

Expression of *UCA1* and *MALAT1* long-chain non-coding RNAs in esophageal squamous cell carcinoma tissues is predictive of patient prognosis

Kai Kang¹, Yong-Hua Huang², Hai-Peng Li², Shu-Mei Guo¹

¹Department of Gastroenterology, the First Hospital of Yulin City, Yulin, Shaanxi Province, China

²Department of Gastroenterology, Xingyuan Hospital of Yulin City, Shaanxi Province, Yulin, Shaanxi Province, China

³Department of Thoracic Surgery, Central Hospital of Baoji City, Baoji, Shaanxi Province, China

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Abstract

Introduction: The long non-coding RNAs (lncRNAs) urothelial cancer associated 1 (*UCA1*) and metastasis associated lung adenocarcinoma transcript 1 (*MALAT1*) are known to impact cancer cell regulation. The aim of the present study was to determine the relationship between the expression of these lncRNAs in esophageal squamous cell carcinoma (ESCC) tissues and disease prognosis.

Material and methods: The expression of *UCA1* and *MALAT1* lncRNAs was assessed in ESCC and adjacent carcinoma tissues (5 cm away from the tumor) and evaluated in relation to overall survival (OS) and disease-free survival (DFS) of patients. This prospective study included 100 ESCC patients who were admitted to the First Hospital of Yulin City between January 2007 and January 2014.

Results: The expression levels of *UCA1* and *MALAT1* lncRNAs in ESCC tissues were significantly higher than those in adjacent carcinoma tissues, and there were statistically significant differences in TNM staging between the patients with high lncRNA expression and low lncRNA expression. The OS and DFS of patients with high *UCA1* and *MALAT1* lncRNA expression levels were significantly shorter than those with low expression levels. Furthermore, the OS and DFS of ESCC patients appeared to be correlated with TNM staging.

Conclusions: These results imply that the up-regulation of *UCA1* and *MALAT1* lncRNAs in ESCC tissues can impact the degree of tumor progression and is predictive of postoperative survival. Therefore, the expression levels of these lncRNAs can be used as measurement indexes to determine the prognosis of ESCC patients.

Key words: long non-coding RNA, urothelial cancer associated 1, metastasis associated lung adenocarcinoma transcript 1, esophageal squamous cell carcinoma, prognosis, survival analysis.

Corresponding authors:

Hai-Peng Li
Department
of Thoracic Surgery
Central Hospital of Baoji City
No. 8 at Jiangtan Road
Baoji 721008
Shaanxi Province, China
E-mail: cyb8888168@126.com

Shu-Mei Guo
Department of
Gastroenterology
the First Hospital of Yulin City
High-tech District
Yulin 719000
Shaanxi Province, China
E-mail: pyp721@163.com

Introduction

Esophageal carcinoma (EC) is one of the most serious malignant tumors of the digestive system. The morbidity rate of EC ranks eighth and

the mortality rate ranks sixth among malignant tumors worldwide [1, 2]. Two histologic types are typical among EC patients: esophageal adenocarcinoma (EAC) and esophageal squamous cell carcinoma (ESCC). Patients in Western countries predominantly develop EAC, while Chinese patients typically develop ESCC. About 50% of ESCCs are formed in the middle esophagus [3]. Surgical removal is currently the most common treatment for ESCC, but the prognosis after a radical operation is less than ideal. According to recent studies, surgical treatment only gives ESCC patients at stages IIA–III a 5-year survival rate of 20–34% and fails to effectively eliminate distal metastasis and local tumor recurrence [4]. Currently, the TNM classification of malignant tumors serves as the primary basis for determining ESCC prognosis. The lack of objective quantitative indicators to accurately predict prognosis lends itself to opportunities for improvement [5].

Long noncoding RNAs (lncRNA) have emerged as influential factors in the occurrence and development of tumors. lncRNAs are more than 200 nucleotides in length and in most cases are produced by RNA polymerase II and undergo post-transcriptional modifications, such as capping and intron splicing. lncRNA genes usually contain few introns and have low levels of transcription, low conservation among different species, and in most cases have spatiotemporal expression specificity [6, 7]. Although this type of RNA does not have protein-encoding function, it can regulate gene expression at the chromosome, chromatin, transcriptional, and post-transcriptional levels and exerts significant regulatory effects through, inter alia, genomic imprinting, cell differentiation, immune reactions, tumor occurrence, and embryonic stem cell multi-potency. Recent studies have shown that multiple lncRNAs can produce small molecular peptides through transcription to become the driving force for the generation of new proteins [8–10]. In multiple large-scale screening studies, the expression of over 100 lncRNAs in ESCC tissues has been found to be altered. The abnormal expression of lncRNAs is mostly present during the evolution of highly differentiated intraepithelial neoplasia into invasive carcinoma, reflecting the important role of lncRNA in the development of ESCC [11–13].

Two genes known to encode lncRNAs are urothelial cancer associated 1 (*UCA1*) and metastasis associated lung adenocarcinoma transcript 1 (*MALAT1*). Li *et al.* reported that the relative level of *UCA1* was significantly higher in ESCC tissues compared to the adjacent non-tumor tissue, and remarkably higher expression of *UCA1* was found in esophageal cancer cell lines compared with the immortalized esophageal epithelial cell line NE1

[14], and it was found to be deregulated in cisplatin-resistant cells compared with their parent cells [15]. *MALAT1* was over-expressed in 46.3% of ESCC tissues. The enhanced *MALAT1* expression levels were positively correlated with clinical stages, primary tumor size, and lymph node metastasis, and inhibition of *MALAT1* suppressed tumor proliferation *in vitro* and *in vivo*, as well as the migratory and invasive capacity [16]. Furthermore, silencing of *MALAT1* could significantly suppress the proliferation of ESCC cells through the arrest of the G2/M cell cycle, which may be due to *MALAT1*-mediated up-regulation of p21 and p27 expression and the inhibition of B-MYB expression [17]. Recently, animal experiments showed that knockdown of *MALAT1* decreased tumor formation and improved survival [18]. Here, we describe the relationship between the expression of *UCA1* and *MALAT1* lncRNAs in ESCC tissues and the associated prognosis.

Material and methods

Tissues samples

The tumor tissues and para-carcinoma tissues were taken from 100 ESCC patients who were admitted to the First Hospital of Yulin City between January 2007 and January 2014 and treated as participants of this case-control prospective study.

Table I. Clinical characteristics of study participants

Characteristics	Number of cases	Percentage
Gender:		
Male	75	75.0
Female	25	25.0
Age [years]:		
≤ 60	68	68.0
> 60	32	32.0
Histological grade:		
1–2	41	41.0
3–4	59	59.0
TNM stage:		
I–II	73	73.0
III	27	27.0
Pathological type:		
Ulcerative carcinoma	65	65.0
Others	35	35.0
Adjuvant chemotherapy or radiotherapy:		
Yes	59	59.0
No	41	41.0

The adjacent carcinoma tissues had been obtained at least 5 cm away from the tumor. Patients were diagnosed with ESCC based on a postoperative pathological examination. Clinical characteristics of participants are shown in Table I. Patients were monitored after surgery by telephone follow-up or visits to the patient's home, and the total survival (OS) and disease free survival (DFS) status was observed, with OS representing the time from surgery to death or the last follow-up visit and DFS representing the time from the surgery to the local recurrence, distal metastasis, or the last follow-up visit. This study was approved by the Ethics Committee of the First Hospital of Yulin City, and informed consent was given by all participants.

Detection of lncRNAs

Expression levels of *UCA1* and *MALAT1* lncRNAs were detected in the tumor tissues and para-carcinoma tissues as follows: total RNA was extracted by RNAiso Plus (code no. 9108Q; TaKaRa Biotechnology Limited Company, Dalian, China), the PrimeScript RT reagent kit (code no. RR037A, TaKaRa Bio.) was used to transcribe and synthesize cDNA, and real-time quantitative PCR (q-RT PCR) was used to quantify target lncRNA with 18S as the internal reference. The primer sequences are shown in Table II. The total reaction volume was 10 μ l, including of 5 μ l of SYBR Green I Master Mix, 1 μ l of RNAiso Plus, 0.6 μ l of forward primers (10 μ mol/l), 0.6 μ l of reverse primers (10 μ mol/l), 1 μ l of cDNA, and 1.8 μ l of dH₂O deionized water. The reaction conditions were: pre-degeneration at 95°C for 10 s, 95°C for 5 s, 60°C for 5 s, and 72°C for 31 s, and amplification for 40 cycles. The differences between the target lncRNA cycle threshold values (*Ct* values) and 18S *Ct* value were determined (Δ *Ct*) and the 2^{- Δ *Ct*} method was used to determine the relative expression of target lncRNA. Compared to the mean expression levels in ESCC tissues, the patients were categorized as having high *UCA1* expression, low *UCA1* expression, high *MALAT1* expression, and low *MALAT1* expression.

Table II. Primer sequences of target lncRNA

lncRNAs	Primer	Sequence
UCA1	Forward	5'-CTCTCCATTGGGTTACCATTC-3'
	Reverse	5'-GCGGCAGGCTTAAGAGATGAG-3'
MALAT1	Forward	5'-CAGTGGGGAACCTGACTCG-3'
	Reverse	5'-GTGCCTGGTCTCTTAC C-3'
18 S	Forward	5'-CAGCCACCCGAGATTGAGCA-3'
	Reverse	5'-TAGTAGCGACGGCGGTGTG-3'

Statistical analysis

Data were analyzed using SPSS 19.0 statistical software (IBM, Armonk, NY). Measurement data were expressed as mean \pm standard deviation and an independent sample *t* test was used to compare groups. Kaplan-Meier survival analysis was used to compare OS and DFS and the log-rank test was used to determine statistical significance. The Cox risk model was used to analyze factors influencing OS and DFS. A *p* level < 0.05 indicated statistical significance.

Results

UCA1 and *MALAT1* lncRNAs are higher in ESCC tissues than in para-carcinoma tissues

The relative expression levels of *UCA1* were 0.485 \pm 0.248 in ESCC tissues and 0.199 \pm 0.122 in adjacent carcinoma tissues, with a significant difference (*t* = 10.348, *p* < 0.05). Similarly, the expression levels of *MALAT1* were 3.842 \pm 0.415 in ESCC tissues and 1.451 \pm 0.136 in adjacent carcinoma tissues, with a significant difference (*t* = 54.750, *p* < 0.05).

UCA1 and *MALAT1* lncRNA expression is correlated with TNM staging in ESCC tissues

Patients were categorized as having high *UCA1* expression (49 cases), low *UCA1* expression (51 cases), high *MALAT1* expression (50 cases), and low *MALAT1* expression (50 cases) compared to the mean expression levels in ESCC tissues. Clinical characteristics were compared between groups and the difference in TNM staging between the high *UCA1* expression group and the low *UCA1* expression group as well as between the high *MALAT1* expression group and the low *MALAT1* expression group was statistically significant (χ^2 = 12.257, 26.839, *p* < 0.05). The difference in other clinical characteristics was not found to be statistically significant (Tables III and IV).

Table III. *UCA1* lncRNA expression in ESCC tissues and clinical characteristics

Characteristics	Number of cases	<i>UCA1</i> high expression (N = 49)	<i>UCA1</i> low expression (N = 51)	χ^2	P-value
Gender:					
Male	75	35	40	0.654	> 0.05
Female	25	14	11		
Age [years]:					
≤ 60	68	31	37	0.990	> 0.05
> 60	32	18	14		
Histological grade:					
1–2	41	22	19	0.603	> 0.05
3–4	59	27	32		
TNM stage:					
I–II	73	28	45	12.257	< 0.05
III	27	21	6		
Pathological type:					
Ulcerative carcinoma	65	33	32	0.233	> 0.05
Others	35	16	19		

Table IV. *MALAT1* lncRNA expression in ESCC tissues and clinical characteristics

Characteristics	Number of cases	<i>MALAT1</i> high expression (N = 50)	<i>MALAT1</i> low expression (N = 50)	χ^2	P-value
Gender:					
Male	75	39	36	0.480	> 0.05
Female	25	11	14		
Age [years]:					
≤ 60	68	36	32	0.735	> 0.05
> 60	32	14	18		
Histological grade:					
1–2	41	19	22	0.372	> 0.05
3–4	59	31	28		
TNM stage:					
I–II	73	25	48	26.839	< 0.05
III	27	25	2		
Pathological type:					
Ulcerative carcinoma	65	31	34	0.396	> 0.05
Others	35	19	16		

UCA1 and *MALAT1* lncRNA expression in ESCC tissues is predictive of patient survival

The OS and DFS of patients with high *UCA1* and *MALAT1* lncRNA expression in ESCC tissues were significantly lower than those of patients with low expression levels ($\chi^2 = 4.880, 5.894, 8.635, 8.906$, $p < 0.05$). OS, DFS, and 95% confidence intervals (CI) are shown in Tables V, VI and VII, and the Kaplan-Meier statistical significance analytic curves are shown in Figure 1.

Multiple factors influence the survival of ESCC patients

Cox survival models revealed that OS and TNM staging (HR = 2.516) were significantly correlated ($p < 0.05$) with *UCA1* lncRNA (HR = 1.638) and *MALAT1* lncRNA (HR = 1.553) in ESCC patients. In addition, DFS and TNM staging (HR = 2.881) were also correlated with *UCA1* lncRNA (HR = 1.662) and *MALAT1* lncRNA (HR = 1.713), as shown in Tables VIII and IX.

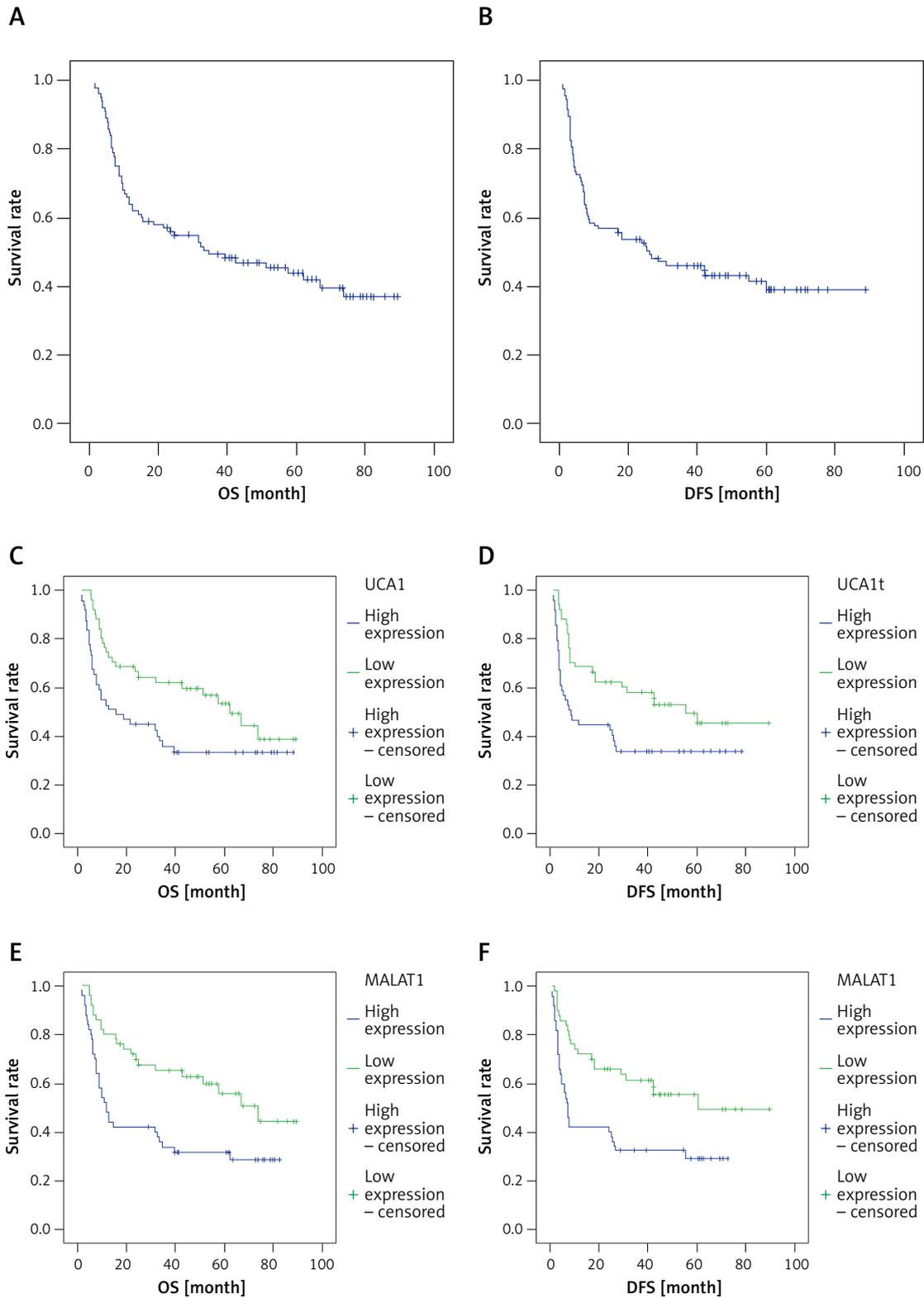


Figure 1. A – Kaplan-Meier survival curve of OS of patients, B – Kaplan-Meier survival curve of DFS of patients, C – Kaplan-Meier survival curve of OS of patients with different *UCA1* lncRNA expression levels, D – Kaplan-Meier survival curve of DFS of patients with different *UCA1* lncRNA expression levels, E – Kaplan-Meier survival curve of OS of patients with different *MALAT1* lncRNA expression levels, F – Kaplan-Meier survival curve of DFS of patients with different *MALAT1* lncRNA expression levels

Table V. OS and DFS of ESCC patients

Survival	Estimate	Std. error	95% CI	
			Lower bound	Upper bound
OS [month]	46.069	3.815	38.591	53.547
DFS [month]	43.457	3.996	35.625	51.288

Table VI. OS and DFS of patients with different *UCA1* lncRNA expression levels

Groups	Survival	Estimate	Std. Error	95% CI	
				Lower bound	Upper bound
UCA1 high expression	OS	53.754	5.096	43.766	63.741
	DFS	51.636	5.403	41.046	62.225
UCA1 low expression	OS	37.538	5.365	27.023	48.052
	DFS	31.855	4.891	22.269	41.442

Table VII. OS and DFS of patients with different *MALAT1* lncRNA expression levels

Groups	Survival	Estimate	Std. error	95% CI	
				Lower bound	Upper bound
MALAT1 high expression	OS	57.089	5.136	47.022	67.156
	DFS	54.440	5.540	43.582	65.299
MALAT1 low expression	OS	32.999	4.760	23.670	42.329
	DFS	27.819	4.289	19.413	36.225

Table VIII. Multiple factors influence OS in ESCC patients

Item	β	χ^2	HR	<i>p</i> -value
TNM stage (stage III)	0.794	5.116	2.516	< 0.05
<i>UCA1</i> high expression	0.436	6.025	1.638	< 0.05
<i>MALAT1</i> high expression	0.237	4.915	1.553	< 0.05

Table IX. Multiple factors influence DFS in ESCC patients

Item	β	χ^2	HR	<i>p</i> -value
TNM stage (stage III)	0.662	4.628	2.881	< 0.05
<i>UCA1</i> high expression	0.168	5.604	1.662	< 0.05
<i>MALAT1</i> high expression	0.339	6.113	1.713	< 0.05

Discussion

In this study, *UCA1* and *MALAT1* lncRNA in ESCC tissues were found to have increased expression levels compared to adjacent carcinoma tissues and were significantly correlated with TNM staging. The results of this study indicate that the expression levels of these two lncRNAs are up-regulated in ESCC tissues and may play important roles in ESCC pathogenicity.

The OS and DFS of patients with high expression of *UCA1* and *MALAT1* lncRNAs in ESCC tissues were found to be significantly lower than those of patients with low expression levels, suggesting that the high expression of these two types of lncRNA played a role in determining OS and DFS. The degree to which *UCA1* and *MALAT1*

lncRNAs changed in ESCC tissues was associated with the progression of the tumors and the patient's postoperative survival, suggesting that the expression of these lncRNAs might be used as auxiliary indicators to evaluate tumor progression and prognosis.

Relevant studies have revealed that *UCA1* can promote the expression of *WNT6* and inhibit the expression of the p27 gene, and it can influence the stage of tumor cells through the PI3K/Akt signaling pathway to exert cancer-promoting effects on proliferation and apoptosis in a variety of cancer types [19, 20]. *UCA1* can also regulate the expression of tumor drug resistance genes [21, 22], giving it the ability to enhance the proliferation, infiltration, and migration of ESCC cells and lower the sensitivity to anti-tumor drugs. Our results

support previous studies suggesting that measuring *UCA1* expression could be a target for the treatment of ESCC and the evaluation of patient condition [23].

MALAT1 can react with multiple proteins in the cell nucleus to influence gene regulation and cancer cell movement capability [24]. Previous studies have found that the inhibition of *MALAT1* can up-regulate the expression of caspase-8, caspase-3 and Bax, and down-regulate the expression of Bcl-2 and Bcl-xL, all of which were related to the development ESCC [25, 26]. The expression of *MALAT1* in ESCC tissues at the middle-advanced stage has been found to be higher than that at the early stage, and the amplitude of the up-regulation is associated with the progression of tumor TNM staging and lymph node metastasis, suggesting that the inhibition of *MALAT1* could stagnate the proliferation of ESCC tumor cells [27]. Therefore, abnormal expression of *MALAT1* in malignant tumor tissues and its involvement with cell cycle stage make it a potential target for the treatment of ESCC [28].

In conclusion, we found that the expression of *UCA1* and *MALAT1* lncRNAs is up-regulated in ESCC tissues and that their expression can impact the degree of tumor progression. This in turn impacts patients' postoperative survival time and can serve as an auxiliary index for predicting the prognosis of ESCC patients. Both lncRNAs are potential targets for ESCC treatments, and future studies will investigate the feasibility of interfering with their expression in a clinical setting.

Conflict of interest

The authors declare no conflict of interest.

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