A PROGNOSTIC FIVE-LNCRNA EXPRESSION SIGNATURE FOR SURVIVAL PREDICTION IN PATIENTS WITH TONGUE SQUAMOUS CELL CARCINOMA

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ABSTRACT

Introduction: *Tongue squamous cell carcinoma (TSCC) is the most common and a highly aggressive malignancy of oral cancer. Deregulated tumorigenic long non-coding RNA (lncRNA) has been reported in several malignancies. However, few studies have investigated systematically the prognostic value of lncRNAs in TSCC patients till date. This study aimed to construct a lncRNA-based prognostic signature to improve the survival prediction for TSCC patients.*

Materials and methods: *We performed a comprehensive analysis for lncRNA expression profiles and corresponding clinical information in a cohort of 122 TSCC patients from The Cancer Genome Atlas (TCGA). Prognostic lncRNAs were identified by employing univariate and multivariate Cox proportional hazards regression analysis, the least absolute shrinkage and selection operator analysis and the Kaplan-Meier curve method. A multi-lncRNA signature and risk score were constructed according to the lncRNA expression and corresponding Cox coefficient. Functional enrichment analysis and drug prediction were also performed using online tools.*

Results: *We established a five-lncRNA expression signature associated with overall survival (OS) based on risk scoring strategy in TSCC. Risk score was dichotomized to result in high and low risk patients. Receiver operating characteristic analysis indicated that this signature exhibited excellent performance for 1-, 3- and 5-year OS event prediction (AUC= 0.856, 0.883 and 0.879 for 1-, 3- and 5-year, respectively). Moreover, multivariate Cox regression analysis revealed that this five-lncRNA signature was an independent predictor for OS when after adjusting T stage, tumor stage and cigarettes history. Functional analysis suggested that the prognostic lncRNAs were mainly involved in apoptosis and sphingolipid signaling pathways. Drug prediction indicated that panobinostat was potentially a promising agent for the treatment of TSCC.*

Conclusion: *Our study demonstrated that the five-lncRNA expression signature may serve as a novel independent biomarker for predicting survival in patients with TSCC.*

Keywords: *Tongue squamous cell carcinoma, lncRNA, prognosis, expression signature.*

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Introduction

Tongue squamous cell carcinoma (TSCC) as the most common type of oral tumors accounts for approximately 25% to 40% of oral cancer patients, usually leading to malfunction of speech, mastication, and deglutition[1]. Epidemiological evidence reveals that major causative factors for the development of TSCC include tobacco consumption, alcohol abuse and viral infection[2]. Taking the consideration that TSCC is generally accompanied by local invasion or even lymph node metastasis at the initial of diagnosis, it could comprehend that the overall mortality rate is high and the 5-year overall survival rate does not exceed 50%[3]. In spite of recent broad progress in surgery, chemotherapy, radiotherapy and targeted therapy, the prognosis remains a considerable challenge due to unsatisfying efficacy and survival outcome. To date, the tumor node metastases (TNM) staging system of the American Joint Committee on Cancer (AJCC) remains an important approach for the treatment and prognosis

of TSCC. However, most patients with early-stage following proper treatment still have a high risk of developing secondary and/or recurrent tumors in the surrounding area[4]. Even among patients with the same TNM staging, the prognosis varied extensively, for which the molecular complexity and biological heterogeneity of TSCC may be responsible[5]. Hence, there is an urgent need to identify potential molecular biomarkers for the individualized therapy and prognosis of TSCC.

Recently, some relatively reliable molecular prognostic biomarkers for TSCC were summarized, including cyclin D1, vascular endothelial growth factors, TP53, E-cadherin, vimentin and cytokines (interleukin-6, interleukin-8, prolactin in liquid samples), etc[6, 7]. Additionally, a 16-gene expression signature was proposed to predict the survival of TSCC[5]. However, all these biomarkers are protein-coding genes (mRNAs). Indeed, mRNAs only constitute a small fraction of the human genome, while more than 98% of the genome may be processed into non-coding RNAs (ncRNAs) which may have more potency for prognostication[8]. Of the ncRNAs, long non-coding RNAs (lncRNAs) are an important class of mRNA-like transcripts greater than 200 nucleotides in length and without coding any proteins[9]. Increasing evidence has demonstrated that lncRNAs play a critical role in cancer biology including cell apoptosis, proliferation, invasion, and migration through chromatin modification, transcriptional modulation, and post-transcriptional regulation, implying its significance in clinics[8, 10]. In TSCC, the biological role of some lncRNAs were investigated more recently. For instance, as oncogenes, H19, HOX transcript antisense RNA (HOTAIR), metastasis-associated lung adenocarcinoma transcript 1(MALAT1) and cancer susceptibility candidate 15(CASC15) were found to be involved in proliferation, invasion, and metastasis; the PCBP2 Overlapping Transcript 1(TUC338), colon cancer-associated transcript 2 (CCAT2) and ADAMTS9 antisense RNA 2 (ADAMTS9-AS2) were found to be involved in proliferation and apoptosis[10]. Whereas, maternally expressed 3 (MEG3) and NF-kappaB interacting LncRNA (NKILA) were found to be involved in the inhibition of invasion and inhibition of metastasis as tumor suppressors[10].

With regard to the survival prediction, up-regulated LINC00152 was reported to predict progression and poor prognosis of TSCC[11]. Overexpression of lncRNA CASC15 was also found to be associated with lower overall survival rate in TSCC patients[12]. However, the functions of most lncRNAs in TSCC have not been elucidated, and the prognostic significance of expression-based lncRNA signature in TSCC remains under-investigated and needs to be systematically evaluated.

In this study, we, for the first time, performed genome-wide analysis of lncRNA expression profiles integrating clinical data of 122 TSCC patients from The Cancer Genome Atlas (TCGA), and developed a reliable five-lncRNA signature acting as a prognostic predictor for TSCC patients. In addition, we proposed some potential drugs against the prognostic lncRNAs by using drug-lncRNA databases. We expected to provide insightful information regarding lncRNAs as biomarkers and therapeutic targets for TSCC patients.

Materials and methods

Patient datasets and processing

Raw expression data (HTSeq-Counts) for Head and Neck squamous cell carcinoma (HNSC) together with the corresponding clinicopathological data were obtained from TCGA by the R-based TCGAbiolinks package[13]. We further screened out TSCC data according to the clinicopathological information. Ensembl IDs of the count data matrix were annotated in the form of gene symbols and biotypes based on GENCODE project gene annotation file (GRCh38) by the online SangerBox software (http://sangerbox.com/) developed by ShengXinRen (https://shengxin.ren), resulting in lncRNA and mRNA expression profiles, respectively. After removing the lowly expressed lncRNAs using the counts-per-million of less than 1, expression levels were normalized using the Trimmed Mean of M-values method from edgeR package and underwent a log2 (x + 1) transformation[14]. Related clinical data (age, gender, survival time, survival status, pathological stage and TNM stage) were collected.

Construction and evaluation of a prognostic lncRNA expression signature

Univariate Cox regression analysis was employed to select candidate prognostic lncRNAs that were significantly associated with overall survival with a p-value less than 0.05. All candidate prognostic lncRNAs were subjected to the least absolute shrinkage and selection operator (LASSO) method to screen out independently prognostic lncRNAs implemented with the survival and glmnet

R packages[15]. Then, the lncRNAs with the lowest Akaike information criterion (AIC) values were retained in the step multivariable Cox proportional hazards model implemented with the survival R package. Subsequently, the prognostic lncRNA expression signature was constructed with the retained lncRNAs. For each patient, the risk score was finally calculated by the normalized expression levels of each lncRNA and corresponding coefficient, which were derived from the step multivariable Cox regression analysis. The risk model was established by using the following formula: Risk score = lncRNAexp1*β1 + lncRNAexp2*β2. + ... +lncRNAexpi*βi, where lncRNAexpi indicates the expression value of each lncRNA and βi indicates the regression coefficient of the step multivariate Cox analysis for the target lncRNAi.

Correlation analysis and enrichment analysis

Pearson correlation coefficients were calculated to identify the co-expression relationship between lncRNAs and genes by using the SangerBox software (http://sangerbox.com/). Genes which have the coefficient with an absolute value of >0.5 and p<0.05 were selected. The correlations between lncRNAs and genes were further illustrated by the Cytoscape software version 3.6.1.

Moreover, the selected genes significantly correlated with the lncRNA signature was forwarded to the enrichment analysis which was performed using an online-based web tool "Metascape" (http://metascape.org/). The significance threshold of false discovery rate (FDR) for the significantly enriched biological processes and pathways was set at 0.05.

Drugs prediction for the lncRNAs targets

We used two online LNCmap (http://bio-bigdata.hrbmu.edu.cn/ LncMAP/) and D-lnc (http://www. jianglab.cn/D-lnc/index.JSP) tools to predict potential drugs targeting lncRNAs in the prognostic model[16, 17]. The LNCmap extracted drug-affected lncRNA profiles by reannotating the microarray data from the Connectivity Map database (http://www.broadinstitute.org/cmap/).

Only the potential drugs with a p-value <0.05 were selected. Moreover, D-lnc provides a comprehensive query and analysis to detect the experimentally validated and computational predicted modifications of drug on lncRNA expression based on the Connectivity Map database. After submitting the lncRNA sequence (FASTA) to the tool, potential drugs targeting the sequence or a highly similar sequence to the submitted were automatically proposed. Then, potential drugs from the LNCmap and D-lnc were integrated, and the drug-lncRNA network was illustrated by the Cytoscape software version 3.6.1.

Statistical analysis

All the data analysis and description were conducted with R version 3.4.3. The patients were dichotomized into high-risk and low-risk groups by the median value of risk score. Chi-squared test was used to detect statistical difference for categorical variables with the table1 R-package. Distribution of risk score, survival status of patients with TSCC, and expression profiles of prognostic lncRNAs were exhibited using the ggplot2 R-package.

Kaplan-Meier curve and log-rank method were carried out to assess the overall survival difference between the high-risk and the low-risk groups with survival and survminer packages. Moreover, receiver operator characteristic (ROC) curves were employed to evaluate the prognostic performance of the lncRNA signature by assessing accuracies and specificities. ROC and area under the curve (AUC) were estimated using the timeROC package[18]. Additionally, we performed multivariate Cox regression to determine the independently prognostic value of the lncRNA signature adjusted by some important clinical variables indicated by the univariate regression analyses with a p-value <0.1. A p<0.05 was considered statistically significant for the multivariate Cox regression.

Results

Patient characteristics

A total of 528 sample data for HNSC were initially downloaded by the TCGAbiolinks. Of which, expression profiles along with clinical information of 122 TSCC patients were picked out. The characteristics of all included patient are summarized in Supplementary Table S1.

Identification of prognostic lncRNAs

After filtration, normalization and Log transformation, the gene expression matrix containing 12606 lncRNAs retained for 122 TSCC patients was generated. The prognostic value of each of these lncRNAs were sequentially analyzed by the univariate Cox regression, resulting in 696 candidate lncRNAs. Then, six lncRNAs were identified by the LASSO analysis with the 696 lncRNAs. For the

six lncRNAs, five lncRNAs (ENSG00000236671/PRKG1-AS1, ENSG00000253930/TNFRSF10A-AS1, ENSG00000235085/AC091153.2, ENSG00000232190/LINC02181, ENSG00000281903/LINC02246) were found to be significantly associated with overall survival (OS) in patients with TSCC by applying multivariate Cox regression. Specially, the results of multivariate Cox regression for the five lncRNAs were shown as forest plot in Figure 1.

	Overall (n=122)		Overall (n=122)
Age (year)		**Gender**	
Mean (SD)	57.7 (13.5)	female	43 (35.2%)
Median [Min, Max]	60.0 [19.0, 87.0]	male	79 (64.8%)
Tumor stage		**T stage**	
I	13 (10.7%)	T1	18 (14.8%)
II	22 (18.0%)	T2	46 (37.7%)
III	28 (23.0%)	T3	34 (27.9%)
IV	59 (48.4%)	T4	24 (19.7%)
N stage		**Radiation**	
N0	53 (43.4%)	no	38 (31.1%)
N1	19 (15.6%)	yes	72 (59.0%)
N2-3	50 (41.0%)	unknown	12 (9.8%)
pharmT		**Alcohol**	
no	74 (60.7%)	No	40 (32.8%)
yes	35 (28.7%)	Yes	79 (64.8%)
unknown	13 (10.7%)	unknown	3 (2.5%)
Cigarettes			
no	57 (46.7%)		
yes	65 (53.3%)		

Table S1: Clinical characteristics of TSCC patients. *pharmT, pharmacotherapy.*

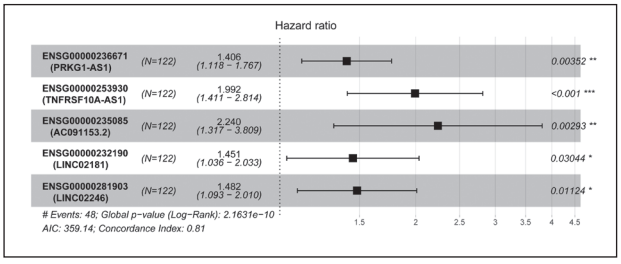

Figure 1: Presentation of risk scores (A), survival status (B) and lncRNA expression pattern (C) in the predicted risk groups by the five-lncRNA signature.

Construction of the prognostic five-lncRNAs signature and risk score

The prognostic signature was constructed by integrating the five lncRNAs expression profiles and corresponding multivariable Cox regression coefficient. As a result, the five-lncRNAs signature model was established, and the risk score was estimated according to the following formula:
risk score=ENSG00000236671*0.3407060+ENSG00000253930*0.6893575+ENSG00000235085*0.8062593+ENSG00000232190*0.3724318+ENSG00000281903*0.3936757.

Based on the five- lncRNAs signature model, the risk score for each patient was calculated.

Survival analysis and evaluation of risk score

To investigate the prognostic value of risk score, the patients were dichotomized into high-risk and low-risk groups by the median value of risk score. Distribution of risk score, survival status of patients with TSCC, and expression profiles of prognostic lncRNAs were shown in Figure 2.

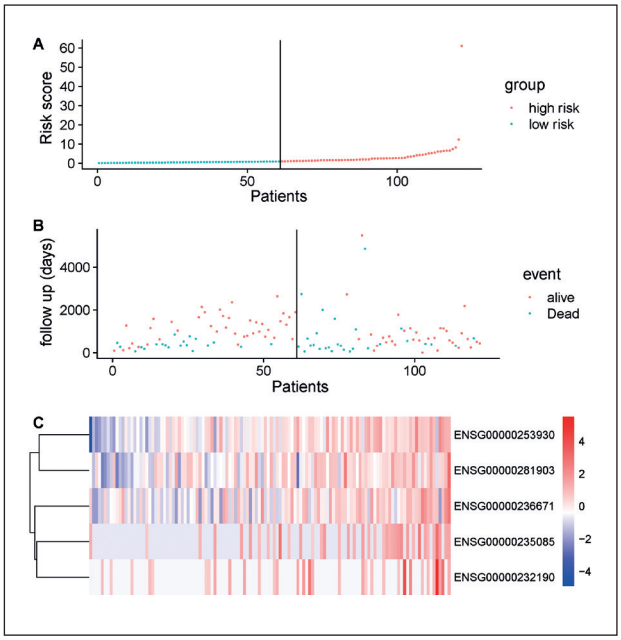

Figure 2: Forest map of the five-lncRNA signature for the survival analysis.

No significant difference regarding clinicopathological data including age, gender, tumor stage, alcohol and cigarettes history was found between high risk and low risk groups (Table 1).

The Kaplan- Meier curve showed that the survival outcome of patients in the high risk group was worse compared to low risk group ($p<0.0001$, Figure 3A). To assess the prognostic performance of the risk score, time dependent ROC analyses were performed for the survival prediction at 1, 3 and 5 years. As shown in Figure 3B, the AUC for 1, 3 and 5 years were 0.856, 0.883 and 0.879 respectively, indicating an excellent and effective performance of our five-lncRNAs signature for predicting survival.

To further determine the independently prognostic value of the risk score, we carried out univariate and multivariate Cox regression analyses using the risk score and other clinicopathological data. The univariate Cox regression analysis showed that risk score and T stage were significantly associated with overall survival (both $p<0.05$), while

the tumor stage and cigarettes history were potentially important factors relevant to prognosis (both p<0.1). In the multivariate Cox regression analysis, the risk score based on the five-lncRNAs signature remained a significant independent predictor of overall survival after adjusting T stage, tumor stage and cigarettes history (Table 2). Additionally, the T stage was also an important independent predictor of the prognosis of TSCC patients.

Variables	High risk (n=61)	Low risk (n=61)	*P*-value
Age (years)			
< 60	33 (54.1%)	24 (39.3%)	0.102
≥ 60	28 (45.9%)	37 (60.7%)	
Gender			
female	23 (37.7%)	20 (32.8%)	0.570
male	38 (62.3%)	41 (67.2%)	
Tumor stage			
I/II	15 (24.6%)	20 (32.8%)	0.317
III/IV	46 (75.4%)	41 (67.2%)	
Alcohol			
no	17 (27.9%)	23 (37.7%)	0.174
yes	44 (72.1%)	35 (57.4%)	
Cigarettes			
no	24 (39.3%)	33 (54.1%)	0.102
yes	37 (60.7%)	28 (45.9%)	

Table 1: Clinical characteristics of TSCC patients in high- and low-risk groups.

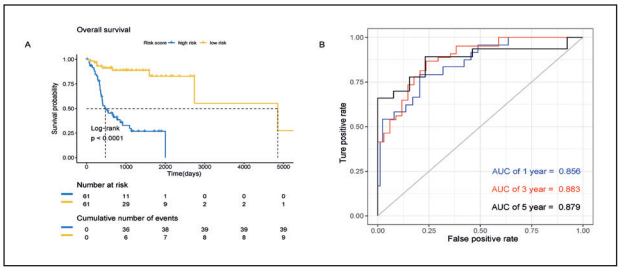

Figure 3: A. Kaplan-Meier analysis for overall survival of patients in the predicted risk groups by the five-lncRNA signature B. Sensitivity and specificity for 1-,3- and 5-year survival prediction by the lncRNA signature.

	Univariate analysis		Multivariate analysis	
Variables	HR(95%CI)	p-value	HR(95%CI)	p-value
Age (≥60 vs <60, year)	0.861 (0.488-1.519)	0.605		
Gender (male vs female)	0.780 (0.436-1.392)	0.400		
pharmT (yes vs no)	1.225 (0.629-2.387)	0.551		
Alcohol (yes vs no)	1.558 (0.806-3.014)	0.188		
Tumor stage (III/IV vs I/II)	1.816 (0.903-3.653)	0.094	0.753 (0.286-1.985)	0.567
T stage (T3-4 vs T1-2)	2.505 (1.368-4.586)	0.003	2.316 (1.008-5.320)	0.048
N stage (N1-3 vs N0)	1.453 (0.803-2.626)	0.217		
Radiation (yes vs no)	0.701 (0.367-1.340)	0.283		
Cigarettes (yes vs no)	1.761 (0.968-3.205)	0.064	1.397 (0.763-2.557)	0.279
Risk score (high vs low)	8.599 (3.791-19.501)	<0.001	7.803 (3.437-17.716)	<0.001

Table 2: Univariate and multivariable Cox regression analyses for overall survival.
pharmT, pharmacotherapy.

Functional enrichment analysis and drug prediction

Functional enrichment analysis of the five-lncRNAs signature was performed using genes significantly correlated with the five lncRNAs. A total of 36 genes were identified with a Pearson coefficient greater than 0.5. The correlations between lncRNAs and genes were illustrated in Figure 4A.

As indicated in Figure 4B, the functional enrichment analysis showed that identified genes were mainly enriched in several biological processes such as cellular response to abiotic stimulus, ncRNA metabolic process, mRNA catabolic process, positive regulation of I-kappaB kinase/ NF-kappaB signaling, negative regulation of defense response, heart morphogenesis and cellular response to organic cyclic compound. For signal pathway enrichment analysis, apoptosis and sphingolipid signaling pathways were the associated pathways. Furthermore, we predicted potential drugs targeting the five lncRNAs with the LNCmap and D-lnc databases, resulting in 19 medications of drug, which was illustrated as a network (Figure 4C). In the network, three medications might be able to target two or more lncRNAs. Particularly, the panobinostat was identified as the hub medication which might target all five lncRNAs.

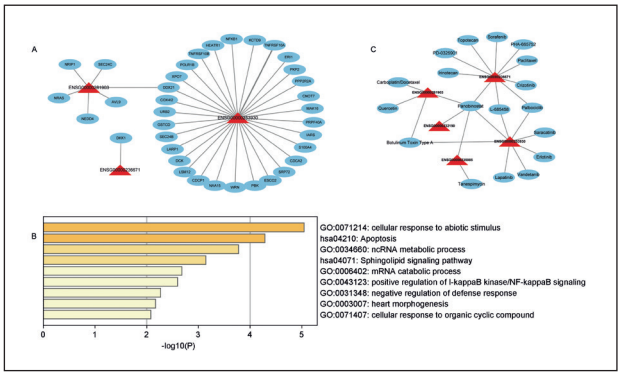

Figure 4: Functional analysis of the five lncRNAs. B. Correlations between lncRNAs and co-expressed genes with an absolute spearman coefficient value of >0.5 and p<0.05. C. The network for the predicted medications potentially targeting a lncRNA.

Discussion

Currently, with the application of high-throughput RNA sequencing and computational techniques, lncRNAs-focus molecular biomarkers were discovered to improve the understanding of the molecular heterogeneity of TSCC and optimize individualized prognosis and treatment[19]. Although the mechanisms for biological behaviors

of most lncRNAs remained under-investigated, their indispensable role was confirmed in the proliferation, invasion, migration, and drug resistance of TSCC, which made them attractive as prognostic biomarkers[10, 20]. With this regard, the study by Yu et al. demonstrated that up-regulation of LINC00152 might serve as a potential biomarker for early detection and prognosis prediction of TSCC[11]. Wu et al. found that overexpression of CASC15 may promote TSCC cell migration and invasion, and predict poor prognosis in TSCC patients[12]. Cheng et al. summarized findings of the published studies and highlighted the predictive role of oncogenic lncRNAs in the development and progression of TSCC by meta-analysis[1]. Nevertheless, the functional and prognostic importance of most lncRNAs remains largely unknown and the single lncRNA may be inefficient to predict the prognosis.

At present, few study has explored systematically the prognostic role of lncRNAs in TSCC patients. Although Zhou et al. performed an integrated analysis of lncRNA-miRNA-mRNA ceRNA network in TSCC using TCGA data and proposed nine potential prognostic lncRNAs, these lncRNAs were only suggested by Kaplan-Meier curve analysis, the independent predictive value was unclear[21]. In the present study, we investigated systematically the prognostic value of lncRNAs at a genome-wide level in a large cohort of patients with TSCC and identified a prognostic five-lncRNA (ENSG00000236671/PRKG1-AS1, ENSG00000253930/TNFRSF10A-AS1, ENSG00000235085/AC091153.2, ENSG00000232190/LINC02181, ENSG00000281903/LINC02246) expression signature for overall survival prediction of TSCC patients. To the best of our knowledge, the current study is the first to systematically evaluate the prognostic significance of lncRNAs in TSCC. The risk score calculated from the expression of five lncRNAs in this signature exhibited excellent performance to separate patients into high-risk and low-risk groups with significantly different OS (AUC= 0.856, 0.883 and 0.879 for 1-, 3- and 5-year OS), suggesting the reliability of the signature in survival prediction of TSCC. To examine the independent prognostic value of the five-lncRNA signature, we performed multivariate Cox regression analyses using the risk score. The five-lncRNA signature was demonstrated to still maintain an independent association with patients' survival after adjustment for several important clinical variables including tumor stage, T stage and cigarettes history. These results indicate that our identified lncRNA signature may be a potential independent predictor to guide patient-tailored treatment in the future clinical trials. Up to date, although there is a rapid increase in the mapping of lncRNA loci, few of them were functionally well-characterized and the biological role of most lncRNAs remains in its infancy[19]. From our literature review, only one of our identified lncRNA signature, PRKG1-AS1 was ever reported for its potential biological mechanism. In line with our results, the study by Wu et al. revealed that PRKG1-AS1 was highly expressed in oral squamous cell carcinoma (OSCC) tissues and high expression of PRKG1-AS1 predicted poor outcomes. Biologically, over-expression of PRKG1-AS1 accelerated cell growth, invasion, and migration and mediated the epithelial- mesenchymal transition (EMT) in OSCC cells, suggesting that PRKG1-AS1 functioned as a facilitator in OSCC by regulating EMT[22]. Previous studies have shown that lncRNAs exert biological functions by regulating or interacting with relevant co-expressed protein-coding genes involved in the same biological processes and pathways[23]. Therefore, we performed in silico analysis for biological process and pathway enrichment to infer potential biological roles of our identified prognostic lncRNAs by correlating a common expression pattern between lncRNAs and protein-coding genes in all TSCC patients.

Functional enrichment analysis revealed that these lncRNAs mainly participated in several biological processes including cellular response to abiotic stimulus, ncRNA metabolic process, mRNA catabolic process, positive regulation of I-kappaB kinase/NF-kappaB signaling, negative regulation of defense response, heart morphogenesis and cellular response to organic cyclic compound, and they also may be mainly implicated in apoptosis and sphingolipid signaling pathways. As indicated by numerous studies, the sphingolipid signaling pathway was deemed important for proliferation, cell cycle regulation, growth and cell death of tumor by modulating the sphingolipid metabolism, mitochondrial outer membrane permeabilization, cell fate and immune responses[24-27].

Furthermore, we also predicted potential medications of drug against the identified lncRNAs by online databases. Nineteen medications were identified, and three of them might be able to target two or more lncRNAs. Of note, panobinostat which potentially targets all five lncRNAs was the most

potential medication for the treatment of TSCC. Indeed, panobinostat is an oral histone deacetylase inhibitor and has acted as an antineoplastic agent in several types of cancers recently. Panobinostat has been approved for use in combination with other agents in refractory or relapsed multiple myeloma by European Medicines Agency[28]. In an open-label, multicentre phase 2 trial, panobinostat in combination with bortezomib therapy was found safe and feasible and showed encouraging activity in patients with relapsed or refractory peripheral T-cell lymphoma[29]. Additionally, a randomized phase-II trial of epigenetic therapy with panobinostat combined with bicalutamide rechallenge demonstrated an increase in radiographic progression-free in castration-resistant prostate cancer resistant to second-line antiandrogen therapy[30]. Collectively, this evidence supports indirectly that panobinostat is potentially a promising agent for the treatment of TSCC. However, the underlying mechanism for our findings and therapeutic value of panobinostat is in need of further validation. Several limitations of the present study should be acknowledged. Firstly, the prognostic expression signature was solely derived from TCGA with limited sample size and constructed based on pure bioinformatics analysis without external validation. Independent cohorts with large sample size are needed to validate the results. Secondly, we were unable to evaluate the impact of some confounders on the survival of TSCC patients, such as histological grade, pharmacotherapy, blood biochemistry, nutritional status, oral hygiene and human papillomavirus status, which may partially affect the robustness of results.

Thirdly, although the performance of the five-lncRNAs was excellent in predicting the prognosis of TSCC, our study is only descriptive and the exact molecular mechanisms to support their prognostic value of identified lncRNAs in TSCCC remains unclear. Future functional researches are required to clarify their biological roles.

Conclusions

In conclusion, we constructed a novel five-lncRNA expression signature (ENSG00000236671/PRKG1-AS1, ENSG00000253930/TNFRSF10A-AS1, ENSG00000235085/ AC091153.2, ENSG00000232190/LINC02181, ENSG00000281903/LINC02246) as independent predictor of survival in patients with TSCC. Further enrichment analyses revealed that the identified lncRNAs were mainly involved in apoptosis and sphingolipid signaling pathways. Drug prediction implied that panobinostat may be a promising agent for the treatment of TSCC.

These findings may facilitate the personalized management and treatment of patients with TSCC. However, the exact molecular mechanisms to support these findings are warranted to be clarified and validated in future studies.

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Ethical Statement:
All data used in the study originated from the TCGA program authorized to the public for research purposes. No ethical permission was needed.

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