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Circular RNA_LARP4 is lower expressed and serves as a potential biomarker of ovarian cancer prognosis

T. ZOU, P.-L. WANG, Y. GAO, W.-T. LIANG

Department of Gynaecology, Guizhou Provincial People's Hospital, Guiyang, Guizhou Province, China

Abstract. – OBJECTIVE: Circular RNAs (circRNAs) have been identified as important regulators in regulating cancer progression. The study aims to investigate the expression of circular RNA_LARP4 (circ LARP4) and clinical significance in ovarian cancer (OC).

PATIENTS AND METHODS: The expression of circ LARP4 was detected in a total of 78 paired ovarian cancer tissue and adjacent normal tissue samples using quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) analyses. The chi-square test was used to assess the association between expression of circLARP4 and clinical-pathological parameters. Survival plot was evaluated using the Kaplan-Meier method. The multivariate Cox analysis model was used for tumor prognosis analysis.

RESULTS: We identified that circLARP4 expression was significantly down-regulated in ovarian cancer tissues compared with corresponding controls. Furthermore, we found that circLARP4 expression was significantly associated with International Federation of Gynecology and Obstetrics (FIGO) stage and lymph node metastases. Lower circLARP4 expression was associated with poor prognosis of OC patients. Moreover, multivariate Cox analysis showed that lower circLARP4 was an independent risk for OC prognosis.

CONCLUSIONS: These results indicated that circLARP4 expression was lower and highlighted that circLARP4 was identified as a potential biomarker of ovarian cancer prognosis.

Key Words:
Circular RNAs, Circular RNA_LARP4, Biomarker, Tumor prognosis.

Introduction

Ovarian cancer (OC) is a highly lethal gynecologic malignancy of the female reproductive system worldwide[1]. Patients who are diagnosed to confine to the ovary have about 90% event-free survival rates. However, more than 70% of cases are diagnosed at an advanced stage and the 5-year survival rates for disease are approximately 20-30%[2,3]. Thus, to identify crucial molecules for predicting and diagnosing of OC is needed.

Circular RNAs (circRNAs) are covalently closed, single-stranded transcripts that may regulate gene expression in mammals[4]. Circular RNAs (circRNAs) are endogenous RNA species formed by spliceosomal joining of the downstream 5' splice site of an exon with the 3'splice site of either the same or an upstream exon, in a process known as back-splicing[4,5]. Recently, circRNAs are identified as biomarkers in some types of tumors[6]. For instance, high circUBAP2 expression is significantly correlated with human osteosarcoma progression and prognosis[7]. CircRNA-MYLK is significantly up-regulated bladder cancer. Importantly, circRNA-MYLK levels are related to the progression of stage and grade of bladder cancer patients[8]. Circular RNA_LARP4 (circLARP4) is identified to sponge miR-424 by circRNA expression profile and bioinformatics analysis and associates with pathological stage and unfavorable prognosis of gastric cancer patients[9]. However, the underlying clinical role of circLARP4 expression in OC remains undetermined.

In this work, we identified that circLARP4 expression was significantly down-regulated in ovarian cancer tissues. Lower circLARP4 expression was significantly associated with poor prognosis of OC patients. Lower circLARP4 expression was identified as an independent risk for OC prognosis. Therefore, these results indicated that circLARP4 was down-regulated and may serve as a potential biomarker of ovarian cancer prognosis.

Patients and Methods

Patients and Tissue Samples

A total of 78 paired ovarian cancer tissue and adjacent normal tissue samples were collected

Corresponding Authors: Pingling Wang, MD; e-mail: ken635jiufenqueta@163.com

from patients (age, ranks from 26 to 68 years) who underwent primary surgical resection at Department of Gynaecology, Guizhou Provincial People's Hospital between July 2010 and December 2014. The normal tissues were located at more than 5 cm away from the tumors. Fresh tissue samples were snap-frozen in liquid nitrogen after surgery and stored at -80°C immediately for the RNA analysis. The investigation was approved by the Ethics Committee of Guizhou Provincial People's Hospital. The written informed patients' consent was obtained from each case before the study. The clinical data are shown in Table I.

RNA Extraction and Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

The total RNA was extracted from frozen ovarian epithelial carcinoma tissue and adjacent normal tissue samples using Trizol (Invitrogen, Carlsbad, CA, USA). The RNA concentration was detected with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Complementary DNA (cDNA) was obtained by reverse transcription with RNA using one step Prime Script miRNA cDNA Synthesis Kit (TaKaRa, Otsu, Shiga, Japan). Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR) reaction was performed using SYBR-Green kit (TaKaRa, Otsu, Shiga, Japan) on an ABI 7500 quantitative PCR instrument (Applied Biosystems, Foster City, CA, USA). Relative mRNA expression of circLARP4 was determined using the $2^{-\Delta\Delta CT}$ methods. The relative primer sequences was as follow: circLARP4-forward: 5'-GGGCATCAGGAGCAAACTTA-3'. circLARP4-reverse: 5'-CTGGCGAATTAAAGCCATTC-3'. GAPDH-forward: 5'-AACTTTGGGATTGTGGAAGG-3', GAPDH-reverse: 5'-ACACATTGGGGGTAGGAACA-3'. GAPDH was used as an internal control.

Statistical Analysis

Statistical analyses were performed using the SPSS software (SPSS, Inc., Chicago, IL, USA). The results were presented as the mean ± standard deviation (SD). The Student's *t*-test was used to assess the statistical significance for comparing two groups. The chi-square test was used to assess the association between expression of circLARP4 and clinical pathological parameters. Survival plot was evaluated using the Kaplan-Meier method and log-rank test. The multivariate Cox analysis model was used for the survival analysis. The $p < 0.05$ was considered as statistical significance.

Table I. The association between circLARP4 expression and clinicopathological parameters.

Clinicopathological parameters	Total (n=78)	circLARP4 expression		*p*-value
		Higher (n=40)	Lower (n=38)	
Age				0.822
≤50	45	23	22	
>50	33	17	18	
Serum CA125				0.962
≤319	31	16	15	
>319	47	24	23	
Tumor size (cm)				0.353
≤4	32	14	18	
>4	46	26	20	
Tumor grade				0.874
higher	33	18	15	
moderately	24	12	12	
lower	21	10	11	
FIGO stage				0.012*
I/II	48	30	18	
III-IV	30	10	20	
Lymph node metastasis				0.002*
Negative	43	29	14	
Positive	35	11	24	

*$p < 0.05$.

Table II. Multivariate Cox regression analysis of parameters determining DFS and OS.

	Disease free survival (DFS)		Overall survival (OS)	
Factors	HR (95% CI)	p	HR (95% CI)	p
Age (years)	0.546 (0.154-1.211)	0.954	0.611 (0.221-1.144)	0.907
Serum CA125	1.108 (0.654-1.766)	0.513	1.211 (0.788-1.987)	0.456
Tumor size (cm)	0.836 (0.467-1.566)	0.698	0.912 (0.599-1.766)	0.619
Tumor grade	1.122 (0.688-1.876)	0.488	1.235 (0.865-1.886)	0.402
FIGO stage	2.155 (1.212-3.669)	0.001*	2.465 (1.611-3.588)	0.001*
Lymph node metastasis	2.443 (1.669-3.808)	0.001*	2.583 (1.884-3.996)	0.001*
Lower circLARP4	2.669 (1.422-4.199)	0.001*	2.818 (1.515-4.899)	0.001*

*$p < 0.05$.

Results

The Expression of circLARP4 is Downregulated in OC Tissue Samples

In the study, we detected the expression of circLARP4 in 78 paired ovarian epithelial carcinoma tissue and adjacent normal tissue samples using qRT-PCR analyses. As shown in Figure 1, the expression of circLARP4 in ovarian cancer tissues was significantly down-regulated compared to adjacent normal tissue samples ($p < 0.01$).

The Expression of circLARP4 Associates With FIGO Stage and Lymph Node Metastases of OC Patients

We further assessed the expression of circLARP4 whether associated with clinical pathological parameters in OC. We divided the patients with ovarian cancer into two groups (higher expression or lower expression group) according to its median expression. The chi square test was used to assess the association between expression of circLARP4 and clinical pathological parameters. As shown in Table I, the lower circLARP4 expression positively associated with tumor advanced FIGO stage ($p = 0.012$; Table I) and lymph node metastases ($p = 0.002$; Table I). However, we did not detect a marked relationship between circLARP4 expression and age, serum CA125, tumor size, or tumor grade ($p > 0.05$, Table I).

Association Between circLARP4 Expression and Prognosis of OC Patients

We then analyzed the association between the expression of circLARP4 and prognosis. Kaplan-Meier curve and log-rank test results showed that patients with lower expression of circLARP4 had significantly worse disease free survival (DFS) ($p < 0.05$; log-rank test, Figure 2A) and overall survival (OS) ($p < 0.05$; log-rank test, Figure 2B).

Afterwards, the multivariate Cox analyses indicated that lower circLARP4 expression (HR, 2.669, $p<0.05$, Table II), International Federation of Gynecology and Obstetrics (FIGO) stage (HR, 2.155, $p<0.05$, Table II), and lymph node metastases (HR, 2.443, $p<0.05$, Table II) were independent prognostic factors of DFS in OC patients. The same was observed for OS survival in OC patients ($p < 0.001$, respective, Table II). Thus, these results indicated that circLARP4 expression was identified as an independent prognostic factor for OC.

Discussion

In recent years, next-generation sequencing techniques, especially RNA-seq, have showed great

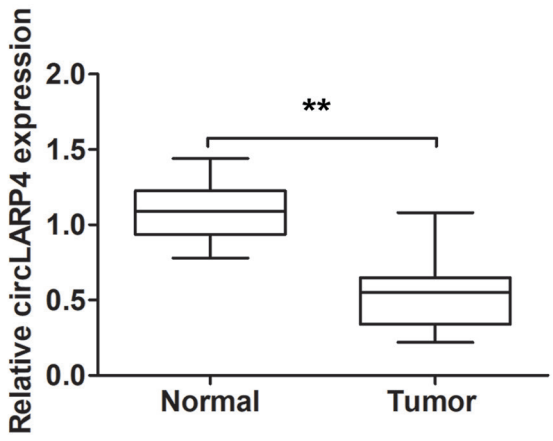

Figure 1. The expression of circLARP4 was downregulated in 78 paired ovarian epithelial carcinoma tissue and adjacent normal tissue samples using qRT-PCR analyses, *$p<0.01$.

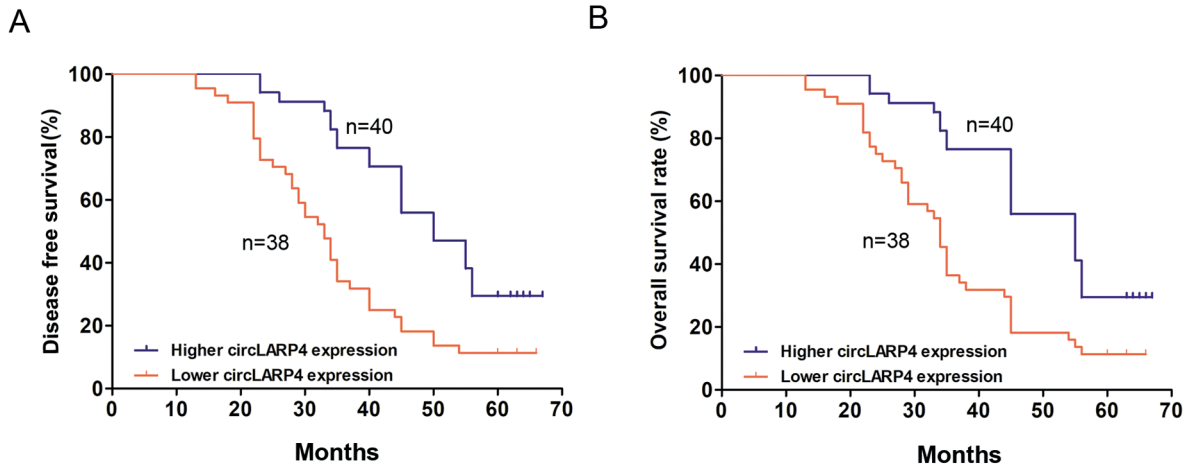

Figure 2. The expression of circLARP4 associated with tumor prognosis. ***(A)*** Kaplan-Meier curve results showed that patients with lower expression of circLARP4 had significantly worse disease free survival and ***(B)*** overall survival, log-rank test.

abundance and dysregulation of many circRNAs in many diseases[10,11]. Aberrant circular RNA is found to play crucial regulators in tumors developments[12]. In the previous study, Zhou et al[13] found that downregulation of hsa_circ_0011946 suppresses the migration and invasion of the breast cancer cell line MCF-7 by targeting RFC3. Kun-Peng et al[14] showed that overexpressed circPVT1 contributes to doxorubicin and cisplatin resistance of osteosarcoma cells by regulating ABCB1. Zhu et al[15] demonstrated that circ_0067934 promotes tumor growth and metastasis in hepatocellular carcinoma through regulation of miR-1324/FZD5/Wnt/β-catenin axis. Circular RNA circ-ITCH inhibits bladder cancer progression by sponging miR-17/miR-224 and regulating p21, PTEN expression[16]. Silencing of hsa_circ_0007534 suppresses proliferation and induces apoptosis in colorectal cancer cells[17]. These studies showed that circRNAs were involved in tumor initiation and progression and may be a prognostic maker or potential therapeutic target of tumors.

In the present work, we first detected the expression of circLARP4 in 78 paired ovarian cancer tissue and adjacent normal tissue samples. The results showed that the expression of circLARP4 in ovarian cancer tissues was significantly downregulated compared to adjacent normal tissue samples. Furthermore, the lower circLARP4 expression positively associated with tumor advanced FIGO stage and lymph node metastases. Patients with reduced expression of circLARP4 had significantly worse disease free survival and overall survival. In the previous study, by circRNA expression profile and bioinformatics analysis, circLARP4 was high expressed in gastric cancer and inhibited biological behaviors of gastric cancer cells by sponging miR-424. The expression of circLARP4 was lower in GC tissues and represented an independent prognostic factor for overall survival of GC patients[9]. In this research, our results also showed the important clinical value of circLARP4 in OC.

Conclusions

We first found that circLARP4 expression was low expression in OC tissues. Lower circLARP4 expression associated with poor prognosis of OC patients. Thus, these results demonstrated that circLARP4 expression may be identified as prognostic maker of OC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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