these 6 circRNAs were associated with various clinicopathological features of EOC. More importantly, we found that circEXOC6B and circN4BP2L2 may act as novel prognostic biomarkers in patients with EOC. On the whole, the results of this study indicate that differentially expressed circRNAs may participate in the pathogenesis of EOC and may thus have potential for use as novel diagnostic and prognostic biomarkers for EOC. Future experiments with larger sample sizes are required to verify the current findings and illuminate the regulatory mechanisms of action of circRNAs in the tumorigenesis of EOC.

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Introduction

Epithelial ovarian cancer (EOC) is the second most common form of gynecological cancer and is currently the first leading cause of gynecological cancer-related mortality (1). The 5-year survival rate of patients with advanced disease is merely 30% (2). Despite the intra-tumor heterogeneity, patients with EOC are usually treated with standard therapy, including cytoreductive surgery and platinum-based chemotherapy (3,4). However, conventional treatment usually causes chemoresistance, resulting in tumor recurrence and even death (3). Therefore, it is imperative to identify novel molecular mechanisms and potential therapeutic targets in order to better control the severity of EOC.

Circular RNAs (circRNAs) are regarded as widespread endogenous non-coding RNAs (5,6). They were discovered >20 years ago, but were mostly misinterpreted as mis-splicing errors due to low abundance (7). Only in 2012/2013, their substantial presence in eukaryotic cells was demonstrated using high-throughput sequencing data (8). circRNAs are characterized by covalently closed loop structures without 5' caps or 3' polyadenylated tails (9). The formation of these structures involves back-splicing events by the spliceosome at the expense of canonical mRNA isoforms (10,11). Such circularization calls for the covalent binding of upstream 5' acceptors to downstream 3' donors (12). Although the concrete mechanisms of back-splicing events remain unclear, flanking introns containing ALU repeats are believed to be essential for the process (13). It is becoming increasingly evident that, instead of being regarded as splicing errors, circRNAs are rather the products of regulated back-splicing events (14). Accordingly, a body of evidence has indicated that circRNAs are of huge regulatory potency (15). Previous studies have

found that a number of circRNAs play crucial roles in human epithelial-mesenchymal transition and porcine embryonic brain development (16,17). Recently, an increasingly number of studies have demonstrated that circRNAs can regulate gene expression by acting as microRNA sponges, such as CDR1as (18).

Of note, a growing body of evidence has indicated that circRNAs are closely associated with the pathogenesis of a number of human diseases, particularly cancer (19). To date, abnormal expression levels of circRNAs have been revealed in various types of cancer, including breast, liver, gastric, colorectal and bladder cancer (20-24). However, the characterization of circRNAs in ovarian cancer remains largely unknown. To date, and at least to the best of our knowledge, only two articles have been published in the literature regarding the association between circRNAs and ovarian cancer (12,25). In the study by Ahmed *et al* (25), differential expression profiles of circRNAs were defined by performing paired-end RNA sequencing of primary sites, peritoneal and lymph node metastases from 3 patients with stage IIIC ovarian cancer. In the study by Bachmayr-Heyda *et al* (12), the expression of circular and linear RNAs and proliferation were analyzed in matched normal colon mucosa and tumor tissues. They then validated the correlation of global circular RNA abundance (the circRNA index) and the proliferation in ovarian cancer cells compared to cultured normal ovarian epithelial cells. However, neither of these two articles investigated the differential expression profiles of circRNAs in EOC specimens compared to normal ovarian tissues. In this study, we aimed to examine the pathogenesis of EOC and to identify potential therapeutic targets and circRNA biomarkers by comprehensively profiling circRNA expression in EOC specimens.

Materials and methods

Human samples. Four EOC specimens and four normal ovarian tissues for circRNA-sequencing analysis were obtained from the Department of Obstetrics and Gynecology in Peking Union Medical College Hospital (PUMCH) between 2013 and 2017. A total of 54 EOC specimens and 54 normal ovarian tissues, including those used for circRNA-sequencing analysis were collected for circRNA validation by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Cancer specimens were collected from patients who had histologically proven to suffer from EOC and had received wedge biopsy of ovaries or cytoreductive surgery. Normal ovarian tissues were collected from patients who underwent adnexectomy due to myoma. Cancer tissues were collected from primary sites, and the normal ovarian tissues were collected from the surface epithelium. Fresh tissues were collected during surgery, frozen in liquid nitrogen within 5 min following resection, and stored at -80˚C until use. All patients were consecutive and did not receive any pre-operative chemotherapy, radiotherapy or target therapy. All the EOC specimens and normal ovarian tissues were confirmed by experienced pathologists. Clinicopathological data, including age at diagnosis, International Federation of Gynecology and Obstetrics (FIGO) stage, residual tumor diameter, lymph node metastasis (LNM), CA125 level, and ascites are presented in Table I. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Ethics Committee of PUMCH. All experiments were performed in accordance with relevant guidelines. Written informed consents were obtained from all patients for research purpose.

RNA-seq analysis. Total RNA was extracted from the tissues using TRIzol reagent (Life Technologies/Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. Briefly, ribosomal RNA was removed from total RNA using the Epicentre Ribo-Zero rRNA Removal kit (Illumina, San Diego, CA, USA). The ribosome-depleted RNA was then treated with RNase R (Epicentre) and fragmented to approximately 200 bp. Subsequently, the purified RNA was subjected to first-strand cDNA synthesis according to the instructions provided with the NEBNext Ultra Directional RNA Library Prep kit for Illumina (NEB, Beverly, MA, USA). The cDNA fragments were repaired using an End-It DNA End Repair kit and then modified by the Klenow fragment to add an A to the 3' end of the DNA fragments, and were finally ligated to adapters. Purified first-strand cDNA was then subjected to 13-15 cycles of PCR amplification . The PCR protocol included an initial denaturation step (98˚C for 30 sec) and 15 cycles of denaturation (98˚C for 10 sec, 65˚C for 75 sec) and annealing (65˚C for 5 min).The library products were evaluated using the Agilent 2200 TapeStation and Qubit 2.0 (Life Technologies, Carlsbad, CA, USA) and then diluted to 10 pM for cluster generation *in situ* on the HiSeq3000 pair-end flow cell followed by sequencing (2x150 bp) on HiSeq3000 (Illumina).

Identification and quantification of circRNAs. Firstly, we used TopHat2 (26) to map the RNA-seq FASTQ reads to the human reference genome (GRCh37/hg19) obtained from the UCSC genome database (http://genome.ucsc.edu/). Subsquently, as previously described, the unmapped reads were used to identify circRNAs (16). In brief, all the unmapped reads were processed to 20-nucleotide anchors from both ends of the sequencing read. Anchors aligning in the head-to-tail orientation indicated a back-spliced junction. Subsequently, the anchor alignment was extended, so that the complete read alignment and the breakpoint were flanked by a GT/AG splice site. The circRNA abundance was measured using the total number of reads that spanned back-spliced junctions. RefSeq was used to annotate the genomic regions that mapped to inferred circRNAs. Gene coordinates were obtained from the RefGene tables in the UCSC Genome Browser (downloaded on 03/28/2014). A custom script was used to determine the host genes of circRNAs. The longest transcript fragment whose boundaries (5' or 3' end) exactly matched both ends of each circRNAs in the same strand was searched. The corresponding gene of this transcript fragment was then defined as the host gene of this circRNA.

RNA preparation and RT-qPCR. Total RNA was extracted from 30-50 mg of the EOC specimens and normal ovarian tissues using TRIzol reagent (Life Technologies/Thermo Fisher Scientific) according to the manufacturer's instructions. RT-qPCR was performed using M-MLV Reverse Transcriptase (Promega, Madison, WI, USA), PowerUp™ SYBR™ Green Master Mix (Thermo Fisher Scientific) according to the manufacturer's

instructions. GAPDH was used as an internal reference gene. The RT-qPCR protocol included an initial denaturation step (95˚C for 20 sec) and 40 cycles of denaturation (95˚C for 3 sec) and annealing (60˚C for 30 sec). The relative expression levels were calculated using $2^{-\Delta\Delta Cq}$ method (27) as previously described (28). The primer sequences were as follows: circBNC2 (forward, 5'-GCAGTTCGGAACCAGAACGAC-3' and reverse, 5'-ATGCTGGCCAGTCTTGCTCAC-3'), circEXOC6B (forward, 5'-TGCCTCAAGTAAGCCACTATC-3' and reverse, 5'-TTCCTTTCCCTCATGTTGTAG-3'), circFAM13B (forward, 5'-GTGAAAGTAACAGAGACTGTTC-3' and reverse, 5'-ACTTACTCTGTAGAGGCTGG-3'), circN4BP2L2 (forward, 5'-CATGGTGTGTCTCGAAAGAAG-3' and reverse, 5'-CTGTACCCATCTTGATGGTGA-3'), circRHOBTB3 (forward, 5'-GCTTTTCAGTGGGAAGAATTG-3' and reverse, 5'-ACATGGCTTACAGCGCAGAGA-3'), circCELSR1 (forward, 5'-CGATTCGACACCATCCATGAAG-3' and reverse, 5'-GTCCACCTGCGTCTCATTGC-3') and GAPDH (forward, 5'-AACGTGTCAGTGGTGGACCTG-3' and reverse, 5'-GAGACCACCTGGTGCTCAGTG-3').

Bioinformatics analysis. Gene Ontology (GO) analysis was performed to explore the functional roles of target genes in terms of biological processes, cellular components and molecular functions. Biological pathways defined by Kyoto Encyclopedia of Genes and Genomes (KEGG), Biocarta and Reactome (http://www.genome.jp/kegg/) were identified by Database for Annotation, Visualization and Integrated Discovery (DAVID; http://www.david.abcc.ncifcrf.gov/).

Statistical analysis. In the circRNA-seq analysis, circRNAs were identified as differentially expressed between the EOC specimens and normal ovarian tissues if the expression change was ≥2-fold with a P-value of <0.05. Data from at least 3 independent experiments are expressed as the means ± standard deviation. The Mann-Whitney U test was used to determine the statistical significance for comparisons between 2 groups, and a P-value less than α which was adjusted by Bonferroni correction (adjusted α was 0.05/6, i.e., 0.0083) was considered statistically significant. Overall survival (OS) and progress-free survival (PFS) were calculated from the date of surgery to the date of death, progress or last follow-up (April 2018). The average follow-up time was 29.0 months (range, 4 to 52 months). Kaplan-Meier survival analysis was used for the analysis of the survival rate; The P-value was calculated by the log-rank test and a P-value <0.05 was considered to indicate a statistically significant difference. Statistical analyses were carried out using SPSS statistical software version 23.0 (SPSS, Chicago, IL, USA) and GraphPad Prism 5.0. All tests were two-sided.

Results

Identification of circRNAs by RNA-seq analysis in EOC. Firstly, circRNAs were characterized using the RNA-seq analysis of 4 EOC specimens and 4 normal ovarian tissues (Fig. 1A). A total of 66,521 distinct circRNA candidates were identified from these samples. Of these, 37,674 circRNAs contained at least 2 unique back-spliced reads (Fig. 1B). The circRNA candidates were annotated using the RefSeq database. More than 50% of these circRNAs were transcribed from protein-coding exons, and a small fraction of these were from introns, non-coding RNAs, 5' UTR, 3' UTR, and other sources (Fig. 1C). The median length of the exonic circRNAs was ~500 nucleotides (nt) (Fig. 1D).

The relative expression analysis of these circRNAs revealed a series of differentially expressed circRNAs in the EOC specimens compared with the normal ovarian tissues (Fig. 2). Based on the RNA-seq results, circRNAs with a signal altered by 2-fold and with value of P<0.05 were identified as statistically altered. On the whole, 4,388 circRNAs were identified to be differentially expressed in the EOC specimens compared with the normal ovarian tissues. Of these, 2,556 circRNAs were upregulated, while 1,832 circRNAs were downregulated in the EOC specimens (data not shown).

Validation of the differential expression levels of circRNAs. In order to verify the reliability of the circRNA-seq results, the 4,388 variable expressed circRNAs were ranked based on calculating fold change and abundance, and the top 6 of the ranked circRNAs were selected for validation in the 54 EOC specimens and 54 normal ovarian tissues by RT-qPCR. Finally, 5 downregulated circRNAs and 1 upregulated circRNA were selected randomly from the 4,388 dysregulated circRNA transcripts. The results of RT-qPCR revealed that these circRNAs were significantly differentially expressed between the 2 groups. In particular, circBNC2 (hsa_circ_0008732) (P<0.0001, Fig. 3A), circEXOC6B (hsa_circ_0009043) (P<0.0001, Fig. 3B), circFAM13B (hsa_circ_0001535) (P<0.0001, Fig. 3C), circN4BP2L2 (hsa_circ_0000471) (P<0.0001, Fig. 3D) and circRHOBTB3 (hsa_circ_0007444) (P<0.0001, Fig. 3E) were significantly downregulated in the EOC specimens, whereas circCELSR1 (hsa_circ_0063809) (P<0.0001, Fig. 3F) was significantly upregulated in the EOC group (Fig. 4). The results of qRT-PCR were consistent with the circRNA-seq results, which further verified the reliability of our circRNA-seq results.

The association between circRNAs and the clinicopathological features of EOC. The association between circRNAs and various clinicopathological features of EOC was evaluated in the 54 patients with EOC. The median expression levels of circRNAs were used to divide the circRNAs into the high level group and the low level group. Our data revealed that the low level of circBNC2 was associated with younger age (P=0.027), an advanced FIGO stage (P=0.045), LNM (P=0.005) and massive ascites (P=0.034). The low level of circEXOC6B was associated with younger age (P=0.018) and LNM (P=0.032). The low level of circFAM13B was associated with younger age (P=0.032), an advanced FIGO stage (P=0.044) and LNM (P=0.034). The low level of circN4BP2L2 was associated with younger age (P=0.047), an advanced FIGO stage (P=0.046) and LNM (P=0.003). The low level of circRHOBTB3 was associated with younger age (P=0.023) and LNM (P=0.009). In addition, the high level of circCELSR1 was associated with an advanced FIGO stage (P=0.043), a larger residual tumor diameter (P=0.035) and massive ascites (P=0.021) (Table I).

Prognostic value of circRNAs in EOC. We further investigated the prognostic value of various clinicopathological features and differentially expressed circRNAs in patients with EOC. The results from univariate analysis revealed that,

Table I. The association between differentially expressed circRNAs and various clinicopathological features of epithelial ovarian cancer.

Variables	N	circBNC2 means ± SD	P-value	circEXOC6B means ± SD	P-value	circFAM13B means ± SD	P-value	circN4BP2L2 means ± SD	P-value	circRHOBTB3 means ± SD	P-value	circCELSR1 means ± SD	P-value
Age, years			0.027[a]		0.018[a]		0.032[a]		0.047[a]		0.023[a]		0.448
<50	23	9.37±2.01		11.10±3.48		12.28±0.96		5.97±1.28		6.95±1.81		11.35±1.63	
≥50	31	10.83±2.50		13.29±2.37		13.62±2.15		7.01±1.56		8.45±2.25		10.56±1.66	
FIGO stage			0.045[a]		0.145		0.044[a]		0.046[a]		0.288		0.043[a]
FIGO I-II	14	10.98±2.04		12.86±2.10		13.94±1.99		7.07±1.24		7.84±1.50		10.44±1.65	
FIGO III-IV	40	9.74±2.82		11.56±2.98		12.52±1.13		6.06±1.50		7.01±2.06		11.67±1.82	
Residual tumor diameter (cm)			0.598		0.849		0.701		0.900		0.873		0.035[a]
<1	43	10.12±2.69		13.11±2.15		13.65±1.94		6.86±1.53		8.01±2.18		10.69±1.92	
≥1	11	9.54±1.55		12.90±1.96		13.24±1.03		6.72±0.96		7.74±1.44		12.13±1.48	
Lymph node metastasis			0.005[a]		0.032[a]		0.034[a]		0.003[a]		0.009[a]		0.172
No	35	10.90±2.72		13.32±2.24		13.71±2.12		7.04±1.70		8.27±2.54		10.70±1.41	
Yes	19	8.80±2.64		11.75±2.02		12.46±1.34		5.69±2.35		6.72±2.98		11.51±1.76	
CA125 level (U/ml)			0.081		0.288		0.438		0.885		0.826		0.425
<600	31	10.82±2.73		12.95±2.46		13.22±1.95		6.43±1.68		8.08±2.37		10.68±1.35	
≥600	23	9.30±1.79		11.70±3.51		12.90±1.79		6.17±1.53		7.41±2.03		11.12±1.74	
Ascites (ml)			0.034[a]		0.211		0.610		0.245		0.434		0.021[a]
<100	13	11.54±3.29		13.73±2.99		13.32±2.75		7.08±2.11		8.45±3.48		9.671±1.86	
≥100	41	9.59±2.30		12.12±2.91		12.65±1.26		6.17±1.48		7.44±2.10		11.21±1.67	

Means ± SD, mean of C, standard definition of C; C, normalized expression of cancer tissues; FIGO, the International Federation of Gynecology and Obstetrics. [a]P<0.05, statistically significant differences.

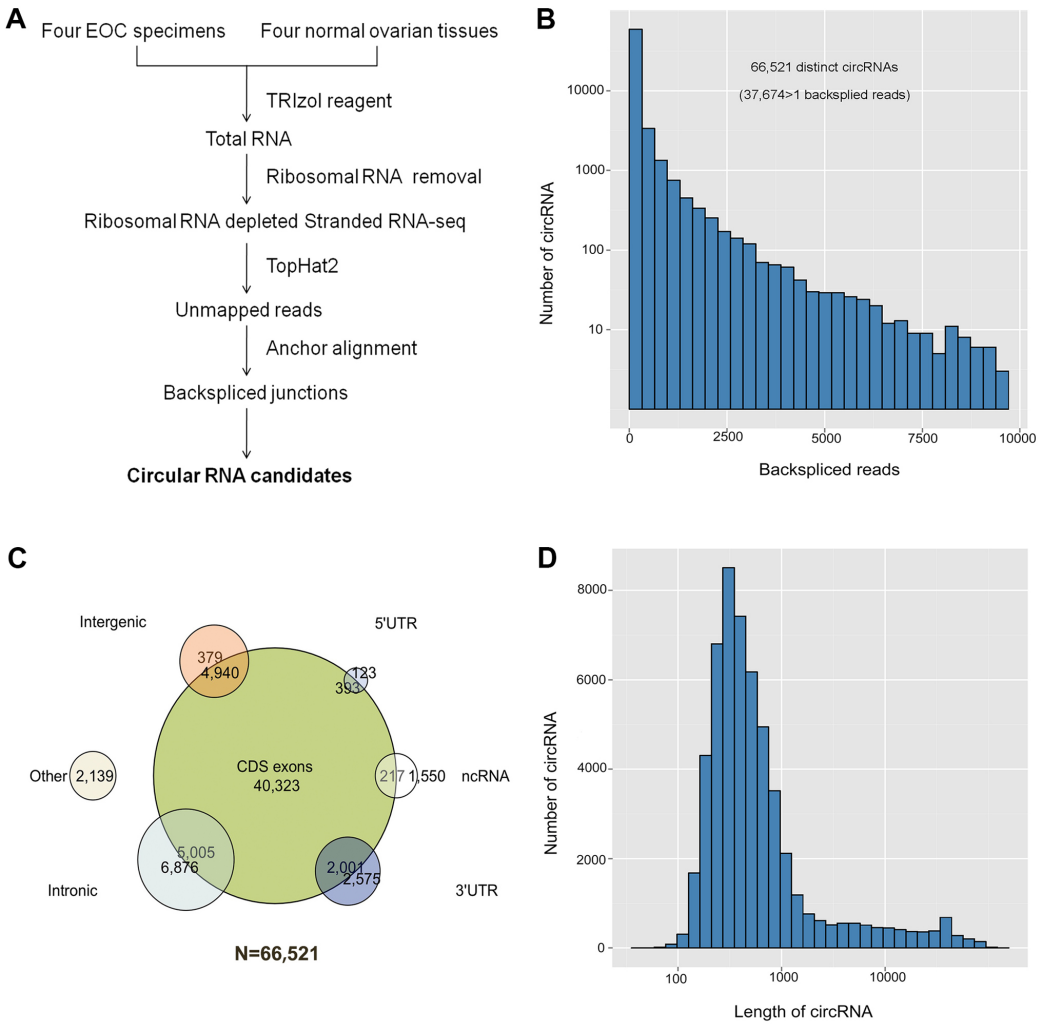

Figure 1. Profiling of circular RNAs (circRNAs) in human epithelial ovarian cancer (EOC) specimens and normal ovarian tissues. (A) circRNA-sequencing analysis in 4 EOC specimens and 4 normal ovarian tissues. (B) The total number of circRNAs and back-spliced reads identified in 4 EOC specimens and 4 normal ovarian tissues. (C) Genomic origin of circRNAs. (D) The length distribution for exonic circRNAs (n=54,351, only known spliced length was considered).

an advanced FIGO stage (P=0.003, log-rank test) and LNM (P=0.024, log-rank test) were predictive of a worse PFS. Moreover, a high level of circN4BP2L2 (P=0.025, log-rank test) was associated with a better PFS. In addition, a high level of circEXOC6B (P=0.045, log-rank test) was associated with a better OS (Table II).

Bioinformatics analyses. The results of GO enrichment analysis of the differentially expressed circRNAs with identified target genes are shown in Fig. 5. GO analysis revealed that numerous target genes were involved in the biological processes, such as cellular process, regulation of biological process, metabolic process, etc. These processes were associated with human tumorigenesis and metastasis. KEGG analysis revealed that there were 23 pathways related to differentially expressed circRNAs, including the ErbB signaling pathway, ECM-receptor interaction and focal adhesion (Fig. 6).

Discussion

In this study, we successfully identified a large number of circRNAs in EOC specimens. We also found that a considerable portion of these circRNAs were differentially expressed in cancer specimens compared with normal ovarian tissues. Our findings suggest that these differentially expressed circRNAs may be regulated and may play an important role in the development of EOC. We further evaluated the prognostic value of circRNAs in EOC and found that circEXOC6B and circN4BP2L2 may have potential for use as prognostic biomarkers in EOC. To the best of our knowledge, we are the first to perform a detailed circRNA-seq analysis of EOC species and normal ovarian tissues.

The circNRA expression profiles in this study demonstrated that 2,556 circRNAs were abnormally upregulated and 1,832 circRNAs were downregulated in the EOC specimens compared with the normal ovarian tissues. In particular, circBNC2, circEXOC6B, circFAM13B, circN4BP2L2, circRHOBTB3 and circCELSR1 were validated to be significantly dysregulated in the EOC specimens compared with the normal ovarian tissues by RT-qPCR. Recently, Bahn *et al* (26) successfully identified >400 circRNAs in human cell-free saliva. It has also been shown that, compared to their corresponding linear counterparts, circRNAs have a higher expression levels in blood. While the liner mRNAs

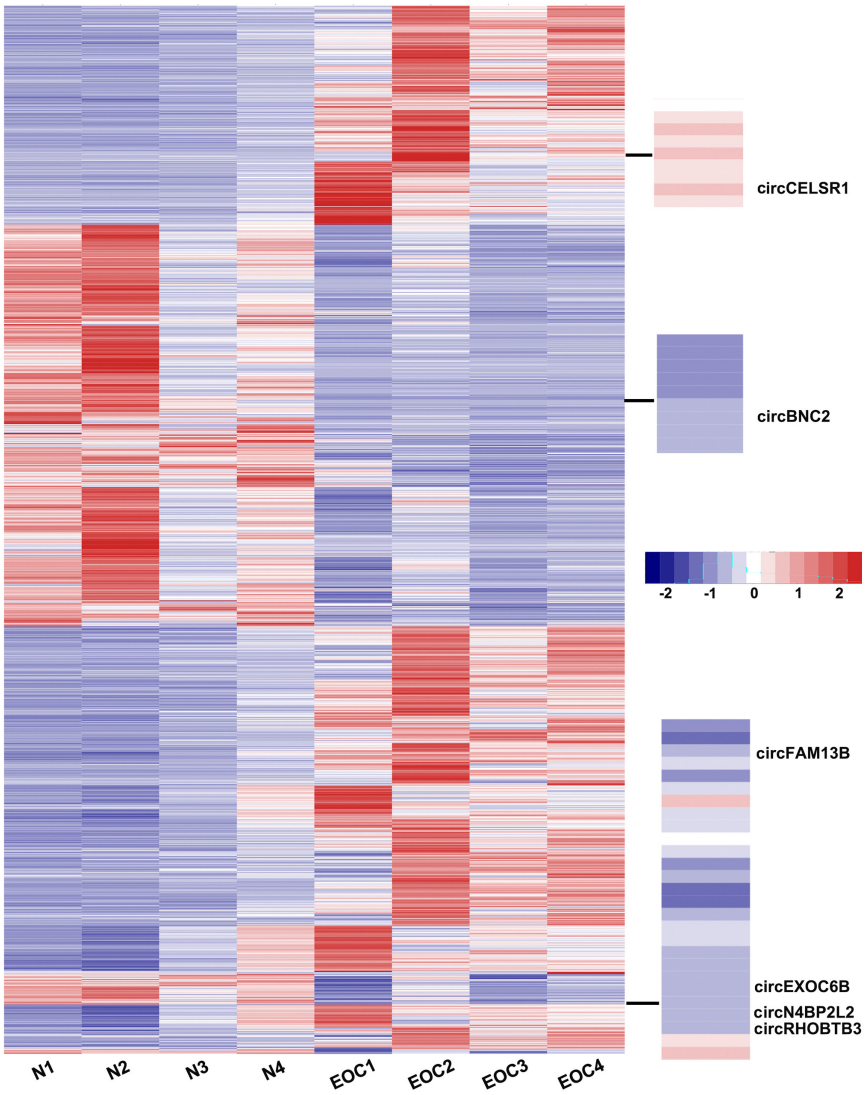

Figure 2. Clustered heatmap of the differentially expressed circular RNAs (circRNAs) in 4 epithelial ovarian cancer (EOC) specimens and 4 normal ovarian tissues. Columns represent tissues, while rows represent circRNAs.

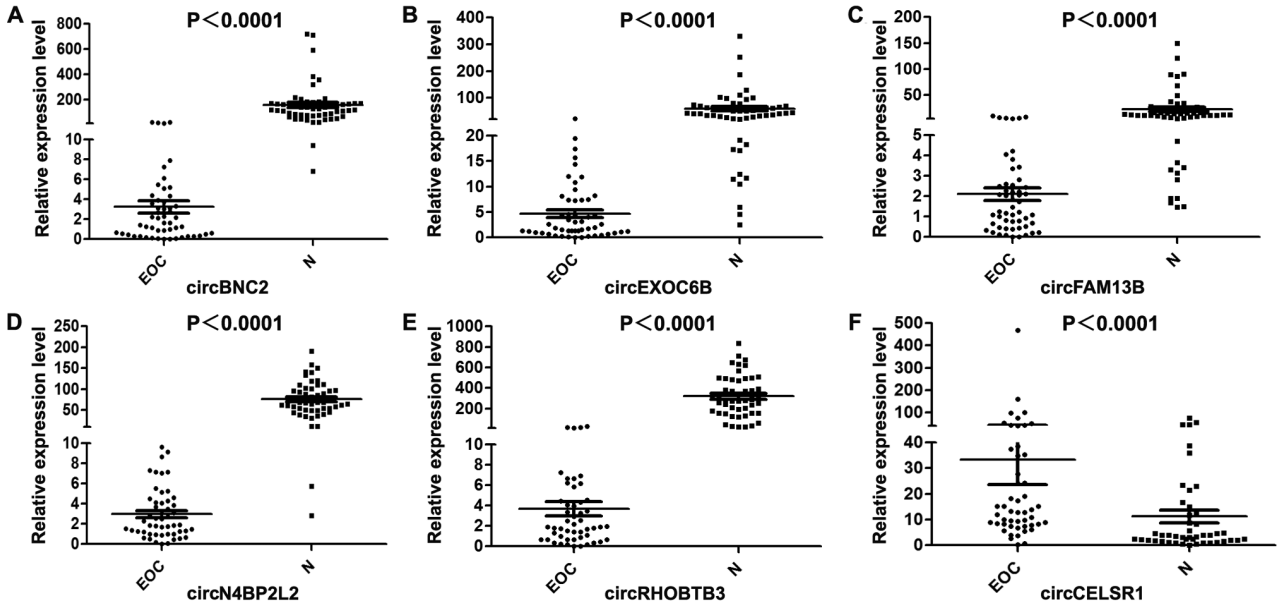

Figure 3. Validation of 6 differentially expressed circular RNA (circRNA) candidates in 54 epithelial ovarian cancer (EOC) specimens and 54 normal ovarian tissues by RT-qPCR. (A) circBNC2, (B) circEXOC6B, (C) circFAM13B, (D) circN4BP2L2, (E) circRHOBTB3 and (F) circCELSR1.

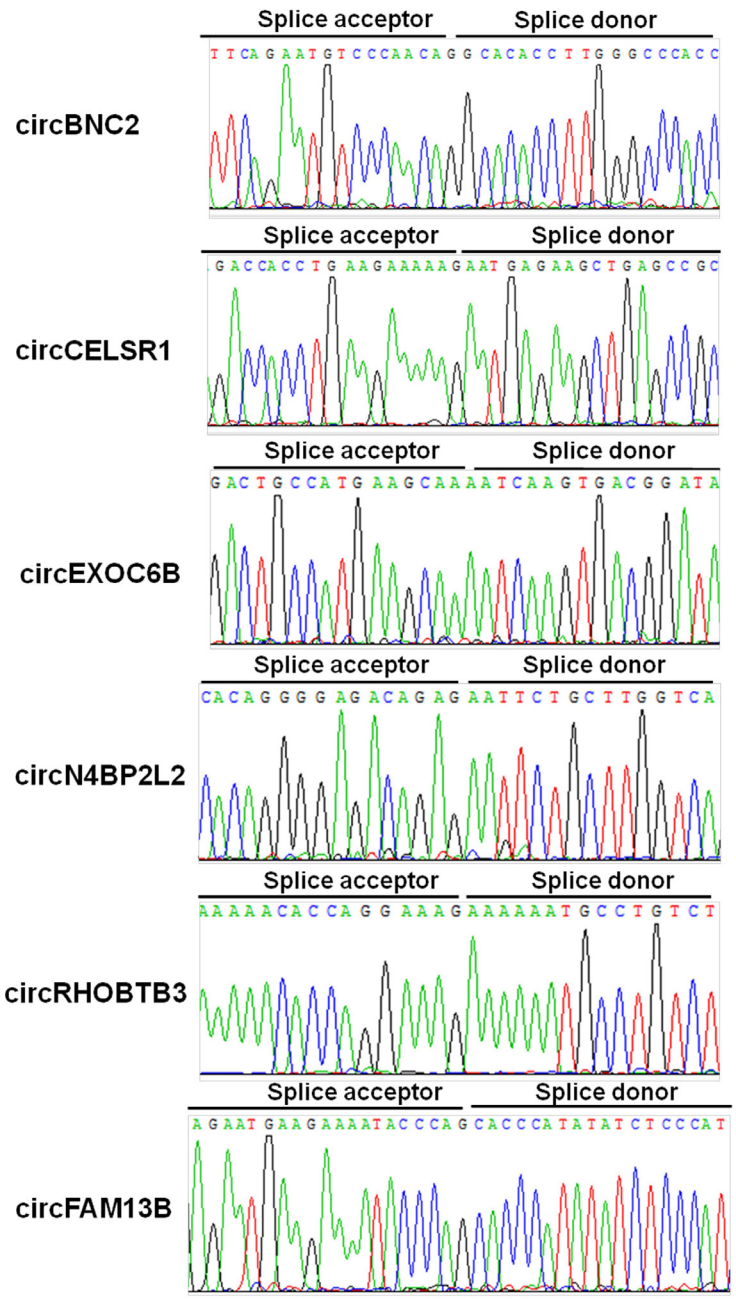

Figure 4. circBNC2, circCELSR1, circEXOC6B, circN4BP2L2, circRHOBTB3 and circFAM13B were validated by sanger sequencing. Splice donor and splice acceptor are indicated.

are actually absent, the circRNA isoforms are easily detected, thus enabling circRNAs to embody disease information which conventional RNA assay fails to determine (19). Given the abundance of circRNAs, their tissue-specific expression patterns and their high stability *in vivo*, circRNAs may be used as promising biomarkers for clinical applications in the future. Accordingly, the results of this study suggest that circBNC2, circEXOC6B, circFAM13B, circN4BP2L2, circRHOBTB3 and circCELSR1 may be potential diagnostic biomarkers for EOC. We further tested these 6 circRNAs in our laboratory with tissues and plasma.

By performing outcome analysis, we found that the expression levels of circEXOC6B and circN4BP2L2 were significantly associated with OS and PFS, respectively. The OS of patients with EOC with a low circEXOC6B expression was significantly worse than that of patients with a high circEXOC6B expression. Moreover, patients with EOC with a low circN4BP2L2 expression had a significantly worse PFS than those with a high circN4BP2L2 expression. These results suggested that circEXOC6B and circN4BP2L2 may act as important and useful prognostic biomarkers in patients with EOC. Our data demonstrated that circEXOC6B and circN4BP2L2 were negatively associated with FIGO stage and/or LNM. In addition, we found that FIGO stage and LNM were negatively associated with the survival of patients with EOC. Thus, circEXOC6B and circN4BP2L2 may play a profound role in regulating tumor invasion and metastasis. Prognostic biomarkers can help in selecting personalized treatment regimens for patients with EOC. circRNAs are highly stable in cells, and their tissue-specific character make them more

Table II. The prognostic values of various clinicopathological features and differentially expressed circRNAs in patients with epithelial ovarian cancer.

Variables	N	OS Rate (%)	P-value	PFS Rate (%)	P-value
All cases (n=54)					
Age (years)					
<50	23	69.6	0.526	39.1	0.334
≥50	31	80.6		51.6	
FIGO stage					
I-II	14	92.9	0.13	78.6	0.003[a]
III-VI	40	70.0		35.0	
Residual tumor diameter (cm)					
<1	43	74.4	0.866	44.2	0.570
≥1	11	81.8		54.5	
Lymph node metastasis					
No	35	80.0	0.354	60.0	0.024[a]
Yes	19	63.2		21.1	
CA125 level (U/ml)					
<600	31	74.2	0.817	48.4	0.860
≥600	23	78.3		43.5	
Ascites (ml)					
<100	13	76.9	0.661	53.8	0.350
≥100	41	75.6		43.9	
circBNC2					
High	27	70.4	0.814	51.9	0.809
Low	27	81.5		40.7	
circEXOC6B					
High	27	88.9	0.045[a]	48.1	0.636
Low	27	63.0		44.4	
circFAM13B					
High	27	77.8	0.956	48.1	0.939
Low	27	74.1		44.4	
circN4BP2L2					
High	27	85.2	0.059	59.3	0.025[a]
Low	27	66.7		33.3	
circRHOBTB3					
High	27	81.5	0.052	51.9	0.121
Low	27	70.4		40.7	
circCELSR1					
High	27	74.1	0.856	44.4	0.944
Low	27	77.8		48.1	

circRNAs, circular RNAs; FIGO, the International Federation of Gynecology and Obstetrics; OS, overall survival; PFS, progress-free survival. [a]P<0.05, statistically significant differences.

sensitive to reflect the real condition *in vivo* (6). Therefore, our findings verified the promising clinical values of circRNAs in tumor diagnosis and treatment.

This study has some limitations. Since the sample size in our study was relatively small and was limited to Han ethnicity Chinese patients from a single institution, the specificity and accuracy of the model cannot be estimated accurately. Therefore, prospectively multicenter studies with a larger sample size and heterogeneous cohorts of patients should be conducted in the future to verify our results. Furthermore, functional experimental analyses of cancer cell lines and experimental animals should be further conducted to

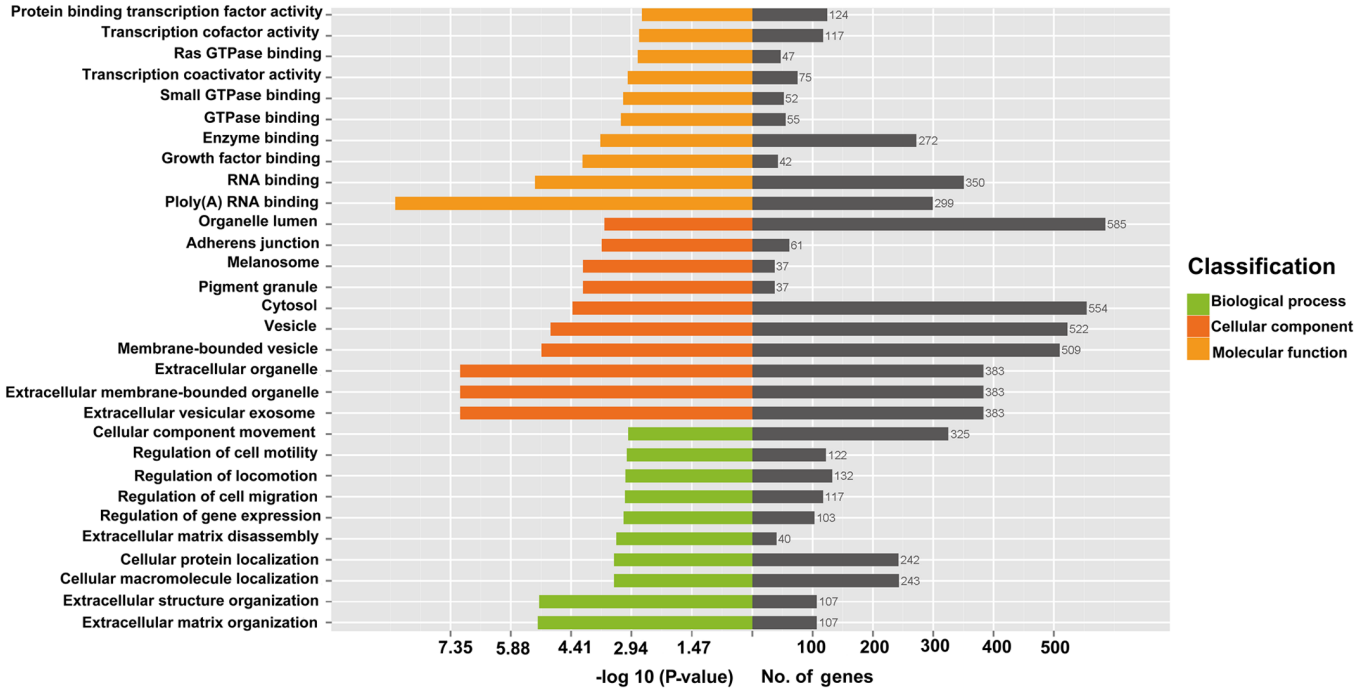

Figure 5. Gene Ontology enrichment analysis on the differentially expressed circular RNAs (circRNAs) with identified target genes.

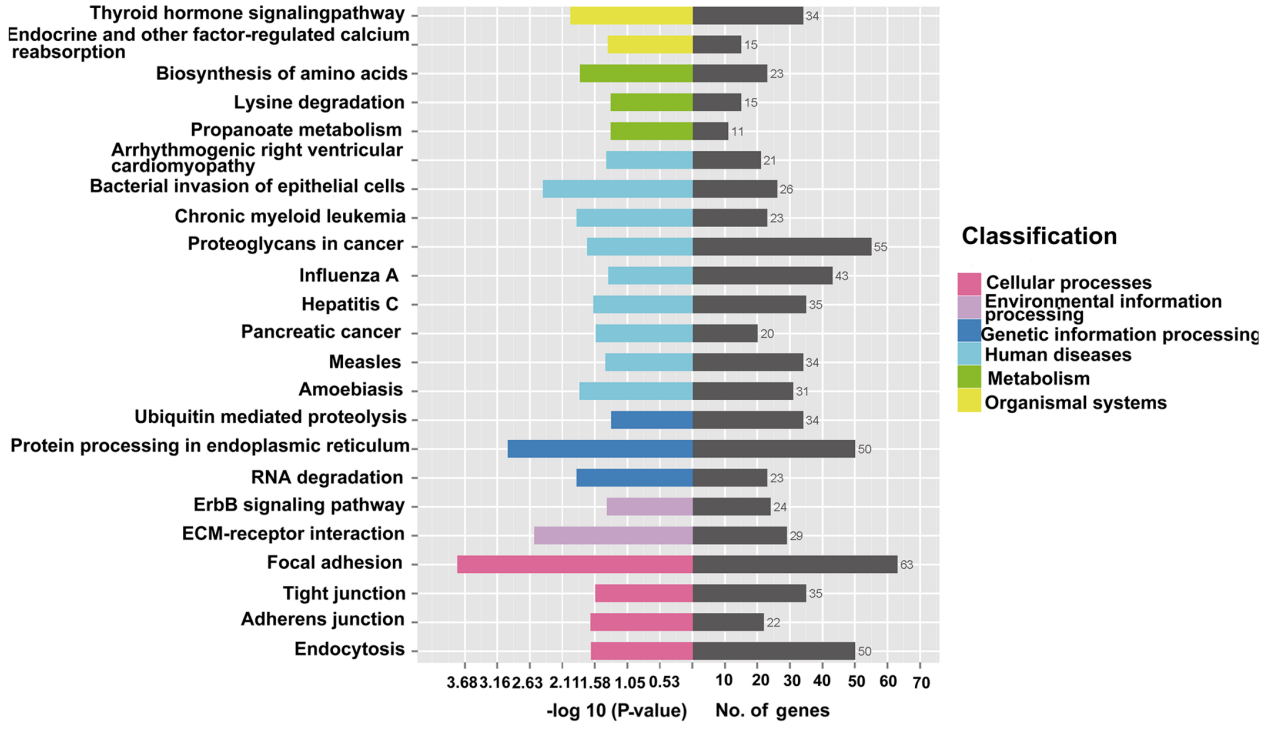

Figure 6. KEGG analysis revealed that there were 23 pathways related to differentially expressed circular RNAs (circRNAs).

determine whether these circRNAs are involved in the tumorigenesis and progression of EOC.

In conclusion, this study identified the circRNA profile of EOC specimens. We demonstrated that circBNC2, circEXOC6B, circFAM13B, circN4BP2L2, circRHOBTB3 and circCELSR1 were significantly dysregulated in EOC specimens and may be potential diagnostic biomarkers for EOC. We also found that circEXOC6B and circN4BP2L2 may serve as novel prognostic biomarkers in patients with EOC. However, future experiments with a larger sample are required to verify the current findings and to elucidate the regulatory mechanisms of action of circRNAs in the tumorigenesis of EOC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LN, BL, YH and JHL contributed to study conception and design, data analysis, and manuscript preparation. WZ contributed to data analysis. MY, SW, DC, JY and KS contributed to data collection and data analysis. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The use of human tissues was approved by the Ethics Committee of PUMCH and patient consent was obtained.

Consent for publication

Not applicable.

Competing Interests

The authors declared that they have no competing interests.

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