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Long non-coding RNA FEZF1-AS1 is up-regulated and associated with poor prognosis in patients with cervical cancer

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Abstract. – OBJECTIVE: Long noncoding RNA FEZF1-AS1 (FEZF1-AS1) has been showed to involve in a variety of cancers. However, its function and clinical significance in cervical cancer (CC) have not been investigated. The aim of this study was to explore the prognostic value of FEZF1-AS1 in CC patients.

PATIENTS AND METHODS: Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was used to determine the expression level of FEZF1-AS1 in CC specimens and adjacent normal cervical tissues. Association between FEZF1-AS1 expression and clinicopathological characteristics was analyzed x2-test. The Kaplan-Meier method was used to estimate survival curves, and the log-rank statistic was used to test the role of FEZF1-AS1 expression. The possibility of FEZF1-AS1 as a prognostic biomarker for CC was examined by Cox proportional hazard regression model.

RESULTS: We found that FEZF1-AS1 expression levels were significantly higher in CC tissues compared with adjacent non-cancerous tissues ($p < 0.01$). High expressions of FEZF1-AS1 were significantly association with poorer histological grade ($p = 0.004$), positive distant metastasis ($p = 0.002$) and advanced FIGO stage ($p = 0.001$). Furthermore, patients with low FEZF1-AS1 expression lived shorter than those with high FEZF1-AS1 expression (Log-rank test, $p < 0.0034$). Cox regression analysis demonstrated that FEZF1-AS1 expression level was an independent prognostic factor for CC overall survival rates ($p = 0.008$).

CONCLUSIONS: We firstly provided clinical evidence that FEZF1-AS1 may be a possible biomarker of poor prognosis in CC.

Key Words:
Long noncoding RNA, FEZF1-AS1, Cervical cancer, Prognosis.

Introduction

Cervical cancer (CC) is a leading cause of cancer death among women, with the large majority of patients living in developing countries[1,2]. Many patients are diagnosed with CC in China every year, accounting for 30% of the global new cases of CC[3]. Currently, CC can be diagnosed in younger patients and at an early stage via using various advanced diagnostic method[4]. However, the outcome of CC patients remains unsatisfactory, especially for those with advanced-stage tumors[5]. Unfortunately, in China, many CC patients experience early cancer metastasis, resulting in poor long-term survival after resection of primary CC[6]. Therefore, the identification of novel biomarkers for the diagnosis and prognosis of CC remains important for guidance of CC treatment. Non-coding RNAs (ncRNAs), once regarded as "transcriptional noise", have recently been shown to act as functional molecules[7]. Long noncoding RNAs (lncRNAs), one of ncRNAs, are evolutionarily conserved noncoding RNAs that are greater than 200 nucleotides in length with no protein-coding capacity[8]. Recently, lncRNAs were reported to be involved in regulating various physiologic progression, including tumor development, cell proliferation and metastasis[9,10]. In addition, additional studies[11-13] confirm that lncRNAs are frequently dysregulated in various tumors, which play oncogenic or tumor suppressive roles during tumorigenesis. Thus, the detection of specified lncRNAs expression levels may help predict the prognosis of cancer patients. Although more and more lncRNAs were identified to be functional biomarkers significantly associated with survival of cancer patients, including CC, the emerging potential role of most lncRNAs in CC is yet unclear[14,15].

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The lncRNA FEZ family zinc finger 1 antisense RNA 1 (FEZF1-AS1) is an lncRNA located in 7q31.32. The expression, function and molecular mechanism of FEZF1-AS1 have been reported in several tumors, including lung adenocarcinoma[16], stomach adenocarcinoma[17] and pancreatic ductal adenocarcinoma[18]. Recently, systematic gene microarray analysis by Chen et al[19] firstly reported that FEZF1-AS1 expression was significantly up-regulated in CC tissues. However, its function and clinical significance in CC have not been investigated.

Patients and Methods

Patients and Tissues Samples

CC tissues and matched adjacent normal cervical tissues were obtained from 196 patients with CC who received surgery at the Linyi People's Hospital. The fresh tissues were immediately frozen in liquid nitrogen and stored at -80°C until use. The 196 enrolled patients were aged from 32 to 67 years, with a median age of 48 years. None of the patients had received chemotherapy or radiotherapy before surgery. The CC stage was classified by two experienced gynecological oncologists according to the International Federation of Gynecology and Obstetrics (FIGO) staging system for CC. Clinical data were obtained from the patients' medical records and shown in Table II. This study was approved by the Research Ethics Committee of Linyi People's Hospital. Written informed consent was obtained from all of participators.

RNA Extraction and Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated with TRizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA was reverse transcribed into cDNAs using a PrimeScript™ one step RT-PCR kit (TaKaRa, Otsu, Shiga, Japan). FEZF1-AS1 levels were measured by qRT-PCR using SYBR Green in an ABI 7500 StepOne Plus Real-time PCR instrument (Biosystems, Foster City, CA, USA). PCR conditions for quantitative RT-PCR were as follows: 94°C for 5 min, 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 20 s. All the primers were obtained from the TaqMan miRNA Assays and the sequences were shown in Table I. The relative amount of FEZF1-AS1 to U6 was calculated using the equation $2^{-\Delta Ct}$.

Table I. The primer sequence of FEZF1-AS1 and U6.

Gene	Sequence
FEZF1-AS1	F: TTAGGAGGCTTGTTCTGTGT R: GCGCAGGTACTTAAGAAAGA
U6	F: GTTGCGTTACACCCTTTCTTG R: GTCACCTTCACCGTTCCAGT

Statistical Analysis

Statistical analyses were performed using SPSS Statistics 13.0 (SPSS Inc., Chicago, IL, USA). Difference of FEZF1-AS1 expression between two groups was compared by Independent-samples *t*-test. Association between expression level of FEZF1-AS1 and each clinicopathological parameter was evaluated using x^2-test. Overall survival curves were plotted according to the Kaplan-Meier method, and the log-rank test was applied for comparison. Cox proportional-hazard modeling was used for univariate and multivariate analysis to determine the role of variables on survival. $p < 0.05$ was considered as statistically significant.

Results

FEZF1-AS1 Expression is Upregulated in CC Tissues

To investigate whether FEZF1-AS1 was dysregulated in CC patients, we detected its expression by RT-PCR assay. We found that FEZF1-AS1 expression was significantly higher in CC tissues compared with their paired normal cervical tissues ($p < 0.01$) (Figure 1). These data indicated that FEZF1-AS1 might be involved in the development of CC in humans.

Clinicopathological Significance of FEZF1-AS1 Expression in Human CC

Next, the association between the expression of FEZF1-AS1 and clinicopathological features was analyzed. The 196 patients with CC were divided into two groups according to the median value of FEZF1-AS1 levels, including high-expression group (n = 104) and low-expression group (n = 92), respectively. The expression of FEZF1-AS1 in CC tissues was significantly associated with histological grade ($p = 0.004$), distant metastasis ($p = 0.002$) and FIGO stage ($p = 0.001$) (Table

Table II. Correlation between the expression of FEZF1-AS1 and clinicopathological parameters in CC patients

Variable	n	FEZF1-AS1 expression		p value
		High	Low	
Age (years)				0.565
≤ 55	81	41	40	
> 55	115	63	52	
Tumor diameter (cm)				0.795
≤ 4.0	85	46	39	
> 4.0	111	58	53	
HPV infection				0.281
Negative	90	44	46	
Positive	106	60	46	
Histological type				0.975
AD	62	33	29	
SCC	134	71	63	
Histological grade				0.004
Well/moderate	111	49	62	
Poor	85	55	30	
Distant metastasis				0.002
Yes	76	51	25	
No	120	53	67	
FIGO stage				
I/II	126	56	70	0.001
III/IV	70	48	22	

I). However, no relationship was found between FEZF1-AS1 and other clinicopathologic features including age, tumor diameter, HPV infection and histological type (all $p > 0.05$).

FEZF1-AS1 Expression is Conversely Associated with Overall Survival in CC Patients

In order to explore whether FEZF1-AS1 expression was associated with prognosis of CC patients, our cohort of CC patients was followed up for 5 years, with a median survival of 44 months. In addition, Kaplan-Meier curve with long-rank analysis were performed. We found that patients with high expression of FEZF1-AS1 had poorer overall survival compared with those with low FEZF1-AS1 group ($p = 0.0034$) (Figure 2). Furthermore, in univariate analysis, histological grade (HR = 3.783, 95% CI: 1.239-6.639, $p = 0.005$), distant metastasis (HR = 4.763, 95% CI: 1.673-8.893, $p = 0.001$), FIGO stage (HR = 4.352, 95% CI: 1.338-7.362, $p = 0.001$, 0.001), and FEZF1-AS1 expression (HR = 3.672, 95% CI: 1.573-6.328, $p = 0.003$) were associated with poor survival (Table III). Further assay by multivariate analysis confirmed that FEZF1-AS1 expression (HR = 3.213, 95% CI: 1.219-5.663, $p = 0.008$) was an independent prognostic factor for overall survival (Table III).

Discussion

As a major public health issue, CC results from the combined effects of environmental factors and genetic alterations[20]. Current therapeutic tools are effective in patients diagnosed at early stages, but there are limited treatment options for

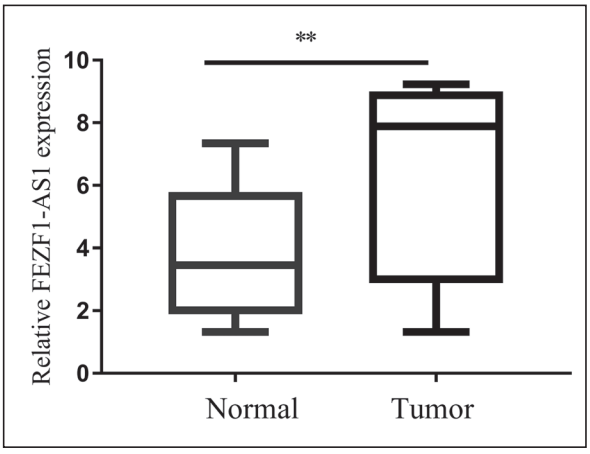

Figure 1. The relative levels of FEZF1-AS1 in 196 paired of CC samples were measured by real-time quantitative RT-PCR, and the U6 small nuclear RNA was used as an internal control. FEZF1-AS1 expression levels were significantly higher in CC tissues compared with adjacent non-cancerous tissues ($p < 0.01$).

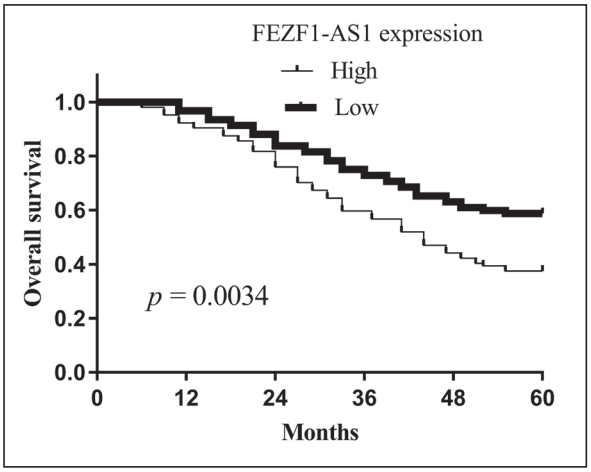

Figure 2. Kaplan-Meier overall survival curves of CC patients according to the level of FEZF1-AS1 expression. High expression of FEZF1-AS1 predicts a poor prognosis in CC patients ($p = 0.0034$)

patients with advanced stages[21]. In order to improve prognosis of CC patients, the investigation about the prognostic factors for CC is especially important, because such predictors are helpful in guiding clinical management[22]. Pathogenesis of CC is very complicated and involved in a large number of genes dysregulation[23]. With the advanced in ncRNA function, lncRNAs as potential biomarkers for diagnosis and prognosis of CC patients become a hot issue.

The year before last, Chen et al[24] firstly reported dysregulation of FEZF1-AS1 expression in colorectal carcinoma tissues by RT-PCR. They performed several *in vitro* and *in vivo* assays and found that FEZF1-AS1 promotes colorectal carcinoma cell proliferation, migration and invasion. Prognostic assay revealed that overexpression of FEZF1-AS1 was significantly associated with poor prognosis of colorectal carcinoma patients. This study firstly suggested FEZF1-AS1 as a functional molecular in cancer. Subsequently, Wu et al[25] found similar results that FEZF1-AS1 expression was highly expressed in gastric cancer and promotes the growth of gastric cancer cells by regulating Wnt signaling pathway. More importantly, Zhou et al[26] confirmed a novel molecular mechanism that FEZF1-AS1 promoted osteosarcoma growth and metastasis by regulating miR-4443/NUPR1 axis, highlighting that FEZF1-AS1 played a critical role in progression of osteosarcoma. Meanwhile, the up-regulation and tumor-promotive role of FEZF1-AS1 in several tumors also reported[18,27]. Although it was confirmed that FEZF1-AS1 expression was significantly up-regulated in CC patients by microarray analysis, the specific function and significance of FEZF1-AS1 in CC have not been reported[19].

In the present work, we performed RT-PCR to determine the expression of FEZF1-AS1 in CC patients. Our results were in line with previous results from microarray analysis. Significant overexpression of FEZF1-AS1 was observed in CC tissues compared to normal cervical tissues. Subsequently, we analyzed the clinical information that was collected by our hospital. Our findings showed that the expression of FEZF1-AS1 in CC tissues was significantly associated with histological grade, distant metastasis and FIGO stage, which indicated that FEZF1-AS1 played a positive role in progression of CC. Furthermore, Kaplan-Meier analysis and log-rank test, suggested that CC patients with up-regulated FEZF1-AS1 expression have shorter overall survival. Then, the Cox proportional hazards model confirmed high FEZF1-AS1 expression level to be an independent predictor of poor prognosis in CC patients. To our best knowledge, we firstly re-

Table III. Univariate and multivariate Cox's hazards analysis on possible prognostic factors for patients with CC.

Factors	Univariate analysis		Multivariate analysis	
	p-value	HR (95% CI)	*p*-value	HR (95% CI)
Age	0.337	1.893 (1.132-2.462)	-	-
Tumor diameter	0.673	1.123 (0.687-1.994)	-	-
HPV infection	0.936	1.452 (0.781-2.123)	-	-
Histological type	0.522	1.213 (0.739-2.445)	-	-
Histological grade	0.005	3.783 (1.239-6.639)	0.008	3.192 (1.023-5.337)
Distant metastasis	0.001	4.763 (1.673-8.893)	0.003	3.736 (1.472-7.132)
FIGO stage	0.001	4.352 (1.338-7.362)	0.006	3.251 (1.138-5.366)
FEZF1-AS1 expression	0.003	3.672 (1.573-6.328)	0.008	3.213 (1.219-5.663)

ported whether FEZF1-AS1 was associated with prognosis of CC patients. However, the biological function and potential mechanism involved in the proliferation and metastasis were not studied in our study. Further functional experiments were needed to elucidate the mechanism underlying the poor clinical prognosis of CC patients.

Conclusions

We demonstrated for the first time that aberrant upregulation of FEZF1-AS1 was significantly correlated with an aggressive phenotype and poor prognosis in CC. A well-designed prospective research was needed to confirm the predictive value of FEZF1-AS1 for prognosis in CC patients.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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