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Overexpression of lncRNA MNX1-AS1 is associated with poor clinical outcome in epithelial ovarian cancer

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Abstract. – OBJECTIVE: Long non-coding RNAs MNX1-AS1(MNX1-AS1) has been proved to be associated with ovarian cancer proliferation and invasion. However, the clinical significance of MNX1-AS1 in epithelial ovarian cancer (EOC) patients remains unknown. The aim of this study was to investigate the prognostic value of the MNX1-AS1 expression in EOC.

PATIENTS AND METHODS: We first measured MNX1-AS1 expression level in 177 paired of EOC and matched normal tissues by Real-time quantitative RT-PCR. The relevance of MNX1-AS1 expression to the clinicopathological factors was assessed. Overall survival (OS) and relapse-free survival (RFS) were analyzed by log-rank test, and survival curves were plotted according to Kaplan-Meier. Univariate and multivariate analyses were performed to analyze the prognostic significance of MNX1-AS1 expression.

RESULTS: We found that the levels of MNX1-AS1 were higher in EOC tissue than in matched normal tissues ($p<0.01$). In addition, MNX1-AS1 expression level was significantly positively correlated with FIGO stage ($p=0.005$), grade ($p=0.040$) and distant metastasis ($p=0.000$). Kaplan-Meier survival curves demonstrated that patients with high-MNX1-AS1 expression showed poorer progression-free survival and overall survival than those with low-MNX1-AS1 expression ($p<0.0001$ and 0.0003, respectively). Then, Cox regression analysis revealed that FIGO stage, distant metastasis, and MNX1-AS1 expression were independent prognostic factors of both overall survival and progression-free survival for patients with EOC.

CONCLUSIONS: Our findings indicated, for the first time, that MNX1-AS1 expression may be a useful marker for predicting the outcome in patients with EOC.

Key Words
Long non-coding RNA, MNX1-AS1, Prognosis. Ovarian cancer.

Introduction

Ovarian cancer is the fifth most common gynecologic malignancy and it has a high mortality rate among women in the world[1,2]. Epithelial ovarian cancer (EOC) is a common entity accounting for 80-90% of ovarian cancer cases[3]. If diagnosed and treated while localized (stages I and II), the 5-year survival rates can reach over 90%[4]. As most of the patients in the early-stage setting are free of any symptoms, most EOC patients are diagnosed with advanced disease (stages III and IV)[5]. Despite recent advances in cancer treatments, the 5-year survival of EOC patients remains poor[6]. In clinical practice, identification of better underlying molecular makers for EOC could guide clinicians in designing personalized treatment strategies.

Non-coding RNAs consist of snRNA, snoRNA, tRNA, rRNA[7]. Long non-coding RNAs (lncRNAs) comprise the mainstream of transcripts that are larger than 200 nucleotides (nt) and not translated into proteins[8]. Although the function and mechanism of most lncRNAs remain unknown, recent evidence shows that lncRNAs are involved in a wide range of biological processes, such as cell growth, tumorigenesis, apoptosis, metastasis, and angiogenesis[9-11]. More importantly, during the last decade, scientists have demonstrated that abnormal expression of lncRNAs may serve as a tumor suppressor or oncogenes according to their targeting genes[12]. Recently, emerging studies reported that functional lncRNAs may be used for diagnosing cancer and determining prognosis. Some lncRNAs have been well studied, such as lncRNA MALAT1[13], lncRNA ANRIL[14], and BCAR4[15]. However, the role and function of most lncRNAs in EOC remains unknown.

MNX1-AS1 (MNX1 antisense RNA1) is an lncRNA located on chromosome 7[16]. To our best knowledge, there is only one work that reported the abnormal expression of MNX1-AS1 in EOC[17]. However, the clinical significance of MNX1-AS1 has not been investigated. We aimed to study the prognostic value of MNX1-AS1 in patients with EOC.

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Patients and Methods

Patients and Specimens

Epithelial ovarian cancer tissues were obtained from 177 patients with EOC who underwent surgical resection at the Linyi People's Hospital from 2004 to 2009. Patients' ages ranged from 24 to 71 years, and the average age was (52.32±11.33) years. All samples were immediately snap-frozen in liquid nitrogen and stored at a bio-freezer at -80°C until they were processed. The histopathologic diagnoses were determined by the hospital's pathologist. None of the patients recruited in this study had undergone preoperative chemotherapy or radiotherapy. The follow-up period ranged from 3 to 60 months with an average of 40 months and a median of 27 months. The clinicopathologic features of all the patients were summarized in Table I. The research was approved by the Ethics Committee of the Affiliated Hospital of Linyi People's Hospital and the inform consents were signed by all the participants.

Quantitative Real-time PCR (qRT-PCR)

Total RNA was extracted using TRIzol Reagent (TaKaRa, Dalian, China). cDNA was synthesized using SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) subsequently. The qRT-PCR was performed on ABI 7500 system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control to normalize the data. Primer sequences were designed as follows: MNX1-AS1, 5'-CCCGCATTTTCAGATTCAC-3' (sense) and 5'- GCTCTCAGCCTCGCCATA-3' (antisense); GAPDH, 5'-GTCAACGGATTTGGTCTGTATT-3' (sense) and 5'-AGTCTTCTGGGTGGCAGTGAT-3' (antisense). the relative expression of MNX1-AS1 was determined by the $2^{-\Delta Ct}$ method.

Statistical Analysis

Statistical analysis was done using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Two-tailed Student's *t*-tests were used to evaluate differences between groups. The correlation of MNX1-AS1 expression with the clinicopathologic data was analyzed using a x^2-test. Kaplan-Meier method was used to calculate the survival rate and the log-rank test was performed to compare survival differences. The univariate and multivariate analysis were carried out using Cox's proportional hazards regression models. A two-tailed *p*-value less than 0.05 was considered to have statistical significance.

Table I. MNX1-AS1 expression and clinicopathologic features in EOC patients.

Clinicopathologic variables	Number of cases	MNX1-AS1 levels		*p*-value
		Low expression	High expression	
Age (years)				NS
<50	88	41	47	
≥50	89	49	40	
Ascites				NS
<100	76	36	40	
≥100	101	54	47	
CA125 level (U/ml)				NS
<600	79	43	36	
≥600	98	47	51	
Tumor size (cm)				NS
≤ 10	124	67	57	
> 10	53	23	30	
FIGO stage				0.005
I + II	112	66	46	
III + IV	65	24	41	
Grade				0.040
G1	115	65	50	
G2 + G3	62	25	37	
Distant metastasis				0.000
Yes	113	69	44	
No	64	21	43	

Results

MNX1-AS1 upregulation in EOC specimens

To investigate whether any difference of MNX1-AS1 expression existed in EOC and matched normal tissues, qRT-PCR was performed. As shown in Figure 1, we found that expression of MNX1-AS1 was increased in EOC samples ($p < 0.01$), compared to normal ovarian tissues. The result hinted that MNX1-AS1 might be a potential tumor promoter in human EOC.

MNX1-AS1 expression in EOC and its relationship with clinicopathological factors

To screen suspicious factors correlated to MNX1-AS1 expression, we further analyzed the correlation between MNX1-AS1 expression and the clinicopathological characteristics of EOC patients. EOC tissues expressing MNX1-AS1 at levels less than the median expression level (3.74) were ascribed to the low expression group (n = 90), and other tissues with expression above the median value were ascribed to the high expression group (n = 87). As indicated in Table I, MNX1-AS1 expression level was significantly positively correlated with FIGO stage ($p = 0.005$), grade ($p = 0.040$) and distant metastasis ($p = 0.000$). However, there were no significant associations between MNX1-AS1 expression and other clinical features including age, ascites, CA125 level and tumor size (all $p > 0.05$).

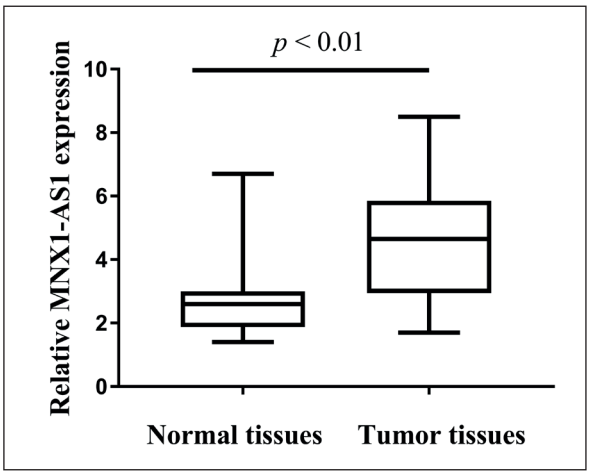

Figure 1. Relative MNX1-AS1 expression levels in EOC tissues and matched normal tissues. MNX1-AS1 expression was and normalized to GAPDH. The expression levels of MNX1-AS1 in EOC samples were much higher than those in normal ovary samples ($p<0.01$).

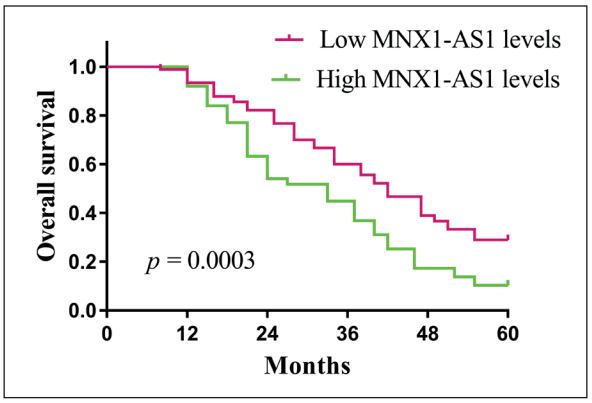

Figure 2. Survival curves in patients with EOC according to MNX1-AS1 expression levels. Patients with high MNX1-AS1 expression had significantly shorter OS compared to low MNX1-AS1 patients (p=0.0003).

High MNX1-AS1 levels are correlated with poor prognosis in EOC patients

To explore whether MNX1-AS1 expression was associated with OS and PFS, we utilized Kaplan Meier survival analysis and log-rank test. Survival information of 177 patients with EOC was obtained through hospital follow-up. As shown in Figure 2 and 3, we found that the group with high level of MNX1-AS1 expression was significantly associated with poor OS ($p = 0.0003$) and PFS ($p < 0.0001$). Subsequently, in univariate analysis of OS, FIGO stage (HR 3.672, 95% CI: 1.671-6.228, $p = 0.001$), distant metastasis (HR 4.138, 95% CI: 1.893-8.224, $p = 0.001$) and MNX1-AS1 expression (HR 3.213, 95% CI: 1.572-5.932, $p = 0.001$) were prognostic indicators

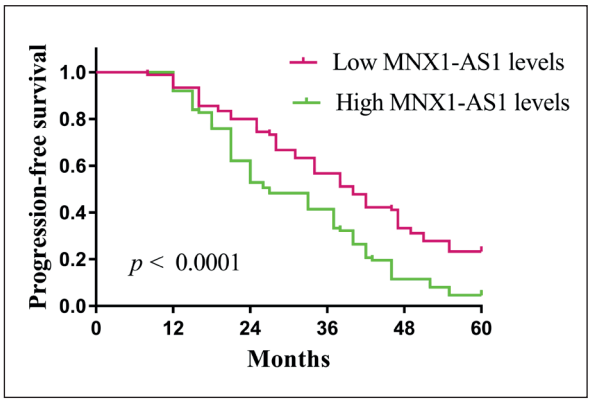

Figure 3. Survival curves in patients with EOC according to MNX1-AS1 expression levels. Patients with high MNX1-AS1 expression had significantly shorter PFS compared to low MNX1-AS1 p atients ($p<0.0001$).

Table II. Univariate and multivariate analyses for OS in EOC patients.

Variable	Univariate			Multivariate		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	0.832	0.527-1.562	0.378	–	–	–
Ascites	1.231	0.711-1.923	0.219	–	–	–
CA125 level	0.918	0.488-1.672	0.201	–	–	–
Tumor size	1.562	0.833-2.213	0.156	–	–	–
FIGO stage	3.672	1.671-6.228	0.001	2.581	1.218-5.129	0.003
Grade	1.983	0.892-2.933	0.083	–	–	–
Distant metastasis	4.138	1.893-8.224	0.001	3.673	1.562-6.893	0.001
MNX1-AS1 expression	3.213	1.572-5.932	0.001	2.672	1.193-4.228	0.002

(Table II). In univariate analysis of PFS, FIGO stage (HR 3.321, 95% CI: 1.482-5.321, $p = 0.001$), distant metastasis (HR 3.729, 95% CI: 1.673-6.323, $p = 0.001$) and MNX1-AS1 expression (HR 2.983, 95% CI: 1.873-5.632, $p = 0.001$) were prognostic indicators (Table III). Furthermore, multivariate analysis confirmed that FIGO stage, distant metastasis, and MNX1-AS1 have the potential to independently predicate OS ($p = 0.002$) and PFS ($p = 0.004$) in EOC (Table II and III).

Discussion

The research for effective molecular markers for diagnosis and prognosis of EOC is very important for prognosis of patients. In the last decade, miRNAs and related genes have been well studied[18,19]. However, most lncRNAs have not been identified. Dysregulation of lncRNAs was reported to be associated with the progression of human malignancies[20]. Recently, lncRNAs were considered to be novel biomarkers for types of human cancer, including EOC. For instance, Bi et al[21] found that lncRNA PCAT-1 over-expression promoted proliferation and metastasis in gastric cancer cells through regulating CDKN1A and was associated with poor prognosis of patients with gastric cancer. Zhou et al[22] reported that forced expression of lncRNA SPRY4-IT1 promoted hepatocellular carcinoma cell proliferation and invasion by activating EZH2, suggesting that lncRNA SPRY4-IT1 might be considered as a therapeutic target in hepatocellular carcinoma. Chen et al[23] reported that lncRNA NEAT1 was significantly up-regulated in ovarian cancer and associated with FIGO stage and distant metastasis; in addition, the NEAT1 expression level was an independent factor in predicting the overall survival of ovarian cancer patients. Those results highlighted the potential of lncRNAs as biomarkers for predicting the prognosis of tumor patients.

Unlike some well-studied lncRNAs, so far, only one paper reported the role of MNX1-AS1 in tumors. Lv et al[17] reported that MNX1-AS1 was significantly highly expressed in EOC and that the

Table III. Univariate and multivariate analyses for PFS in EOC patients.

Variable	Univariate			Multivariate		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	0.721	0.672-1.842	0.322	–	–	–
Ascites	1.523	0.821-2.231	0.271	–	–	–
CA125 level	0.821	0.517-1.772	0.251	–	–	–
Tumor size	1.821	0.727-2.342	0.183	–	–	–
FIGO stage	3.321	1.482-5.321	0.001	2.931	1.132-4.273	0.006
Grade	1.723	0.791-2.673	0.091	–	–	–
Distant metastasis	3.729	1.673-6.323	0.001	3.218	1.382-5.931	0.001
MNX1-AS1 expression	2.983	1.873-5.632	0.001	2.362	1.362-4.213	0.004

expression level of MNX1-AS1 was correlated with EOC stage. Furthermore, they showed that down-regulation of MNX1-AS1 suppressed cell proliferation, colony formation, and cell migration ability. Those results indicated that MNX1-AS1 served as a tumor promoter in EOC. However, the prognostic value of MNX1-AS1 in EOC remains unknown.

In the present study, it was identified that MNX1-AS1 expression in EOC was much higher than the normal ovarian tissues. Also, we found that high MNX1-AS1 expression was significantly associated with FIGO stage, grade, and distant metastasis. Moreover, our clinical results revealed that MNX1-AS1 level was inversely correlated with EOC patient survival time. To assess whether the expression of MNX1-AS1 was a tumor prognostic biomarker, univariate and multivariate analysis was performed using Cox proportional hazards model. The results indicated that MNX1-AS1 expression were independent prognostic factors of both OS and PFS for patients with EOC. However, the present study was not related to the potential mechanism by which MNX1-AS1 impacted the outcome of EOC patients. Further studies should focus on exploring the contribution of MNX1-AS1 in EOC through molecular mechanisms.

Conclusions

To our best knowledge, this work firstly demonstrated that increased MNX1-AS1 expression predicts poor OS and PFS of patients with EOC. Therefore, MNX1-AS1 may be an independent prognostic marker for EOC patients.

Conflict of Interest

The authors declare no conflicts of interest.

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