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CircHIPK3 is upregulated and predicts a poor prognosis in epithelial ovarian cancer

N. LIU[1], J. ZHANG[1], L.-Y. ZHANG[1], L. WANG[2]

[1]Department of Gynaecology in Weifang Yidu Central Hospital, Wei Fang, ShanDong, China
[2]Department of Gynaecology in Weifang People's Hospital, Wei Fang, ShanDong, China

Ning Liu and *Jie Zhang* contributed equally to this work

Abstract. – OBJECTIVE: CircRNAs have been recently identified as important regulators in tumors biological functions. However, the clinical significance of circHIPK3 in epithelial ovarian cancer (EOC) remains unknown.

PATIENTS AND METHODS: The expression of circHIPK3 in EOC tumor tissues and adjacent noncancerous tissues was analyzed by quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). The association between circHIPK3 expression and clinicopathological factors was analyzed by using Chi-square test. Kaplan-Meier method and log-rank test were used to analyze the association of circHIPK3 expression with disease-free survival (DFS) and overall survival (OS) time of EOC patients. Univariate and multivariate Cox analysis was also performed.

RESULTS: We found that circHIPK3 was higher expressed in EOC tissues and cells compared to adjacent normal tissue and ovarian epithelium cell line, respectively. Higher circHIPK3 expression associated with lymph node invasion, FIGO stage, and worse DFS and OS of patients. Moreover, multivariate Cox analysis showed that higher circHIPK3 was an independent predictor of DFS and OS in EOC patients.

CONCLUSIONS: Thus, circHIPK3 may be a novel biomarker for predicting EOC prognosis.

Key Words:
CircRNAs, circHIPK3, Ovarian cancer, Predictor, Prognosis.

Introduction

Ovarian cancer (OC), the most common gynecological malignancy, causes large cancer-related mortality in female worldwide[1]. Epithelial ovarian cancer (EOC) accounts for about 90% of all ovarian cancers. More than 225,000 new cases are diagnosed, and approximately 100,000 cases die of this disease[2]. Although large advances including surgical resection and chemotherapy have improved, the overall 5-year survival rate for patients is about 35-38%[3,4]. Thus, to investigate novel prognostic markers and therapeutic targets is urgent.

Recently, circular RNAs (circRNAs), a novel class of noncoding RNAs, emerged as crucial regulators of tumor cell proliferation, differentiation, apoptosis, invasion, and metastasis[5,6]. For example, increased circular RNA UBAP2 acts as a sponge of miR-143 to promote osteosarcoma progression[7]. The circGFRA1 and GFRA1 act as ceRNAs in triple negative breast cancer by regulating miR-34a[8]. Hsa_circ_0067531 may affect the biological functions of CD90+ hepatocellular carcinoma (HCC) cells and may be a promising candidate for diagnosis and therapy of HCC[9]. CircRNA_100782 regulates pancreatic carcinoma proliferation through the IL6-STAT3 pathway[10].

Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs in tumors[11]. In the previous study, circHIPK3 contains two critical binding sites for the microRNA miR-558 and can abundantly sponge miR-558 to suppress the expression of heparanase (HPSE)[12]. In the study, we found that circHIPK3 was higher expressed in EOC tissues compared to adjacent normal tissues. Higher circHIPK3 expression is associated with lymph node invasion, FIGO stage, and worse prognosis. Thus, circHIPK3 may be a novel biomarkers of predicting EOC prognosis.

Patients and Methods

Patient Tissues

A total of 69 EOC tissues and adjacent noncancerous tissues were obtained from primary EOC patients at Department of Gynecology in Weifang People's Hospital. None of the patients had re-

Corresponding Author: Li Wang, MD; e-mail: ke79muhuangyi@163.com

ceived any therapy before surgery. After surgery, all specimens were immediately frozen in liquid nitrogen and stored at -80°C until use. The study was approved by the Ethics Committee of Weifang People's Hospital. Written informed consent was obtained from all patients in the study. The clinicopathological factors were shown in Table I.

Cell Lines Culture

The ovarian cancer cell lines (A2780, HO-8910, SKOV3 and CAOV3 cells) and a human ovarian epithelium cell line (HOEC) were purchased from the American Type Culture Collection (Manassas, VA, USA). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS, Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA). All the cells were cultured at 37°C in a humidified incubator containing 5% CO_2.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was isolated from tissues and cells using Invitrogen TRIzol reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturers' instructions. RNA concentration was assessed spectrophotometrically at 260 nm (Thermo ND 2000; Thermo Fisher Scientific, Inc. Wilmington, DE, USA). The RNA was reversed to complementary DNA (cDNA) by using reverse transcription kit (Qiagen, Bayern, Germany). SYBR® Premix Ex Taq™ II (Takara, Dalian, China) was used to analyze the mRNA expression. GAPDH was used as internal controls. The primers used for qRT-PCR in this study were as follow: circHIPK3-F: 5'-TGGAGACTGGGGGAAGATGA-3'; circHIPK3-R5'-CACACTAACTGGCTGAGGGG-3'. The mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

Statistical Analysis

The data was analyzed using SPSS 19.0 for Windows (IBM Corp. Released 2010. IBM SPSS Statistics for Windows, Armonk, NY, USA).

Results were expressed as mean ± standard deviation (SD). The association between the circHIPK3 expression and clinicopathological features was analyzed by using Chi-square test. Survival plots were calculated using Kaplan-Mei-

Table I. The correlation between circHIPK3 expression and clinicopathological factors.

Characteristic	Patients (n = 69)	circHIPK3 expression		*p*-value
		Lower (n = 36)	Higher (n =33)	
Age (years)				0.402
≤ 50	35	20	15	
> 50	34	16	18	
Position				0.431
Bilateral	40	23	17	
Unilateral	20	8	12	
Unknown	9	5	4	
Tumor size (mm)				0.817
≤ 20	20	10	10	
> 20	49	26	23	
Differentiation				0.406
Higher and moderately	49	24	25	
Lower	20	12	8	
Lymph node invasion				
0.043[a]				
Negative	44	27	17	
Positive	25	9	16	
Tumor grade				0.200
G1+G2	41	24	17	
G3+G4	28	12	16	
FIGO stage				
0.003[a]				
I	42	28	14	
II-III	27	8	19	

[a] $p < 0.05$.

er methods and log-rank test. Univariate and multivariate analysis Cox regression model was also used. The p-value < 0.05 is identified as statistically significant.

Results

The circHIPK3 Expression is Higher in EOC Tissues and Cells

In the study, we examined the expression of circHIPK3 in 69 paired of EOC tissue samples and matched adjacent noncancerous tissues. As shown in Figure 1A, the qRT-PCR analysis results demonstrated that circHIPK3 expression is significantly higher in EOC tissue samples compared to matched adjacent noncancerous tissues ($p < 0.05$). We also found that circHIPK3 expression is significantly higher in four ovarian cancer cell lines (A2780, HO-8910, SKOV3 and CAOV3 cells) compared to a human ovarian epithelium cell line (HOEC) (Figure 1B). Thus, these results indicated that the circHIPK3 expression was higher in EOC tissues and cells, which may be involved in tumor progression.

Association of circHIPK3 Expression with Clinicopathological Factors in EOC Patients

Furthermore, we analyzed the association between circHIPK3 expression and clinicopathological factors of EOC patients. According to the median expression of circHIPK3 in EOC tissues, patients were divided into two groups (higher circHIPK3 expression group and lower circHIPK3 expression group). The results demonstrated that higher circHIPK3 expression was positively associated with lymph node invasion ($p = 0.043$, Table I) and advanced FIGO stage ($p = 0.003$, Table I) in EOC patients. However, no association was found between circHIPK3 expression and age, tumor size or tumor position and so on in patients (all of $p > 0.05$, Table I).

Higher circHIPK3 Expression Predicts a Poor Prognosis of EOC Patients

Subsequently, we analyzed the association of circHIPK3 expression with prognosis of EOC patients, the Kaplan-Meier method and log-rank test showed that higher circHIPK3 expression showed a shorter DFS (log rank=9.589, Figure 2A, $p < 0.05$) and OS (log rank=10.731, Figure 2B, $p < 0.05$) of patients compared to lower circHIPK3 expression in EOC patients. Moreover, univariate and multivariate Cox analysis showed that higher circHIPK3 expression (HR, 2.226; 95% CI, 1.445-3.444; $p < 0.05$), FIGO stage (HR, 2.019; 95% CI, 1.206-3.318; $p < 0.05$), and lymph node invasion (HR, 1.894; 95% CI, 1.211-3.055; $p < 0.05$) were independent poor prognostic factors for DFS patients (Table II). Consistently, higher circHIPK3 expression (HR, 2.188; 95% CI, 1.065-

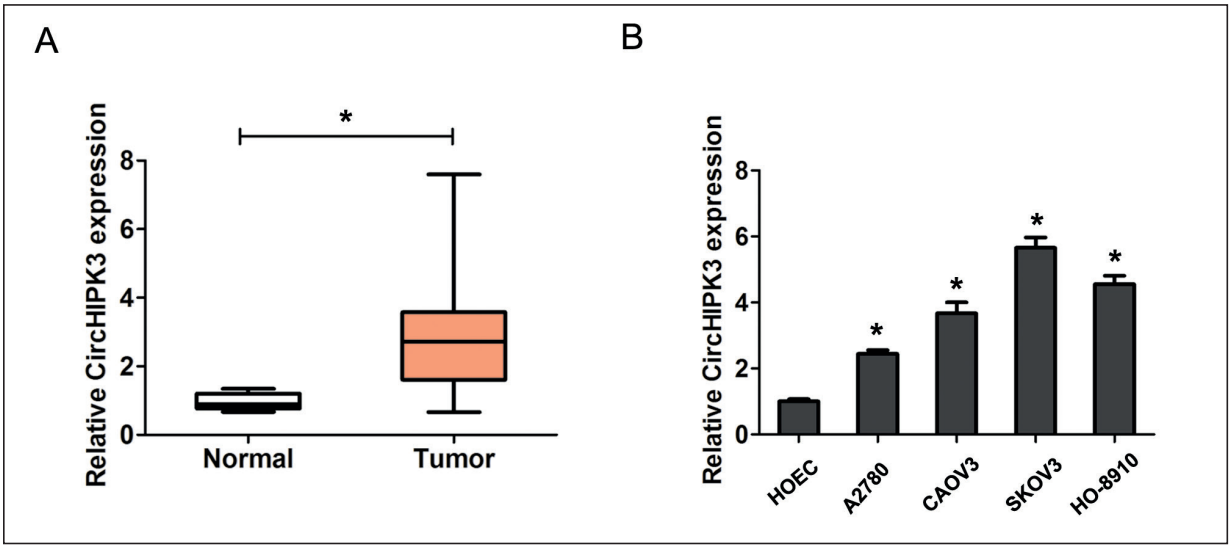

Figure 1. The circHIPK3 expression is higher in EOC tissues and cells. **A,** The circHIPK3 expression was examined in 69 paired of EOC tissues and adjacent normal tissues using qRT-PCR analysis. GAPDH was used as an internal control. **B,** The circHIPK3 expression was examined in three ovarian cancer cell lines (A2780, HO-8910, SKOV3, and CAOV3 cells) compared to a human ovarian epithelium cell line (HOEC) using qRT-PCR analysis. GAPDH was used as an internal control. *$p < 0.05$.

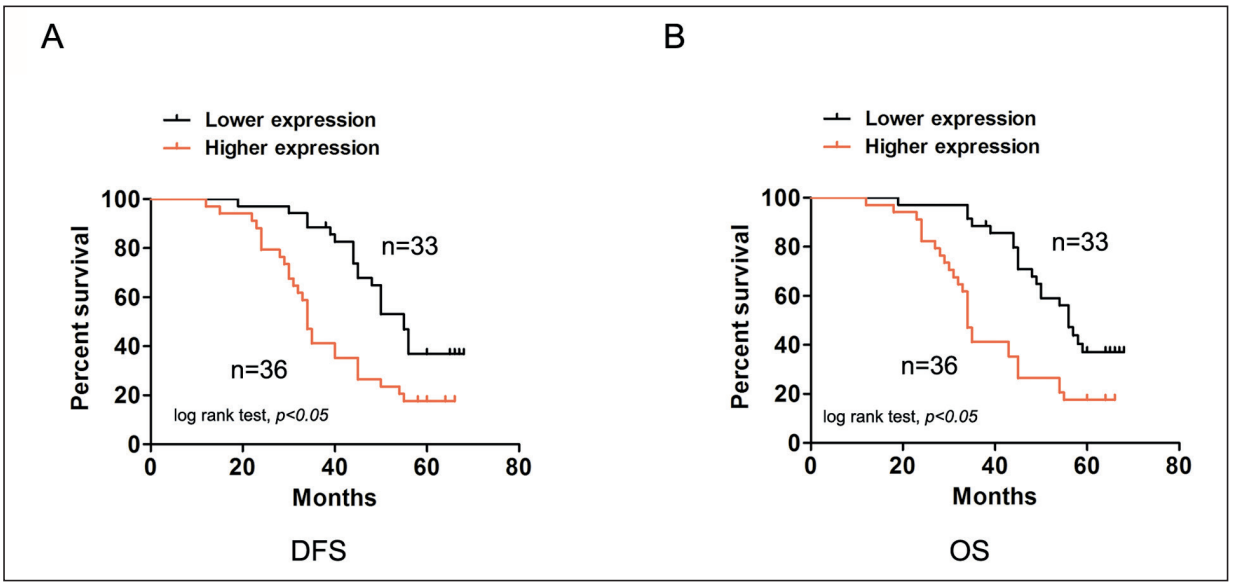

Figure 2. The circHIPK3 expression predicts poor DFS and OS of EOC patients. **A,** The higher circHIPK3 expression groups showed a poor DFS compared to lower circHIPK3 expression groups using Kaplan-Meier method and log rank test in EOC patients. **B,** The higher circHIPK3 expression groups showed a poor OS compared to lower circHIPK3 expression groups using Kaplan-Meier method and log rank test in EOC patients.

3.788; $p < 0.05$), FIGO stage (HR, 2.112; 95% CI, 1.105-3.655; $p < 0.05$), and lymph node invasion (HR, 1.966; 95% CI, 1.044-3.556; $p < 0.05$) were also independent poor prognostic factors for OS patients (Table III). Thus, these results indicated that circHIPK3 acted as a prognostic marker for EOC.

Discussion

Due to the lack of efficient screening programs, more than 70% of patients are diagnosed at an advanced stage, resulting in a poor 5-year overall survival (OS) rate[13]. Thus, to explore diagnostic and prognostic biomarker for EOC patients with the early stage is important.

Circular RNAs (circRNAs) are a class of non-coding RNAs (ncRNAs) that form covalently closed continuous loop structures, lacking the terminal 5' and 3'ends. Although limited studies about the involvement of circRNAs in cancer development and progression, some findings have described that circRNA may serve as novel cancer diagnostic and prognostic biomarkers[14]. Circ-LDLRAD3 was up-regulated in pancreatic

Table II. Univariate and multivariate Cox proportional hazards analysis of the association between clinical characteristics and circHIPK3 expression and DFS are shown.

Factor	Univariate Cox analysis		Multivariate Cox analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age (years)	0.557 (0.344-1.066)	0.987		
Position	0.788 (0.424-1.366)	0.768		
Tumor size (mm)	0.955 (0.577-1.684)	0.621		
Differentiation	0.907 (0.512-1.588)	0.661		
Lymph node invasion	2.155 (1.499-3.356)	0.001[a]	1.894 (1.211-3.055)	0.002[a]
Tumor grade	0.686 (0.462-1.268)	0.798		
FIGO stage	2.334 (1.553-3.655)	0.001[a]	2.019 (1.206-3.318)	0.001[a]
circHIPK3 expression	2.575 (1.626-4.014)	0.001[a]	2.226 (1.445-3.444)	0.001[a]

[a]$p < 0.05$.

Table III. Univariate and multivariate Cox proportional hazards analysis of the association between clinical characteristics and circHIPK3 expression and OS are shown.

Factor	Univariate Cox analysis		Multivariate Cox analysis	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Age (years)	0.654 (0.424-1.135)	0.841		
Position	0.818 (0.424-1.553)	0.671		
Tumor size (mm)	0.899 (0.533-1.566)	0.665		
Differentiation	0.984 (0.433-1.752)	0.697		
Lymph node invasion	2.214 (1.284-3.712)	0.001[a]	1.966 (1.044-3.556)	0.001[a]
Tumor grade	0.799 (0.452-1.258)	0.724		
FIGO stage	2.456 (1.412-3.811)	0.001[a]	2.112 (1.105-3.655)	0.001[a]
circHIPK3 expression	2.675 (1.244-4.342)	0.001[a]	2.188 (1.065-3.788)	0.001[a]

[a]$p < 0.05$.

cancer cell lines, pancreatic cancer tissues, and plasma samples from patients with pancreatic cancer. High expression of circ-LDLRAD3 was significantly associated with venous invasion, lymphatic invasion, and metastasis, which may be a biomarker in the diagnosis of pancreatic cancer[15]. The circRNA0003906 expression level was markedly downregulated in both colorectal cancer tissues and cell lines. The downregulation of circRNA0003906 level significantly correlated with lymphatic metastasis and poor differentiation[16]. CircRNA_100782 regulates BxPC3 cell proliferation by acting as miR-124 sponge through the IL6-STAT3 pathway[10]. Hsa_circ_0074362 levels were significantly downregulated in gastric cancer tissues, gastritis tissues, and gastric cancer cell lines. Its levels were associated with lymphatic metastasis[17].

In the study, we found that circHIPK3 expression is significantly higher in EOC tissue samples compared to adjacent noncancerous tissues. In addition, we also found that circHIPK3 expression is significantly higher in EOC cells. Furthermore, higher circHIPK3 expression was positively associated with advanced FIGO stage, lymph node invasion in EOC patients. To analyze the association of circHIPK3 expression with prognosis, the Kaplan-Meier method and log-rank test showed that higher circHIPK3 expression showed a shorter survival time compared to lower circHIPK3 expression in EOC patients. Multivariate Cox analysis showed that higher circHIPK3 was an independent poor prognostic factor for EOC patients. Thus, our finding indicated that circHIPK3 acted as a predictor of EOC, which indicated an important clinical value.

Conclusions

We showed that higher circHIPK3 expression was correlated with poor prognosis of EOC patients, indicating that circHIPK3 acted as a potential biomarker for predicting the prognosis of EOC.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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