

RESEARCH ARTICLE

Serum lncRNA LOXL1-AS1 is a diagnostic and prognostic marker for epithelial ovarian cancer

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Abstract

Background: The present study aimed to examine the levels of circulating LOXL1-AS1 in epithelial ovarian cancer (EOC) patients and to analyze its diagnostic and prognostic value.

Methods: The levels of LOXL1-AS1 in 185 EOC patients and 43 healthy volunteers were evaluated by a quantitative reverse transcriptase-polymerase chain reaction. The potential of LOXL1-AS1 as a biomarker for EOC diagnosis was determined by receiver-operating characteristic (ROC) curve assays. The associations between clinicopathological parameters and LOXL1-AS1 expression were analyzed using a chi-squared test. The influence of LOXL1-AS1 on overall survival was analyzed by the use of Kaplan–Meier. A Cox proportional hazards assays were conducted for the determination of the prognostic value of LOXL1-AS1.

Results: The expression of LOXL1-AS1 was dramatically higher in EOC patients compared to healthy controls ($p < 0.01$). LOXL1-AS1 yielded an area under the ROC curve of 0.843 with 65.3% sensitivity and 68.2% specificity in discriminating high-grade EOC from healthy controls. It was also shown that LOXL1-AS1 expression was associated with advanced FIGO stage ($p = 0.004$) and positively distant metastasis ($p = 0.013$). Kaplan–Meier assays revealed that patients with high LOXL1-AS1 expression had a shorter overall survival than those with low expression ($p = 0.0006$). By performing multivariate assays, LOXL1-AS1 was confirmed to be an independent prognostic factor for predicting the prognosis of EOC patients.

Conclusions: We provide evidence indicating that LOXL1-AS1 expression is correlated with a poor clinical outcome in EOC patients and may act as an independent prognostic indicator, as well as a new diagnostic biomarker.

KEYWORDS

circulating LOXL1-AS1, diagnosis, EOC, lncRNA, prognosis

1 | INTRODUCTION

Epithelial ovarian cancer (EOC) remains one of the most prevalent and destructive gynecological neoplasms with an increased incidence in

China.^{1,2} EOC is classified into four histologic grades in agreement with World Health Organization (WHO) criteria.³ Despite the clinical application of the effective treatment approaches for EOC, including surgery, radiotherapy and chemotherapy, the average survival of patients with

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this tumor has improved only slightly.^{4,5} Thus, it is always of great interest to identify novel and efficient cancer biomarkers for use in the diagnosis and prediction of prognosis for EOC patients.

It has been confirmed that the majority of human transcripts are non-coding RNAs (ncRNAs) initially considered to represent spurious transcriptional noise.⁶ Long non-coding RNAs (lncRNAs) comprise a class of ncRNAs that are greater than 200 nucleotides in length and lack the potential of coding proteins.⁷ Growing studies in recent years have shown that lncRNAs play a critical role in the modulation of a series of cellular processes in the shape of RNAs and display less evolutionary constraint.^{8,9} In addition, lncRNAs are also reported to be involved in gene regulation at different levels, especially epigenetic modification.¹⁰ Interestingly, in cancer research, lncRNAs are confirmed to act as tumor suppressors or oncogenes in the progression of tumors.^{11,12} Furthermore, the dysregulation of various lncRNAs has been documented in EOC, which has promoted growing interest in their possible potential as prognostic and diagnostic biomarkers.^{13,14} Meanwhile, further reports assessing the involvement of lncRNA in EOC are scarce.

lncRNA LOXL1-antisenseRNA (LOXL1-AS1) is located on chromosome 15q24.1 and is approximately 480 nucleotides in size.¹⁵ Previously, LOXL1-AS1 was reported to be a tumor promoter of glioblastoma and was also shown to be expressed abnormally in prostate cancer.^{16,17} However, the clinical impact of LOXL1-AS1 in EOC has remained largely unclear. In the present study, we aimed to investigate the expression profiles of circulating LOXL1-AS1 and its diagnostic and prognostic value in EOC patients.

2 | MATERIALS AND METHODS

2.1 | Patients and specimens

In total, 43 healthy volunteers and 185 EOC patients were enrolled from Linyi Cancer Hospital between December 2011 and May 2014. The ages of all the patients ranged from 29 to 68 years, with an average age of 51 years. No radiotherapy or chemotherapy was received before surgery for all EOC patients. The follow-up information on these cases was also integrated. The clinical parameters of EOC patients in this study are presented in Table 1. Ethical approval was obtained from the Ethics Committee of Linyi Cancer Hospital, and every subject provided their written informed consent before surgery.

2.2 | Collection of samples

Blood samples from all subjects were collected in gel separator tubes (TransGen Biotech, Haidian, Beijing, China). After the collection of blood samples, centrifuging was used to separate the plasma and surplus elements at 1200 g for 30 minutes. Then, separate components were stored in 1.5-mL RNase free tubes (TransGen Biotech) at -80°C for further reverse transcriptase-polymerase chain reaction (RT-PCR) assays.

TABLE 1 Relationship between circulating LOXL1-AS1 expression and clinicopathological variables in EOC patients

Variable	Number	Circulating LOXL1-AS1 expression		p value
		High	Low	
Age (years)				0.190
< 50	116	62	54	
≥ 50	69	30	39	
Tumor size (cm)				0.139
≤ 8	119	64	55	
> 8	66	28	38	
Ascites				0.266
<100	73	40	33	
≥ 100	112	52	60	
FIGO stage				0.004
I + II	118	68	50	
III + IV	67	24	43	
Distant metastasis				0.013
Yes	127	71	56	
No	58	21	37	

2.3 | Quantitative qRT-PCR

Total RNAs were extracted from 600 μL of plasma from all samples using Trizol Reagent (Ambion, Pudong, Shanghai, China). The concentration and purity of total RNA were examined using a NanoDrop ND-1000 spectrometer (NanoDrop Technologies, Haidian, Beijing, China). First-strand cDNA was synthesized using Reverse EasyScript One Step gDNA Removal and cDNA Synthesis SuperMix (Xinghan Tech, Pudong, Shanghai, China). Next, RT-PCR was carried out by manipulating a SYBR Premix Ex Taq II (TaKaRa, Otsu, Shiga, Japan) on a CFX96 real-time PCR Systems (TaKaRa, Otsu, Shiga, Japan). The amplification of PCR was started with 10 minutes of denaturation at 95°C , followed by 50 amplification cycles (12 seconds at 95°C , 20 seconds at 60°C and 10 seconds at 72°C). These data were further analyzed employing the comparative Ct methods with GAPDH as the endogenous control for the normalization of experimental data. The PCR primers for LOXL1-AS1 or GAPDH were designed as: LOXL1-AS1 forward, 5'-TTCCATTTACCTGCCGAAG-3' and reverse, 5'-GTCAGCAAACACATGGCAAC-3'. GAPDH forward, 5'-CAATGACCCTTCATTGACC-3' and reverse, 5'-GACAAGCTTCCCGTTCTCAG-3'.

2.4 | Statistical analysis

All statistical analyses were performed using SPSS, version 18.0 (SPSS Inc., Chicago, IL, USA) or Prism, version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical differences were examined using Student's *t* test. The diagnostic value of circulating LOXL1-AS1 for EOC was examined by calculating the area under the receiver-operating characteristic (ROC) curve (AUC). A chi-squared test was performed to determine

correlations between LOXL1-AS1 overexpression and clinicopathologic variables of the EOC samples. Survival curves were constructed with the Kaplan–Meier methods and compared by log-rank tests. The prognostic impact of survival variables was evaluated using a multivariate analysis. $p < 0.05$ was considered statistically significant.

3 | RESULTS

3.1 | Circulating LOXL1-AS1 expression is up-regulated in EOC tissues

To study the specific function of circulating LOXL1-AS1 in EOC patients, the expression pattern of circulating LOXL1-AS1 in EOC patients and healthy volunteers was detected with qRT-PCR. As shown in Figure 1A, it was found that the expression of circulating LOXL1-AS1 was distinctly higher in EOC patients compared to healthy controls ($p < 0.01$). In addition, we also found that patients with advanced stages displayed a higher expression of LOXL1-AS1 (Figure 1B). Overall, our findings revealed that dysregulation of LOXL1-AS1 may participate in the pathogenesis of hepatocellular carcinoma.

3.2 | The diagnostic value of circulating LOXL1-AS1 in EOC patients

We next explored the diagnostic value of circulating LOXL1-AS1 in EOC patients using ROC analysis. As shown in Figure 2, the results showed that circulating LOXL1-AS1 effectively differentiated primary EOC patients from normal controls with an AUC of 0.843 (95% confidence interval [CI] = 0.756–0.931; $p < 0.001$). At the optimal cut-off value of 4.52 for circulating LOXL1-AS1, the sensitivity was 63.7% and the specificity was 85.3%, with an AUC of 0.745 with respect to distinguishing EOC patients from healthy individuals. However, when we explored the diagnostic value of circulating LOXL1-AS1 with respect to distinguishing low-grade patients from healthy individuals, the diagnostic effects were limited. Thus, our findings suggested that the detection of circulating LOXL1-AS1 may help with the diagnosis of EOC patients.

FIGURE 1 Up-regulation of circulating LOXL1-AS1 in EOC tissues detected by qRT-PCR. (A) The expression of circulating LOXL1-AS1 in patients and healthy individuals. (B) Circulating LOXL1-AS1 levels among patients with different FIGO stages

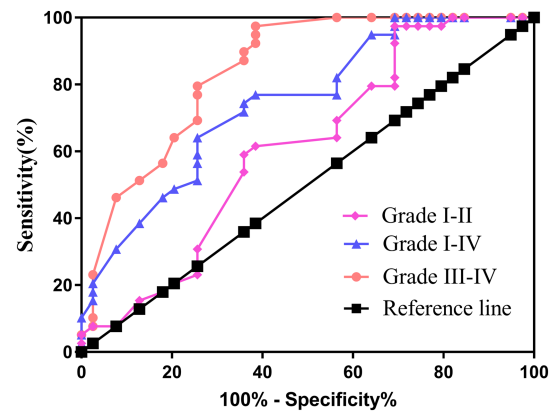
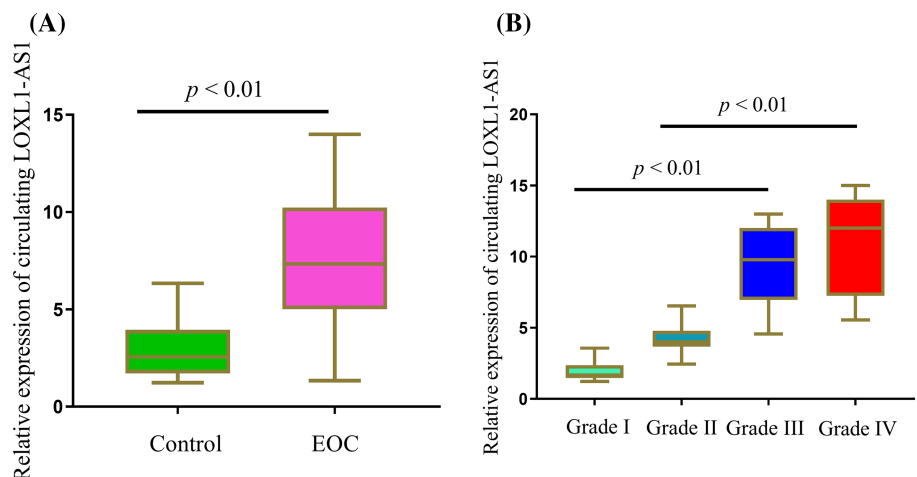


FIGURE 2 ROC curve analysis of circulating LOXL1-AS1 in the diagnosis of all stages, low-grade and high-grade patients

3.3 | Relationship between clinicopathological factors and circulating LOXL1-AS1 expression in EOC patients

To further analyze the clinical significance of circulating LOXL1-AS1 in clinical progression of EOC, all subjects were classified into a high expression group ($n = 93$) and a low expression group ($n = 92$). The relationships between circulating LOXL1-AS1 expression levels and clinical parameters are presented in Table 1. We found that a high circulating LOXL1-AS1 level was remarkably correlated with advanced FIGO stage ($p = 0.004$) and positively distant metastasis ($p = 0.013$) (Table 1). However, no significant correlations between circulating LOXL1-AS1 expression and other parameters were observed, such as gender, age and tumor size (all $p > 0.05$).

3.4 | Circulating LOXL1-AS1 expression is an independent predictor for overall survival

Moreover, Kaplan–Meier assays were conducted to detect the prognostic value of LOXL1-AS1 in EOC patients. As shown in Figure 3, it was observed that the 5-year overall survival of high-circulating

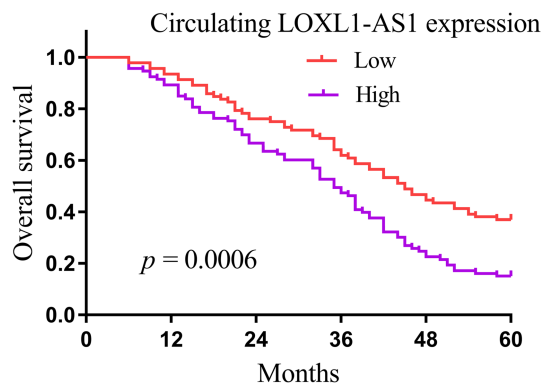


FIGURE 3 Kaplan–Meier overall survival analyses were carried out for the exploration of the relationships between circulating LOXL1-AS1 expression and EOC patient survival

LOXL1-AS1 group (85.9%) was distinctly lower than that of low-circulating LOXL1-AS1 group (62.4%; $p = 0.006$). Furthermore, in the univariate Cox model, we showed that FIGO stage, distant metastasis and circulating LOXL1-AS1 expression were correlated with the survival rate of EOC patients (All $p > 0.05$) (Table 2). More importantly, using multivariate Cox regression analyses, our group demonstrated that circulating LOXL1-AS1 expression could be regarded as an independent predictor with respect to predicting the prognosis of EOC patients (relative risk = 3.053, 95% CI = 1.154–4.784, $p = 0.007$), in addition to FIGO stage and WHO grade (Table 2).

4 | DISCUSSION

EOC is the most common gynecological tumors in women worldwide. Despite the safe surgical resection followed by adjuvant chemotherapy and radiotherapy, the long-term overall survival remains short. The lack of effective methods for the detection of EOC early is one of the major factors contributing to the high mortality in patients with EOC.^{18,19} Thus, the identification of useful biomarkers for the screening of patients with EOC might result in a significant improvements in their survival rates. Previously, lncRNAs were reported to be dysregulated in EOC and to be involved in the metastasis of tumor cells, suggesting that lncRNAs may be used as potential biomarkers. In addition, several studies have focused on identifying circulating lncRNAs

as cancer biomarkers in serum or plasma.²⁰ Importantly, several important lncRNAs such as lncRNA MALAT1 and lncRNA HIF1A-AS1 were identified as candidates in the exploration of novel biomarkers.^{21,22} However, a large number of lncRNAs remain to be studied.

Previous studies on the non-coding genomes have developed objection against the original central dogma of molecular biologies. Non-coding RNAs were demonstrated to be involved in the modulation of the expression of several genes, which suggested that they may influence the progression of tumors when they regulated tumor-related genes.^{23,24} LOXL1-AS1 was a newly discovered lncRNA first reported by Hauser *et al.*¹⁵ Subsequently, several studies reported its effects on prostate cancer and medulloblastoma. For example, Long *et al.*¹⁷ showed that LOXL1-AS1 expression was distinctly up-regulated in prostate cancer and its knockdown suppressed tumor cell proliferation via regulating miR-541-3p and CCND1. Gao *et al.*²⁵ showed that LOXL1-AS1 was highly expressed and associated with advanced clinical stages in medulloblastoma patients, and its suppression inhibited tumor cells proliferation and metastasis by modulating the PI3K/AKT pathway. In recent years, circulating lncRNAs being used as novel biomarkers became a research hotspot, and several circulating lncRNAs have been confirmed to be associated with the clinical prognosis of tumor patients. Although the biological function of LOXL1-AS1 has been investigated in previous studies, its clinical significance has remained largely unclear.

In the present study, we first showed that circulating LOXL1-AS1 expression was distinctly up-regulated in EOC patients compared to healthy volunteers, and its higher expression was strongly correlated with advanced clinical stages, which suggested that the detection of circulating LOXL1-AS1 may have a diagnostic value. The results of ROC assays confirmed our hypothesis that detecting the levels of circulating LOXL1-AS1 can be used to distinguish low-grade patients from healthy individuals. Then, we divided all patients into two groups to explore its clinical significance, finding that a high level of circulating LOXL1-AS1 was correlated with advanced FIGO stage and positively distant metastasis, suggesting that the overexpression of LOXL1-AS1 acted as a positive factor contributing to the clinical progression of this tumor. Moreover, the association between circulating LOXL1-AS1 and overall survival was analyzed using Kaplan–Meier assays, and the results showed that patients with higher levels of circulating LOXL1-AS1 may have a poorer overall survival. Based on

TABLE 2 Univariate and multivariate survival analysis of influencing factors

Variable	Univariate analysis			Multivariate analysis		
	HR	95% CI	<i>p</i>	HR	95% CI	<i>p</i>
Age	1.572	0.624–2.441	0.221	–	–	–
Tumor size	1.423	0.852–2.668	0.432	–	–	–
Ascites	1.744	0.761–2.334	0.156	–	–	–
FIGO stage	3.342	1.451–4.776	0.011	2.894	1.216–4.327	0.016
Distant metastasis	3.542	1.538–5.446	0.003	3.125	1.233–4.852	0.008
Circulating LOXL1-AS1 expression	3.342	1.368–5.138	0.002	3.053	1.154–4.784	0.007

multivariate Cox analysis, high expression of circulating LOXL1-AS1 was confirmed to be an independent prognostic indicator for the survival of EOC patients, and the relative risk (95% CI) was 3.053 (1.154–4.784). Taken together, circulating LOXL1-AS1 could be used as a potential marker to screen high-risk patients with EOC who may achieve a poor clinical prognosis and thus require aggressive clinical treatment.

We determined that circulating LOXL1-AS1 expression is significantly up-regulated in EOC and is correlated with several clinical pathologies. Higher serum circulating LOXL1-AS1 levels could distinguish patients with EOC from healthy controls. Accordingly, this lncRNA can function as an ideal indicator with respect to the diagnosis and clinical outcome of EOC.

AUTHOR CONTRIBUTIONS

CNL designed and performed all of the experiments and analyzed the data. HYZ wrote the manuscript. All authors read and approved the final version of the manuscript submitted for publication.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

DATA AVAILABILITY STATEMENT

All data and materials regarding the present study are available from the corresponding author.

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