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The down-regulation of long non-coding RNA LINC01088 is associated with the poor prognosis of epithelial ovarian cancer patients

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Abstract. – OBJECTIVE: Long intergenic non-coding RNA 1088 (LINC01088) has been suggested to act as a tumor suppressor in epithelial ovarian cancer (EOC); however, the prognostic role of LINC01088 has not been evaluated in cancer patients. This study aimed to investigate the expression of LINC01088 in EOC, along with evaluating its clinical-pathological and prognostic importance.

PATIENTS AND METHODS: A bioinformatics tool (GEPIA) was used to screen the dysregulated lncRNAs. Quantitative Real-time PCR (qRT-PCR) was used to measure expression level of LINC01088 in EOC tumor samples and adjacent non-tumor tissues. Then, the association between LINC01088 expression and pathological parameters were further evaluated. Overall survival (OS) was estimated using the Kaplan-Meier method, and the differences in survival were compared using the log-rank test. Univariate and multivariate analysis were performed using the Cox proportional hazard analysis.

RESULTS: We found that LINC01088 expression was significantly down-regulated in EOC tissues via "GEPIA". Then, the results of RT-PCT confirmed that the expression levels of LINC01088 were significantly lower in EOC tissues compared to adjacent noncancerous tissues ($p < 0.01$). Interestingly, lower LINC01088 expression levels were associated with FIGO stage ($p = 0.000$), grade ($p = 0.003$) and distant metastasis ($p = 0.006$). Moreover, Kaplan-Meier analysis indicated that patients with low LINC01088 expression had a poor overall survival ($p = 0.0013$). Finally, univariate and multivariate analysis show that LINC01088 expression is an independent predictor for overall survival.

CONCLUSIONS: Low LINC01088 expression was associated with the progression of EOC and could serve as a potential independent prognostic biomarker for patients with EOC.

Key Words:
LncRNA LINC01088, Epithelial ovarian cancer, Prognosis.

Introduction

Ovarian cancer is the leading cause of mortality among all gynecological malignancies[1]. As we known, ovarian cancer can be divided into three subgroups: epithelial, stromal and germ cell tumor and 75% of all ovarian cancer types are epithelial ovarian cancer (EOC)[2]. In China, EOC has become a serious and under-recognized threat to women's health with more than 52000 newly diagnosed cases[3]. Despite the improvements in therapeutic intervention with combinations of surgery, chemotherapy, radiotherapy and other novel biological therapies, the survival rate of EOC patients has not sufficiently improved because EOC progression is often asymptomatic and is detected at a late stage[4-6]. Therefore, major efforts have focused on the identification of sensitive biomarkers for the early diagnosis or predicting the clinical outcome in EOC patients. Emerging studies[7] have shown that ncRNAs play important roles in the occurrence and progression of various tumors. Long non-coding RNAs (lncRNAs) are a class of noncoding RNA more than 200 nucleotides in length[8]. LncRNAs regulate the expression of protein-coding genes on epigenetic, transcriptional post-transcriptional levels[9]. It has

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been confirmed[10,11] that lncRNAs play functional roles in many biological processes such as cell development, proliferation, metastasis and differentiation. In addition, accumulative evidences establish the participation of lncRNAs in human disease pathogenesis including malignant neoplasm[12,13]. Moreover, the dysregulation of lncRNAs correlates with malignancy grade and histological differentiation, which have important clinical implications in EOC diagnosis of sub-classification[14]. Up to date, more and more lncRNAs have been reported to function as oncogenes, tumor suppressor genes or both, depending on the conditions[15-17]. However, the expression pattern, function and clinical significance of most of lncRNAs remain unknown. Long intergenic non-coding RNA 1088 (LINC01088) was a newly identified lncRNA, which had been reported to be down-regulated in EOC by microarray analysis[18]. The biological function of LINC01088 in tumors remains largely unknown. Up to date, only one study by Zhang et al[18] reported that LINC01088 function as a tumor suppressor in EOC. However, the clinical significance of LINC01088 has not been investigated. In this study, we further demonstrate whether LINC01088 expression was down-regulated in EOC and firstly reported its prognosis value in EOC patients.

Patients and Methods

Patients and Tissue Samples

EOC tissues and adjacent morphologically normal tissues were collected from 184 EOC patients who underwent surgery at First Affiliated Hospital of Jinzhou Medical University between 2009 and 2013. All samples were processed by two professional pathologists. The fresh tissue specimens were snap frozen in liquid nitrogen. The histopathologic diagnoses were determined by the hospital's pathologist using International Federation of Gynecology and Obstetrics (FIGO). No patients received chemotherapy or radiotherapy prior to surgery. These patients received surgery in our department and were followed for at least 5 years. Clinical follow-up data were obtained by telephone or from outpatient records. The study was approved by the Medical Ethics Committee of First Affiliated Hospital of Jinzhou Medical University (Liaoning, China) and informed written consents were obtained from all cases.

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

Total RNA was extracted from tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Reverse transcription was conducted with Revertra Ace aPCR RT master mix with gDNA remover (TaKaRa, Otsu, Shiga, Japan). qRT-PCR assays were performed to detect LINC01088 expression using the PrimeScript RT reagent Kit and SYBR Premix Ex Taq (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's instructions. U6 was employed to be the control. We assessed the RNA expression according to relative quantification using the $2^{-\Delta\Delta Ct}$ method to determine the fold change in the expression. Primers used for target amplification are listed in Table I.

Statistical Analysis

All statistical analyses were performed using SPSS version 20.0 (IBM, Armonk, NY, USA). Values were expressed as mean ± standard error. Differences between groups were analyzed by the two-tailed Student's *t*-test. Associations between LINC01088 expression and clinicopathologic characteristics were determined by the x^2-test. Survival analysis was evaluated by the Kaplan-Meier method, and the log-rank test was used to compare survival times between groups. The Cox proportional hazards regression model was employed for univariate and multivariate analyses to estimate the prognostic factors for survival prediction. A $p < 0.05$ was considered statistically significant.

Table I. The primer sequence of LINC01088 and U6.

Genes	Sequences
LINC01088 (F)	5'-GCTGGAAACTCCGACGCCATA-3'
LINC01088 (R)	5'-GAGTTGGCCTAGTAGGCCGC-3'
U6 (F)	5'-CTCGCTTCGGCAGCACA-3'
U6 (R)	5'-AACGCTTCACGAATTTGCGT-3'

Table II. Correlation between LINC01088 expression and clinicopathological features in EOC patients.

Parameters	Group	Total	LINC01088 expression		*p*-value
			High	Low	
Age (years)	< 55	90	40	50	NS
	≥ 55	94	54	40	
Histological subtypes	Serous	119	61	58	NS
	Others	76	33	32	
Tumor size	< 8 cm	112	63	49	NS
	≥ 8 cm	72	31	41	
FIGO stage	I + II	111	69	42	0.000
	III + IV	74	25	48	
Grade	G1	114	68	46	0.003
	G2 + G3	70	26	44	
Distant metastasis	Yes	60	22	38	0.006
	No	124	72	52	

Results

LINC01088 Expression is Frequently Downregulated in Human EOC Tissues

In order to identify dysregulated lncRNAs in EOC, we used a online Bioinformatics tools (GEPIA), which is a web server for cancer and normal gene expression profiling and interactive analyses[19]. As shown in Figure 1A, we identified LINC01088 as a frequently downregulated lncRNA in EOC tissues. Next, we further detect the expression of LINC01088 in EOC patients from our hospital. As shown in Figure 1B, the results showed that the level of LINC01088 expression was significantly decreased in EOC tissues compared with adjacent normal tissues ($p < 0.01$). Taken together, our results indicated LINC01088 was down-regulated in EOC patients and might serve as a tumor suppressor.

The Relationship Between LINC01088 Expression and Clinical Features

To analyze whether LINC01088 was associated with the development and progression of EOC, the expression levels of LINC01088 in EOC tissues were categorized as low or high in relation to the

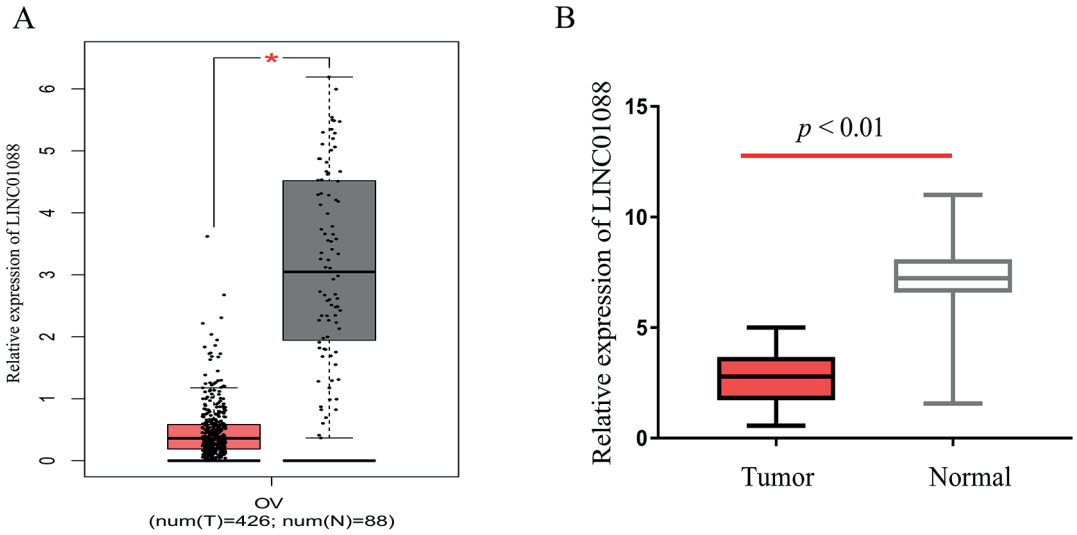

Figure 1. LINC01088 expression was significantly down-regulated in EOC tissues. Relative expression of LINC01088 in EOC tissues in comparison with adjacent non-tumor tissues. LINC01088 expression was examined by qRT-PCR and normalized to U6 expression.

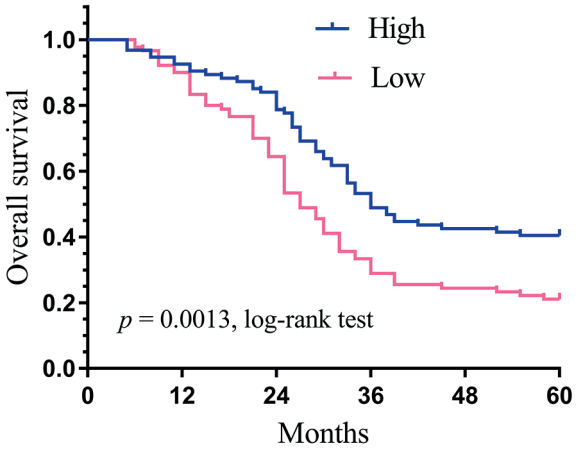

Figure 2. Kaplan-Meier curves for overall survival of 184 EOC patients, divided according to LINC01088 expression levels. Low LINC01088 expression was significantly associated with poor survival ($p = 0.0013$, log-rank test).

mean value. As shown in Table II, it was observed that LINC01088 downregulation was correlated with FIGO stage ($p = 0.000$), grade ($p = 0.003$) and distant metastasis ($p = 0.006$). However, there was no association between LINC01088 expression and age, histological subtypes or tumor size (all $p > 0.05$). Taken together, these observations indicated LINC01088 may serve as a negative regulator in clinical progression of EOC.

LINC01088 Downregulation Predicts Poor Prognosis in Patients With EOC

To clarify the impact of LINC01088 expression on the survival of patients with EOC, log-rank test-based survival analysis of BC patients was performed. As shown in Figure 2, overall survival was better in patients in the LINC01088 high-expression group compared with those in the LINC01088 low-expression group ($p = 0.0013$). Then, univariate analyses were utilized to evaluate whether the LINC01088 expression level and various clinicopathological features are influencing factor for the five-year survival of EOC patients. As shown in Table III, we found that LINC01088, FIGO stage, grade and distant metastasis were significantly associated with EOC prognosis. Furthermore, Cox multivariate survival analysis were done, including those significantly statistic parameters, and indicated that that FIGO stage (HR = 2.348, 95% CI: 1.277-4.238; $p = 0.013$), grade (HR=2.427, 95% CI: 1.338-5.439; $p = 0.009$), distant metastasis (HR = 2.236, 95% CI: 1.156-4.066; $p = 0.0016$) and LINC01088 expression (HR = 2.893, 95% CI: 1.337-5.347; $p = 0.005$) were independent risk prognostic factors for EOC (Table III).

Discussion

Every year, there are many cases diagnosed with EOC and the overall 5-year survival for EOC is approximately 45%[20]. In order to improve prognosis of EOC patients, it is essential to predict the prognosis of EOC patients because it can guide the individualized treatment[21]. Up to date, several clinicopathological variables, such as FIGO stage, histological grade and distant metastasis, were used for prediction of prognosis of EOC patients. However, those factors have been used with feedback of unsatisfactory predictive value[22,23]. Thus, it is of significant importance to find some precise prognostic markers improving the survival of EOC patients. Recently, lncRNAs became hot a candidate because of its observe dysregulated expression and critical biological function in progression of EOC[24]. Therefore, identification of EOC-associated lncRNAs may facilitate the development of lncRNA-directed

Table III. Univariate and multivariate analysis of prognostic parameters in EOC patients by Cox regression analysis.

	Univariate analysis			Multivariate analysis		
Variable	RR	95% CI	*p*-value	RR	95% CI	*p*-value
Age	1.322	0.569-1.873	0.321	-	-	-
Histological subtypes	1.633	0.745-2.438	0.219	-	-	-
Tumor size	1.437	0.834-1.782	0.531	-	-	-
FIGO stage	2.879	1.453-5.338	0.007	2.348	1.277-4.238	0.013
Grade	3.132	1.648-6.337	0.005	2.427	1.338-5.439	0.009
Distant metastasis	2.673	1.449-5.673	0.007	2.236	1.156-4.066	0.016
LINC01088 expression	3.556	1.648-6.783	0.001	2.893	1.337-5.347	0.005

diagnosis and predication of prognosis. Several reports have shown that dysfunction of lncRNAs was associated with EOC. For instance, lncRNA HOTAIR was reported to be highly expressed in EOC and associated with poor prognosis of EOC patients[25]. In addition, its knockdown suppressed EOC cell metastasis and EMT both *in vitro* and *in vivo*. LncRNA ANRIL was observed to be up-regulated in EOC and its knockdown inhibited EOC cell proliferation *in vitro* and *in vivo*[26]. LncRNA CCAT2, a well-studied lncRNA, was found to be overexpressed in EOC tissues and its knockdown inhibit proliferation and promote apoptosis and induce cell cycle arrest by modulating miR-424[27]. All those findings highlighted the potential of lncRNAs as potential prognostic biomarkers and therapeutic targets of EOC. More importantly, the potential mechanism by which lncRNAs regulated the tumor behavior was involved in the expression of miRNAs, which expanded our knowledge on the molecular mechanism of tumor progression[28,29]. Recently, Zhang et al[18] firstly screen a novel lncRNA (LINC01088) in EOC tissues which was significantly down-regulated by microarray analysis. Moreover, *in vitro* works showed that overexpression of LINC01088 significantly suppressed EOC cells proliferation by targeting miR-24-1-5p, indicating that LINC01088 served as a tumor suppressor in development of EOC. However, to our best knowledge, the clinical significance of LINC01088 in EOC patients has not been investigated. In this study, we used "GEPIA" to screen abnormally expressed lncRNA and found that LINC01088 expression was significantly down-regulated in EOC patients. Furthermore, we used our samples to confirm these results, finding LINC01088 expression was significantly up-regulated in EOC tissues compared with matched normal tissues. Our results were in line with previous study by Zhang et al[18]. Then, we analyzed the correlation between LINC01088 overexpression and clinicopathological features of EOC patients, and found that LINC01088 expression was associated with FIGO stage, grade and distant metastasis. Moreover, the overall survival time of patients with lower LINC01088 expression levels was shorter than that of patients with higher LINC01088 expression levels. More importantly, multivariate Cox regression analyses also indicated that LINC01088 could serve as an independent prognostic factor for EOC patients. Our findings suggested that the low expression of LINC01088 could be used as an independent predictor to indicate the poor prognosis of EOC patients.

Conclusions

We showed that LINC01088 expression was associated with advanced clinical progression, survival time, and prognosis of EOC patients. These results suggested that LINC01088 could prove to be useful prognostic biomarkers and therapeutic strategies for patients with EOC.

Conflict of Interest

The Authors declare that they have no conflict of interest.

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