Original Paper

Predictive Value of LINC01133 for Unfavorable Prognosis was Impacted by Alcohol in Esophageal Squamous Cell Carcinoma

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Key Words

Esophageal squamous cell carcinoma (ESCC) • LINC01133 • lncRNA expression • Prognosis • Alcohol

Abstract

Background/Aims: Considerable evidence indicates that long noncoding RNAs (lncRNAs) exert importantly regulatory functions during human cancer initiation and progression and are promising biotargets in the fight against cancer. **Methods:** In this study, we evaluated the role of the lncRNA *LINC01133* in esophageal squamous cell carcinoma (ESCC). *LINC01133* expression in ESCC was examined by quantitative real-time PCR. The correlations between *LINC01133* expression and clinicopathological variables and survival were examined by the χ^2 test, Kaplan–Meier method, log-rank test, and univariate Cox regression analysis. **Results:** *LINC01133* expression levels were frequently lower in ESCC tissues and cell lines than in paired normal tissues and an immortalized esophageal epithelial cell line, respectively. The expression of *LINC01133* decreased in a TNM stage- and lifestyle-independent manner. *LINC01133* was an independent protective factor and had an anti-tumor effect in the early stage of ESCC development. More importantly, we discovered that drinking status in our cohort impaired the predictive accuracy of *LINC01133* for patients with ESCC. Furthermore, a new risk model combining *LINC01133* expression, drinking status, and TNM stage provided better survival discrimination compared with three other predictors. **Conclusions:** Our data indicate that a loss of *LINC01133* expression is a potential poor prognostic biomarker and therapeutic target for ESCC and provide additional prognostic information to improve the outcomes of ESCC patients.

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Introduction

Esophageal carcinoma (EC) is one of the most common cancers and a leading cause of cancer-related deaths according to the latest global and Chinese cancer statistics [1, 2]. Approximately 50% of EC cases occur in China, and 90% of cases are esophageal squamous cell carcinoma (ESCC), the predominant histological type of EC [3, 4]. The mortality rates of ESCC have begun to decline due to recent advances in diagnostic and therapeutic techniques, but the 5-year overall survival (OS) for patients with resectable ESCC is only 35–45%, which is still dismal [5, 6]. This can likely be explained by the inability of the TNM staging system to accurately predict the prognosis of patients with resectable ESCC [7]. Some patients with early-stage ESCC rapidly develop local and regional recurrences and distant metastases after esophagectomy [8]. Therefore, it is imperative to identify novel prognostic biomarkers to assist oncologists in improving the predictive accuracy of the TNM staging system for ESCC.

Over the past decade, long noncoding RNAs (lncRNAs) have attracted considerable research attention [9]. Although lncRNAs lack protein-coding capacity, they drive many critical biological processes, including tumor growth, invasion, and metastasis [10–12]. For instance, the lncRNA *HOTAIR* (HOX antisense intergenic RNA) is one of the most important oncogenic lncRNAs in several human tumor types, including breast cancer, hepatocellular carcinoma, and colorectal cancer [10]. *HOTAIR* regulates tumor proliferation, migration, and chemotherapeutic sensitivity by modulating gene expression and chromatin dynamics [13]. In addition, the lncRNA *XIST* (X-inactive specific transcript), involved in genetic imprinting, appears to be dysregulated in various cancers, including lung cancer, gastric cancer, and ESCC [4, 14, 15]. Owing to their powerful functions and tissue-specific expression, lncRNAs are considered highly promising biomarkers in cancer diagnosis, therapy, and prognosis [16].

LINC01133 is a novel lncRNA located on human chromosome 1q23 that encodes a long intergenic noncoding RNA (Table 1). High expression of *LINC01133* was first reported in lung squamous cell cancer [17]. Knockdown of *LINC01133* significantly inhibits the migration and invasion capacity of lung cancer cells and osteosarcoma cells by targeting *EZH2*, *LSD1*, and sponging miR-422a [18, 19]. Interestingly, two recent studies have shown that *LINC01133* expression is lost in colorectal cancer, and the silencing of *LINC01133* suppresses metastasis and epithelial–mesenchymal transition by interacting with *SRSF6* [20, 21]. These contradictory results suggest that *LINC01133* exerts both a positive and negative effect on migration and invasion in a tumor type-dependent manner. However, very little is known about the expression level and prognostic significance of *LINC01133* in ESCC.

Materials and Methods

ESCC tissue specimens

ESCC tissues and paired normal esophageal epithelial tissues from 149 patients were surgically collected at Sun Yat-Sen University Cancer Center between January 2007 and December 2009. The eligibility criteria for patients with ESCC were (1) no distant metastases and no local and/or systemic treatment before the operation, (2) histological diagnosis of samples by two pathologists, and (3) no serious complications or other malignant disease. The clinical stage was determined according to the 7th edition of the TNM classification of the International Union against Cancer (UICC). Overall survival (OS) was defined as the time from the date of radical surgery to the date of death or last follow-up, and progression-free survival (PFS) was the time from the date of radical surgery to the date of relapse, metastasis, or last follow-up. The study was reviewed and approved by the medical ethics committee of Sun Yat-Sen University Cancer Center. Informed consent was obtained from each patient before surgery.

Table 1. Gene annotations of LINC01133. HGNC, HUGO Gene Nomenclature Committee. ID, identity

Characteristics	
HGNC gene symbol	Homo sapiens long intergenic non-protein coding RNA 1133
Alternative gene name	RP11-48020.4
Ensembl version	ENSG00000224259
Gene type	lincRNA
Location	Chromosome 1: 159,961,224-159,979,061
Transcript ID	ENST00000423943.1
Transcript size	1113 nucleotides

ESCC cell lines and cell culture

Nine ESCC cell lines (KYSE-140, KYSE-150, KYSE-180, KYSE-30, KYSE-410, KYSE-510, KYSE-520, EC-18, and HKESC1) and an immortalized esophageal epithelial cell line, NE-1, were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). Cancer cells were cultured at 37°C and 5% CO₂ in RPMI-1640 medium (Gibco, Carlsbad, CA) supplemented with 10% fetal bovine serum. The NE-1 cell line was maintained in a 1:1 mixture of defined keratinocyte serum-free medium with growth supplements (Gibco) and EpiLife medium (Cascade Biologics, Portland, OR). All experiments were performed during the exponential phase of cell growth.

Quantitative real-time PCR (qRT-PCR)

Total RNA from ESCC tissues and cells was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Total RNA quality and concentration were examined by absorbance at 260/280 nm using a NanoDrop-2000 spectrophotometer (NanoDrop Products, Wilmington, DE). RNA was reverse transcribed to cDNA using PrimeScript™ RT Master Mix (Takara, Dalian, China). For quantitative real-time PCR (qRT-PCR), the cDNA was quantified in triplicate using SYBR Green Mix (Promega, Madison, WI) on a Light Cycler 480 instrument (Roche, Basel, Switzerland). The relative expression of *LINC01133* was calculated using the 2^{-ΔΔC_t} method and *GAPDH* was used as the reference gene. The following primers were used for qRT-PCR. *LINC01133* forward: 5'- TGGTGGAGAGAATGGAGG -3'; reverse: 5'- AACCCAGTTCCTTAGAATCTTC -3'. *GAPDH* forward: 5'- CTCCTCCTGTTCGACAGTCAGC -3'; reverse: 5'- CCCAATACGACCAATCCGTT -3'.

Statistical analysis

All statistical analyses were implemented in SPSS version 23.0 (SPSS, Chicago, IL). Relative expression of *LINC01133* in ESCC tissues and paired normal tissues was evaluated by the paired-sample *t*-test. The chi-squared test or Fisher's exact test was used to examine the correlation between *LINC01133* expression and various clinicopathological variables. Survival analysis was performed using the Kaplan–Meier method, and the log-rank test was used to assess the differences between patient groups or subgroups. Univariate and multivariate Cox proportional hazards regression analyses were used to identify the independent prognostic factors that predict OS and PFS. All *P*-values were two-sided and *P*-values of less than 0.05 were defined as statistically significant.

Results

LINC01133 is expressed at low levels in ESCC tissues and cell lines

Given the important role of *LINC01133* in the pathogenesis of several human cancers, we first examined its expression level in 149 pairs of ESCC tissues (T) and matched adjacent non-cancerous esophageal tissues (N) by qRT-PCR. We detected reduced expression of *LINC01133* in the majority of ESCC tissues (*P* < 0.0001, Fig. 1A and 1B). *LINC01133* expression was downregulated in 66.4% of tumor tissues, with 3-fold lower relative expression levels in tumors than in paired normal tissues (Fig. 1B). We also examined *LINC01133* expression in a panel of ESCC cell lines. Compared with the immortalized normal epithelial cell line NE-1, *LINC01133* expression was generally lower in 6 of 9 ESCC cell lines (Fig. 1C). These results indicate that *LINC01133* was significantly downregulated in ESCC and imply that it functions as a tumor suppressor in ESCC.

Reduced expression of LINC01133 is positively correlated with cancer progression and unhealthy lifestyles in patients with ESCC

Next, we evaluated the relationship between *LINC01133* expression and widely recognized clinicopathological features of ESCC patients. The clinicopathological and demographic data of our cohort are shown in Table 2. These patients were divided into *LINC01133* high- and low-expression groups according to their relative expression in ESCC tissues with the median cancer/normal fold change (0.512) as the cutoff value. Chi-squared analysis indicated that low *LINC01133* expression in ESCC is remarkably associated with

Fig. 1. LINC01133 expression is significantly downregulated in ESCC tissues and cell lines. (A) The expression of LINC01133 in 149 pairs of ESCC tissues (T) and corresponding adjacent normal esophageal tissues (N) determined by qRT-PCR. Results are presented as the difference in Δ cycle threshold (Δ Ct) in tumor tissues compared with normal tissues. (B) A scatter plot of the ratio of LINC01133 expression in ESCC tissues versus paired normal tissues representing the relative expression of LINC01133 in individual samples. (C) Relative expression of LINC01133 in a panel of ESCC cell lines and an immortalized normal epithelial cell (NE-1). Data are means of triplicate experiments $\pm$ SD.

ever smoking, ever drinking, large tumor size (≥ 5 cm in length), greater depth of tumor invasion (stage T3–T4), lymph node metastasis (stage N1–3), and advanced TNM stage (stage III) (all $P < 0.05$, Table 2). No significant statistical association was observed between *LINC01133* expression and other clinicopathological variables (Table 2).

We further assessed the relative expression of *LINC01133* in different subgroups. *LINC01133* expression decreased strikingly with increases in TNM stage, T stage, and N stage (Fig. 2A–2C). However, no statistically significant difference was found between stages T2 and T3, among stages N1, N2, and N3, and between tumors of different sizes (Fig. 2B–2D). Surprisingly, although there was a marginally significant difference in expression between patients with different smoking statuses, *LINC01133* was markedly lower in patients who reported ever drinking than in those who reported never drinking (Fig. 2E and 2F). Taken together, these results suggest that *LINC01133* is intimately involved in cancer progression and is related to unhealthy lifestyles in patients with ESCC.

LINC01133 downregulation is associated with poor prognosis in ESCC

To evaluate the clinical significance of *LINC01133* in this malignancy, we analyzed the correlation between clinicopathologic variables and clinical outcomes, including OS and PFS. Univariate analysis revealed that drinking status, T stage, N stage, TNM stage, and *LINC01133* expression were significant prognostic factors for OS and PFS in ESCC patients (all $P < 0.05$, Table 3). Multivariate analysis indicated that *LINC01133* is an independent favorable predictor of OS (HR: 0.459; 95% CI: 0.260–0.810; $P = 0.007$) and PFS (HR: 0.500; 95% CI:

Table 2. Correlation between LINC01133 expression levels and clinicopathological parameters in patients with ESCC. NOTE: ESCC, esophageal squamous cell carcinoma. TNM, tumor node metastasis. Bold type indicates $P < 0.05$

Variables	No. (%)	LINC01133 Expression		P value
		Low (N, %)	High (N, %)	
Gender				
Female	32 (21.5%)	12 (37.5%)	20 (62.5%)	0.101
Male	117 (78.5%)	63 (53.8%)	54 (46.2%)	
Age (years)				
< 60	75 (50.3%)	34 (45.3%)	41 (54.7%)	0.119
≥ 60	74 (49.7%)	41 (55.4%)	33 (44.6%)	
Smoking status				
Never	50 (33.6%)	18 (36.0%)	32 (64.0%)	0.013
Ever	99 (66.4%)	57 (57.6%)	42 (42.4%)	
Drinking status				
Never	80 (53.7%)	32 (40.0%)	48 (60.0%)	0.007
Ever	69 (46.3%)	43 (62.3%)	26 (37.7%)	
Tumor location				
Upper and Middle	110 (73.8%)	55 (50.0%)	55 (50.0%)	0.891
Lower	39 (26.2%)	20 (51.3%)	19 (48.7%)	
Tumor size (cm)				
< 5 cm	101 (67.7%)	45 (44.6%)	56 (55.4%)	0.041
≥ 5 cm	48 (32.3%)	30 (62.5%)	18 (37.5%)	
Pathological Grade				
I–II	111 (74.5%)	53 (47.7%)	58 (52.3%)	0.280
III	38 (25.5%)	22 (57.9%)	16 (42.1%)	
T stage				
T1	3 (2.0%)	0 (0.0%)	3 (100.0%)	0.015
T2	23 (15.4%)	8 (34.8%)	15 (65.2%)	
T3	118 (79.2%)	62 (52.5%)	56 (47.5%)	
T4	5 (3.4%)	5 (100.0%)	0 (0.0%)	
N stage				
N0	67 (45.0%)	22 (32.8%)	45 (67.2%)	0.001
N1	49 (32.9%)	30 (61.2%)	19 (38.8%)	
N2	22 (14.8%)	15 (68.2%)	7 (31.8%)	
N3	11 (7.3%)	8 (72.7%)	3 (27.3%)	
TNM stage				
I	6 (4.0%)	1 (16.7%)	5 (83.3%)	< 0.001
II	69 (46.3%)	23 (33.3%)	46 (66.7%)	
III	74 (49.7%)	51 (68.9%)	23 (31.1%)	

0.307–0.812; $P = 0.005$) for ESCC patients (Table 3). Additionally, N stage was an independent predictor of poorer OS and PFS, and T stage was an independent predictor of only poorer OS in ESCC patients (Table 3).

To further explore the predictive value of *LINC01133*, the log-rank test and Kaplan–Meier survival analysis were used to investigate the association between *LINC01133* expression and prognosis in our patient cohort. The *LINC01133* low-expression group had a significantly shorter 5-year OS rate (34.3% vs. 71.9%) and 5-year PFS rate (21.6% vs. 56.7%) compared with those in the *LINC01133* high-expression group (all $P < 0.0001$, Fig. 3A), suggesting that the underexpression of *LINC01133* is significantly associated with poor prognosis in ESCC.

We further stratified the 149-patient cohort into subgroups according to tumor size, pathological grade, and TNM stage. *LINC01133* maintained its predictive value for OS and PFS in all of the subgroups, excluding PFS in patients with tumor size ≥ 5 cm ($P = 0.0683$) or TNM stage III ($P = 0.1032$) (Fig. 3B–3G). Interestingly, the discriminative power of *LINC01133* was obviously stronger for patients with small tumors, pathological grade I–II, and TNM stage I–II than for patients with large tumors, grade III, and stage III (Fig. 3B–3G). These observations suggest that *LINC01133* is a novel prognostic marker for ESCC and could be implicated in the early stage of ESCC development.

Fig. 2. LINC01133 expression was markedly decreased in patients with advanced ESCC and unhealthy lifestyles. Relative expression of LINC01133 in ESCC tissues with different (A) TNM stages, (B) T stages, and (C) N stages. qRT-PCR was performed to assess the relative expression of LINC01133 in (D) ESCC tissues with tumor sizes of < 5 cm and ≥ 5 cm, (E) tissues with different smoking statuses, and (F) tissues with different drinking statuses. Results presented as Δ cycle threshold (ΔCt) values in tumor tissues relative to the corresponding normal tissues.

Table 3. Univariate and multivariate Cox regression analysis of LINC01133 and survival in patients with ESCC. NOTE: TNM, tumor node metastasis. ESCC, esophageal squamous cell carcinoma. HR, hazard ratio. CI, confidence interval. Bold type indicates $P < 0.05$

Clinical variables	Univariate analysis HR	95% CI	P value	Multivariate analysis HR	95% CI	P value
Overall Survival						
Gender (Male vs. Female)	1.269	0.676-2.380	0.459			
Age (≥ 60 y vs. < 60 y)	0.976	0.595-1.600	0.923			
Smoking status (Ever vs. Never)	1.236	0.722-2.118	0.440			
Drinking status (Ever vs. Never)	1.949	1.178-3.224	0.009	1.508	0.899-2.530	0.119
Tumor location (Lower vs. Upper/Middle)	1.289	0.752-2.208	0.355			
Tumor size (≥ 5 cm vs. < 5 cm)	1.492	0.892-2.495	0.127			
Pathological Grade (III vs. II/I)	1.121	0.642-1.97	0.687			
T stage (T4 vs. T3 vs. T2 vs. T1)	3.061	1.406-6.662	0.005	2.508	1.054-5.967	0.038
N stage (N3 vs. N2 vs. N1 vs. N0)	1.769	1.394-2.245	< 0.001	1.709	1.190-2.455	0.004
TNM stage (III vs. II vs. I)	2.427	1.492-3.946	< 0.001	0.690	0.315-1.511	0.353
LINC01133 expression (high vs. low)	0.334	0.197-0.566	< 0.001	0.459	0.260-0.810	0.007
Progression-Free Survival						
Gender (Male vs. Female)	1.378	0.787-2.414	0.262			
Age (≥ 60 y vs. < 60 y)	0.959	0.622-1.479	0.851			
Smoking status (Ever vs. Never)	1.332	0.828-2.144	0.238			
Drinking status (Ever vs. Never)	1.660	1.072-2.568	0.023	1.234	0.787-1.934	0.359
Tumor location (Lower vs. Upper/Middle)	1.103	0.676-1.798	0.695			
Tumor size (≥ 5 cm vs. < 5 cm)	1.376	0.871-2.175	0.171			
Pathological Grade (III vs. II/I)	1.052	0.635-1.742	0.844			
T stage (T4 vs. T3 vs. T2 vs. T1)	1.861	1.069-3.240	0.028	1.431	0.762-2.690	0.265
N stage (N3 vs. N2 vs. N1 vs. N0)	1.877	1.495-2.356	< 0.001	1.781	1.257-2.523	0.001
TNM stage (III vs. II vs. I)	2.412	1.590-3.659	< 0.001	0.823	0.415-1.633	0.577
LINC01133 expression (high vs. low)	0.381	0.243-0.599	< 0.001	0.500	0.307-0.812	0.005

Fig. 3. Low expression of LINC01133 was positively correlated with a poor overall survival (OS) and progression-free survival (PFS). Kaplan–Meier curves of OS and PFS based on LINC01133 expression in (A) all 149 patients with ESCC, (B and C) patients with tumor sizes of <5 cm and ≥5 cm, (D and E) patients with pathological grade I–II and III, and (F and G) patients with TNM stage I–II and III. P-values were obtained by log-rank tests.

Prognostic significance of LINC01133 is influenced by drinking status in ESCC patients

Tobacco and alcohol use are major risk factors for ESCC [3]. Based on the negative correlation between LINC01133 expression and smoking or drinking status (Table 2 and Fig. 2), patients with ESCC were stratified into two subgroups. A Kaplan–Meier survival analysis verified that LINC01133 maintained its independent prognostic value, irrespective of whether patients had smoked or not (Fig. 4A and 4B), and similar results were observed in the patient subgroup stratified by drinking status (all $P < 0.05$) (Fig. 4C and 4D). More interestingly, patients with low LINC01133 expression had similarly poor OS and PFS, regardless of their drinking status (all $P > 0.05$, Fig. 4E). However, in the subgroup with high LINC01133 expression, patients who reported ever drinking had significantly shorter OS ($P = 0.0261$) and marginally significantly worse PFS ($P = 0.0532$) than those of patients

who reported never drinking (Fig. 4F). Collectively, our results indicate that alcohol could attenuate the antitumor effects of *LINC01133* on ESCC, indicating that *LINC01133* expression combined with alcohol consumption may supply additional prognostic information for oncologists and patients.

LINC01133 expression combined with drinking status and TNM stage better predicts patient survival in ESCC

The current TNM staging system has a well-defined and validated role in the management of human cancers. However, the TNM staging system in our cohort was not adequate for discriminating survival outcomes between patients with stage I and II ESCC (OS, $P = 0.5705$; DFS, $P = 0.4978$) (Fig. 5A). Hence, feasible biomarkers are urgently needed to improve the discriminatory power of the TNM staging system.

According to the results shown in Fig. 4, we hypothesized that the combination of *LINC01133* expression and drinking status might improve the prediction of survival in patients with ESCC. To this end, we established a combined group based on the sum of *LINC01133* expression (1 or 0) and drinking status (0 or 1) for each patient. Kaplan–Meier survival analysis indicated that patients in group 2 (score 1) had significantly worse OS ($P = 0.0011$) and PFS ($P = 0.0042$) than those of patients in group 1 (score 0), while the prognostic difference

Fig. 4. Drinking status affected the prognostic significance of *LINC01133* in patients with ESCC. Kaplan–Meier survival analysis and log-rank tests were conducted to evaluate the prognostic significance of *LINC01133* in ESCC patients with (A and B) different smoking statuses and (C and D) different drinking statuses. Kaplan–Meier survival analysis of the correlation between drinking status and OS/PFS outcome in patients with (E) low *LINC01133* expression and (F) high *LINC01133* expression. P-values were calculated by log-rank tests.

between group 2 and group 3 (score 2) was not statistically significant (Fig. 5B). In this case, group 2 and 3 were combined into a new group 1 (score 1). TNM stage I–II and stage III were scored 0 and 1, respectively. As illustrated in Fig. 5C, a new combined risk score was calculated as the sum of the scores for the new group (0 or 1) and TNM stage (0 or 1) for each case. Patients with combined scores of 0, 1, or 2 were classified as low risk (LR), moderate risk (MR), or high risk (HR), respectively. As expected, statistically significant differences were observed among three new combined risk groups (Fig. 5C).

In addition, we performed a receiver operating characteristic (ROC) curve analysis to compare the discriminative power of *LINC01133* expression, *LINC01133* combined with drinking status (LINC+Drink), TNM stage, and the combination of the three predictors (LINC+Drink+TNM) for predicting patient survival. Compared with TNM stage, the ROC curves for *LINC01133* and LINC+Drink did not illustrate strong separation for OS (area under the ROC curves (AUC): 0.626 vs. 0.614 vs. 0.655, Fig. 5D) and PFS (AUC: 0.617 vs. 0.591 vs. 0.630, Fig. 5D). However, LINC+Drink+TNM, which yielded AUC values of 0.679 for OS and 0.651 for PFS, performed best among the four predictors and significantly improved the predictive accuracy compared with that of the TNM stage for OS, but not for PFS (Fig. 5D). Altogether, these findings suggest that *LINC01133* expression combined with drinking status and TNM stage provided greater discriminative power for the prognosis of ESCC. This combined risk model could supply more prognostic information to help oncologists develop effective treatment strategies.

Discussion

Owing to unsatisfactory survival results for patients with resectable

Fig. 5. Combination of *LINC01133* expression, drinking status, and TNM stage has the best predictive value in patients with ESCC. (A) OS and PFS of 149 ESCC patients were predicted by the TNM staging system. (B) All patients were stratified into three groups according to the combined score of *LINC01133* expression and drinking status. OS and PFS of patients were predicted by the combined groups. (C) OS and PFS outcomes of ESCC patients were predicted according to the combination of *LINC01133* expression, drinking status, and TNM stage, indicating that the combined risk model significantly improves the predictive value of the TNM staging system. (D) Receiver operating characteristic (ROC) analysis compares OS (left panel) and PFS (right panel) of ESCC patients by TNM staging system, *LINC01133* expression, *LINC01133* combined with drinking status (LINC+Drink), and the combination of three predictors (LINC+Drink+TNM). The result shows that the area under the curve (AUC) of LINC+Drink+TNM is largest among the four predictors.

ESCC, there is a pressing need to identify key molecular mechanisms underlying the development of this disease in order to improve diagnosis and prognosis [3, 22]. Recent research focusing on lncRNAs has improved our understanding of the complex regulatory mechanisms underlying tumorigenesis and metastasis in human cancers [10]. In ESCC, the dysregulation of some lncRNAs, such as *MALAT1* [23, 24], *91H* [25], *HOTAIR* [26], *ANRIL* [27, 28], and *MEG3* [29, 30], promotes or inhibits the progression and metastasis of tumors and is closely associated with poor survival [31]. These results confirm that lncRNAs are potential diagnostic and therapeutic biomarkers in ESCC.

In this study, we focused on the novel lncRNA *LINC01133*, whose role in ESCC was unclear. Our results indicated that *LINC01133* expression was significantly reduced in ESCC tissues, as determined by qRT-PCR, and low expression was predictive of poor survival in patients with ESCC, consistent with previous observations [20, 21]. One previous study found that *LINC01133* is distributed in the cytoplasm and nucleus based on a FISH assay [20]. *LINC01133* may act as pivotal regulator for epigenetic modifications and transcriptional and post-transcriptional processes. Intriguingly, *LINC01133* downregulation in our study was positively correlated with large tumors, greater depth of invasion, lymph node metastasis, and advanced TNM stage. Furthermore, a survival analysis in different clinical subgroups indicated that the predictive power of *LINC01133* was superior in patients with small tumors, pathological grade I–II, and TNM stage I–II than in patients with large tumors, grade III, and stage III. The clinical results suggested that *LINC01133* has a tumor suppressive function in the early stage of ESCC development, and a lack of *LINC01133* may induce esophageal squamous cell tumorigenesis.

Increasing reports have established that unhealthy lifestyles (alcohol abuse, tobacco use, and micronutrient deficiency) are risk factors for the initiation and progression of ESCC worldwide [32, 33]. In our study, drinking status, and not smoking status, was significant in OS and PFS by a univariate analysis, but the independent effect of drinking status on the risk of ESCC was not substantiated by a multivariate Cox proportional analysis, consistent with previous results [34, 35]. A potential explanation is that tobacco use impairs the predictive accuracy of drinking status in our cohort, in which only 6.04% of patients were pure drinkers.

Because studies of lncRNAs and environmental risk factors are relatively new, few known lncRNAs (PCGEM1 and lnc-PSD4) are associated with alcohol consumption in human cancer [36, 37]. To our knowledge, lncRNAs correlated with alcohol consumption have not been reported in ESCC. In this study, the expression level of *LINC01133* was negatively associated with drinking status. In a stratified analysis, we further found that the discriminative power of *LINC01133* was dramatically greater in patients who reported never drinking than in patients who reported ever drinking, and the predictive value of drinking status in patients with low *LINC01133* expression was not significant. These results demonstrated that the expression and functions of *LINC01133* may be influenced by alcohol. Although alcoholic beverages or ethanol is not classified as a carcinogen, we tentatively hypothesized that acetaldehyde, the metabolite of ethanol and an established carcinogen, regulates the expression and functions of *LINC01133* via interfering with epigenetic processes, including DNA repair mechanisms, DNA damage, and histone modifications. This hypothesis warrants further investigation in the near future.

In clinical practice, many oncologists have realized that the TNM staging system alone does not have the optimal predictive value in several human cancers, including ESCC [7, 38, 39], consistent with our findings. Consequently, additional prognostic biomarkers are required to improve the risk assessment of the TNM staging system. In this regard, we evaluated the impact of *LINC01133* or *LINC01133* combined with drinking status on patient outcomes. Unfortunately, neither *LINC01133* alone nor *LINC01133* combined with drinking status had higher sensitivity and specificity than those of TNM stage alone. However, a new risk model based on a combination of *LINC01133*, drinking status, and TNM stage had the best sensitivity and specificity among the four predictors evaluated, suggesting that this risk model could provide more effective discriminative power for predicting patient survival than the TNM staging system alone. Thus, the simultaneous assessment of *LINC01133* expression,

drinking status, and TNM stage can provide oncologists with more accurate prognostic information for patients with ESCC.

Conclusion

In summary, our findings indicated that *LINC01133* was significantly underexpressed in ESCC. *LINC01133* functions as a tumor suppressor and plays a critical role in the early stage of ESCC development. More importantly, our results demonstrated for the first time that drinking status can influence the predictive value of *LINC01133* in ESCC. The combination of *LINC01133* expression, drinking status, and TNM stage better identified patients with ESCC at high risk after esophagectomy; more aggressive postoperative therapy is recommended for these patients. However, future studies are needed to elucidate the mechanism by which alcohol regulates the biological functions of *LINC01133* in ESCC.

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Disclosure Statement

There are no conflicts of interest to disclosure for any author.

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