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Overexpression of LncRNA FER1L4 in endometrial carcinoma is associated with favorable survival outcome

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Abstract. – OBJECTIVE: Long noncoding RNA Fer-1-like protein 4 (FER1L4) is a novel cancer-related lncRNA and functions as a tumor suppressor in several cancers. However, the clinical significance of FER1L4 has not been investigated. The purpose of this study was to investigate whether the increased expression of FER1L4 can be used as a prognostic biomarker in endometrial carcinoma (EC).

PATIENTS AND METHODS: Expression levels of FER1L4 in 191 pairs of EC and adjacent normal tissues were detected by TaqMan Real-time quantitative RT-PCR assay. The association between FER1L4 expression and clinicopathological parameters was subsequently determined. The clinical and prognostic significance of FER1L4 expression was analyzed statistically by Kaplan-Meier estimate and Cox regression model.

RESULTS: We observed that FER1L4 in EC tissues was significantly down-regulated compared with adjacent non-tumor tissues ($p<0.01$). Low expression of FER1L4 was significantly correlated with distant metastasis ($p=0.002$), lymph node metastasis ($p=0.010$) and FIGO stages ($p=0.006$). Furthermore, Kaplan-Meier survival curves showed that overall survival rate in patients with high FER1L4 expression level was markedly higher than those with low FER1L4 expression level ($p=0.0071$). Finally, multivariate analysis of the prognosis factors confirmed that low FER1L4 expression was a significant independent predictor of poor survival in EC (HR=2.782, 95% CI: 1.144-5.123, $p=0.004$).

CONCLUSIONS: We provided the significant clinical relevance of FER1L4 in EC and suggested that FER1L4 may act as an independent prognostic indicator for EC patients.

Key Words
LncRNA FER1L4, Endometrial carcinoma, Prognosis.

Introduction

Endometrial carcinoma (EC) is the most frequently diagnosed uterine cancer, and the fourth most common cancer among women[1,2]. Although the incidence of EC is lower in East Asian than in Western countries, it tends to increase markedly in recent years[3]. The cause of endometriosis is multifactorial, including immunity, environment and treatment with tamoxifen during breast cancer therapy[4]. In spite of advances in diagnostics and new therapeutic strategies that have been applied in the treatment of EC, survival rates of patients with advanced-stage and recurrent EC remain poor[5,6]. Therefore, it is of great significance to search for novel markers for EC, which can accurately identify the biological characteristics of tumors and predict clinical outcome. Long noncoding RNAs (lncRNAs) are another class of ncRNAs containing > 200 nucleotides and lacking protein-coding ability[7]. Growing studies[8,9] show that lncRNAs play essential roles in many cellular and developmental processes, including cell proliferation, apoptosis, and differentiation. In addition, more and more lncRNAs have been found being dysregulated in a range of caners and contributing to tumorigenesis and tumor progression[10]. For instance, Jiang et al[11] reported that SNHG1 expression was significantly up-regulated in osteosarcoma and its overexpression was found to promote cell proliferation and cell migration through sequestration of miR-577 and activation of WNT2B/Wnt/β-catenin pathway. Bi et al[12] found that lncRNA PCAT-1, a carcinogenic lncRNA, was highly expressed in gastric cancer patients, and promoted proliferation and metastasis in gastric cancer cells through regulating CDKN1A. In addition, Wang et al[13] showed that lncRNA BANCR expression was significantly overexpressed in patients with EC and significantly correlated with FIGO stage, pathological grade, myometrial invasion, and lymph node metastasis. On the other hand, *in vitro* assay showed that inhibition of lncRNA BANCR suppressed EC cells proliferation and invasion through modulating MMP2

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and MMP1 via ERK/MAPK signaling pathway. These findings highlighted the important role of lncRNAs as potential therapeutic targets and cancer-related biomarkers for prognosis of cancer patients. Fer-1-like protein 4 (FER1L4), a newly identified tumor-associated lncRNA, has been reported to be dysregulated in several tumors. Recently, several studies have suggested that FER1L4 exhibited tumor anti-oncogenic activity in glioblastoma, and may also serve as a positive predictor for prognosis in patients with colon cancer and osteosarcoma[14-16]. More importantly, Qiao et al[17] found that FER1L4 served as a tumor suppressor because its overexpression suppressed cancer cell proliferation and cycle in EC. However, to our best knowledge, the potential role of FER1L4 as a prognostic biomarker in EC patients has not been investigated. Thus, the aim of the current study was to explore the clinical significance of FER1L4 for EC.

Patients and Methods

Patients and Tissue Samples

Tumor samples and the adjacent normal tissues were obtained from 191 EC patients who underwent hysterectomy at Dalian Third People's Hospital. Only patients diagnosed with a pure adenocarcinoma without other histological elements were included. Before surgery, no patient had undergone hormone therapy, radiotherapy or chemotherapy. EC tissues were staged according to the 2009 International Federation of Gynecology and Obstetrics (FIGO) guidelines[18]. The tissue samples were immediately snap-frozen in liquid nitrogen and stored at -80°C until RNA extraction. The clinicopathologic features of all the patients were summarized in Table I. This study was approved by the Institutional Review Board and informed consent was obtained from each patient prior to biopsy or surgery.

Table I. Correlation between FER1L4 expression and different clinicopathological features in patients with endometrial carcinoma.

Clinicopathological features	No. of cases	FER1L4 expression		*p*-value
		Low	High	
Age				NS
< 55	111	50	61	
≥ 55	80	44	36	
Pathology				NS
Adenocarcinoma	150	73	77	
Non-adenocarcinoma	41	21	20	
ER				NS
Negative	111	60	51	
Positive	80	34	46	
PR				NS
Negative	97	43	54	
Positive	94	51	43	
Vessel invasive				NS
Negative	123	54	69	
Positive	68	40	28	
Pathology classification				NS
Well + Moderate	116	50	66	
Poor	75	44	31	
Distant metastasis				0.002
Negative	117	47	70	
Positive	74	47	27	
Lymph node metastasis				0.010
Negative	125	53	72	
Positive	66	41	25	
FIGO stages				0.006
I-II	128	54	74	
III/IV	63	40	23	

Table II. Sequence of the primers used in this study.

Gene	Sequences
FER1L4	F: 5'-ACACAGTCCTTGTGGGTTCC-3' R: 5'-CCTGTCTCCTCCATCTCTCC-3'
GAPDH	F: 5'-GTCAACGGATTTGGTCTGTATT-3' R: 5'-AGTCTTCTGGGTGGCAGTGAT-3'

Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from tissues specimens using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instruction. The concentration and purity of RNA were determined using NanoDrop 1000 spectrophotometer (Thermo-Fisher Scientific, Waltham, MA, USA). cDNA synthesis was performed with 2 mg of total RNA, using the miScript II RT Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. qRT-PCR was performed with Power SYBR Green (TaKaRa, Otsu, Shiga, Japan). Results were normalized to the expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The relative expression of FER1L4 was calculated and normalized using the $2^{-\Delta\Delta Ct}$ method. All primers were purchased from Genewiz Biotechnologies (Haidian, Beijing, China) and shown in Table II.

Statistical Analysis

Statistical analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). The quantitative data were compared between groups using the Student's *t*-test. The x^2-test was used to analyze correlations between FER1L4 expression and clinicopathologic characteristics. Survival analysis was performed using Kaplan-Meier curves, and the differences were revealed by log-rank test. Univariate and multivariate Cox regression models were used to evaluate prognostic significance. The difference was considered statistically significant at $p<0.05$.

Results

FER1L4 was Down-Regulated in EC Tissues

To determine whether FER1L4 was involved in the tumorigenesis of EC, the expression level of FER1L4 was detected in EC tissues and matched normal tissues obtained from 191 patients by qRT-PCR. As shown in Figure 1, the results showed that FER1L4 expression was markedly downregulated in EC tissues than in corresponding adjacent normal tissues ($p<0.01$). These results indicate that FER1L4 is lowly expressed in EC.

Correlation Between FER1L4 Expression and Clinicopathological Characteristics of EC

In order to assess the clinical role of FER1L4 in EC, the correlation between its expression level and clinicopathological parameters were analyzed. We firstly divided all EC patients into two groups according to the median value of FER1L4 expression level, including high-expression group (n=97) and low-expression group (n=94), respectively. The results were shown in Table I. We found that low expression of FER1L4 was significantly correlated with distant metastasis ($p=0.002$), lymph node metastasis ($p=0.010$) and FIGO stages ($p=0.006$). However, no significant associations were observed between FER1L4 level and patients' age, pathology, ER, PR, vessel invasive or pathology classification ($p>0.05$).

Prognostic Value of FER1L4 for EC Patients

The association between FER1L4 expression and survival of EC patients was investigated by Kaplan-Meier analysis and log-rank test. As shown in Figure 2, we found that the 5-year overall survival of low FER1L4 expression group was significantly shorter than that of high FER1L4 expression group ($p=0.0071$). Our results suggested that down-regulation of FER1L4 might be associated with poor survival of EC patients. Then, Cox regression analyses were applied to further

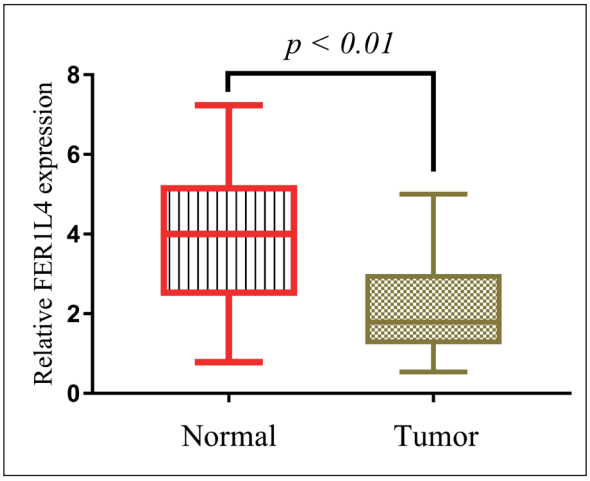

Figure 1. FER1L4 expression in 191 pairs of clinical EC and adjacent normal tissues were detected by qRT-PCR. The expression level of FER1L4 in EC tissues was significantly lower than that in non-tumor tissues ($p<0.01$).

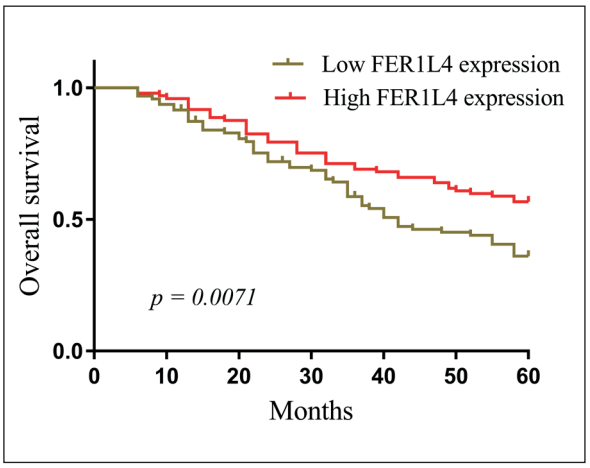

Figure 2. Kaplan-Meier curves for overall survival of 191 EC patients, divided according to FER1L4 expression levels. High FER1L4 expression was significantly associated with poor survival (p=0.0071, log-rank test).

evaluate the prognostic value of FER1L4 in EC. As shown in Table III, univariate survival analyses showed that distant metastasis, lymph node metastasis, FIGO stages and FER1L4 expression were significantly associated with overall survival of EC patients (all p<0.05). Notably, the results of multivariate logistic regression analysis showed that FER1L4 expression (HR=2.782, 95% CI: 1.144-5.123, p=0.004) was an independent poor prognostic factor for EC.

Discussion

EC is a common malignancy of the female genital tract and its overall 5-year survival is approximately 80% for all stages[19]. However, the prognosis for patients diagnosed at an advanced stage remains very poor. Up to date, chemotherapy is the mainstay of treatment and radiation or hormone therapy could be used as an adjuvant treatment for patients with EC[20]. However, these treatment methods are not effective enough for EC patients with advanced stage. Therefore, it is necessary to screen useful biomarkers and novel therapeutic targets of EC. Up to date, more and more biomarkers have been ascertained in the diagnosis or prognosis of EC patients[21-23]. Among them, lncRNAs are considered as a hot candidate. Previously, several researches[24] have reported the expression pattern and biological function of FER1L4 in various tumors. FER1L4 was firstly found to be downregulated in gastric cancer by lncRNA microarray[25]. Then, Liu et al[26] further confirmed that FER1L4 was lowly expressed in gastric cancer and significantly associated with tumor size, distant metastasis and TNM stage. In addition, Yue et al[15] found that FER1L4 was down-regulated in colon cancer and correlated with adverse clinical features and unfavorable survival. Further *in vitro* experiments showed that overexpression of FER1L4 significantly suppressed colon cancer cells proliferation and metastasis by associating with miR-106a-5p in colon cancer. Wu et al[27] also identified FER1L4 as a tumor suppressor in hepatocellular carcinoma because knockdown of FER1L4 was found to promote the malignancy of hepatocellular carcinoma cells by modulating miR-106a-5p. More importantly, in endometrial carcinoma, FER1L4 was found to be down-regulated in endometrial carcinoma and its up-regulation suppressed cancer cell proliferation by regulating PTEN expression, showing that FER1L4 served as a tumor suppres-

Table III. Univariate and multivariate analyses of prognostic factors in EC patients.

Variables	Univariate analysis			Multivariate analysis		
	HR	95% CI	*p*-value	HR	95% CI	*p*-value
Age	1.032	0.664-1.832	0.516			
Pathology	0.933	0.518-1.537	0.344			
ER	0.744	0.348-1.342	0.211			
PR	0.842	0.839-1.563	0.317			
Vessel invasive	1.123	0.557-2.326	0.121			
Pathology classification	1.327	0.677-2.179	0.119			
Distant metastasis	3.138	1.567-5.377	0.005	2.347	1.258-4.458	0.009
Lymph node metastasis	2.567	1.328-4.337	0.008	2.137	1.038-3.263	0.025
FIGO stages	2.984	1.663-5.873	0.005	2.534	1.328-4.163	0.012
FER1L4 expression	3.562	1.217-6.623	0.002	2.782	1.144-5.123	0.004

sor in EC[17]. However, whether the aberrant expression of FER1L4 was associated with poor prognosis of EC patients has never been investigated in EC before. In this study, we performed RT-PCR to detect the expression of FER1L4 in EC tissues, finding that FER1L4 expression was significantly down-regulated in EC tissues compared to their matched non-tumor tissues. Then, the association between FER1L4 and clinicopathological parameters was analyzed and the results indicated that low expression of FER1L4 was significantly correlated with distant metastasis, lymph node metastasis and FIGO stages, indicating that FER1L4 may play critical roles in the pathogenesis of EC. Of note, results from survival analyses suggested that the patients with low FER1L4 showed poorer overall survival than those with high FER1L4, suggesting FER1L4 as a potential prognostic biomarker for EC patients. Of note, in univariate and multivariate analysis stratified for known prognostic variables, FER1L4 was identified as an independent prognostic marker. Further investigations should be performed to validate the clinical implications of FER1L4 in the personalized therapeutic strategies.

Conclusions

We demonstrated that FER1L4 was down-regulated in human EC tissues and could be considered an independent prognostic factor in EC patients. These findings suggested that FER1L4 might act not only as a novel diagnostic and prognostic marker, but also as a potential therapeutic target for EC.

Conflict of Interests

The Authors declare that there are no conflicts of interest.

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