Prognostic Value of Immune Associated Long Non-Coding RNAs In Laryngeal Squamous Cell Carcinoma

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Primary research

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Abstract

Background: Increasing studies have demonstrated that immune associated lncRNAs (IALs) take an important part in the occurrence and development of multiple cancers. However, the prognosis value of IALs in laryngeal squamous cell carcinoma (LSCC) remains unexplored. This study aimed to evaluate the importance of IALs in LSCC.

Methods: RNA sequencing data profiles of LSCC and clinical information of patients were obtained from TCGA dataset. Correlation analysis was performed to screen IALs. Then, a IALs based prognostic signature was constructed through univariate and multivariate Cox regression. The uncover molecular mechanisms of these selected IALs were explored by the bioinformatics analyses.

Results: a total of seven differentially expressed survival-associated IALs were found in LSCC patients. a six IALs (LINC02154, SNHG12, CHKB-DT, AL158166.1, AC027307.2 and AL121899.1) based prognostic signature was established, which was a reliable tool to predict the prognosis of LSCC. The area under the curve (AUC) were 0.817 (one-year), 0.847 (three-year) and 0.895 (five-year). Further analysis, there were different infiltration of immune cells between low-risk and high-risk group patients. Additionally, a lncRNA-miRNA-mRNA regulatory network based on six IALs, 75 miRNAs, and 156 differentially expressed mRNAs was constructed.

Conclusions: IALs may play critical role in the occurrence and progression of LSCC, and the IALs based prognostic signature can predict the overall survival rate of LSCC.

Background

Laryngeal squamous cell carcinoma (LSCC) is a common malignant tumor in the world, and approximately 100,000 cases deaths from LSCC in 2020 [1]. In particular, more than 50% of LSCC cases are diagnosed at advanced stage [2]. Although the treatment of advanced LSCC continue to improve, the overall prognosis remains poor [3]. At present, clinical decision-making and prediction of treatment outcomes for LSCC patients mainly rely on the tumor-node-metastasis (TNM) stage system [4]. However, similar treatment outcomes can vary greatly for patients at the same TNM stage. Therefore, there is an urgent need for the identification of novel diagnostic and prognostic biomarkers to improve LSCC patient prognosis.

Long non-coding RNAs (lncRNAs) are a group of longer than 200 nucleotides RNA molecules with limited protein-coding ability [5]. lncRNA play crucial roles in diverse cellular processes, including proliferation, apoptosis and differentiation [6]. Moreover, lncRNA have immune response, oncogenic and tumor suppressor functions [7]. Previous research has suggested that lncRNA MIR31HG functions as a carcinogenic lncRNA and facilitates tumor progression in HNSCC [8]. In addition, prognostic signature based on immune associated lncRNA (IALS) has been evaluated in many cancers, including breast cancer, pancreatic cancer and Head and neck cancer (HNC) [9–11]. Nevertheless, the prognostic value of IALs in LSCC has not been well known until now.

In this study, we aimed to evaluate the prognostic value of IALs expression in LSCC and try to develop individualized prognostic signature. This study could provide valuable clinical utility for LSCC prognostic prediction.

Methods

Data collection

lncRNA and mRNA expression and clinical profiles of LSCC samples from the Cancer Genome Atlas (TCGA) database (<https://tcga-data.nci.nih.gov/tcga/>), including LSCC (n=110) and normal samples (n=12). Patients with survival time less than 30 days were omitted. Then, the immune associated genes (IRGs) list was obtained via the Immunology Database and Analysis Portal (ImmPort) database [12], and IRGs list was used to screen immune associated lncRNAs (IALs) by a co-expression strategy.

Identification of differentially expressed genes and lncRNAs

“limma” package in R software was used to screen the differentially expressed genes and lncRNAs, which were screened using the threshold of a log |fold change| > 1 and an adjusted *P*-value < 0.05. Correlation analysis was performed between IRGs and differentially expressed lncRNAs. Those with immune gene correlation coefficients greater than 0.4 and p value less than 0.001 were considered as IALs. The “gplots” package in R software were used to draw volcano maps.

Immune associated lncRNA based prognostic signature (IALPS)

The survival data of included patients were downloaded from TCGA database. Then, univariate cox regression was used to select survival-associated IALs (SAIALs) through the R software “survival” package and *P* < 0.01 was considered significant. SAIALs were submitted for multivariate Cox regression analysis. The IALPS was constructed based on expression data multiplied by the Cox regression coefficient.

Validation of the Constructed Risk Signature

The LSCC patients were divided into high and low risk groups based on the median risk score. Then, the Kaplan–Meier method with log-rank test was used to establish survival curve of the two groups. The one-year, three-year, and five-year survival receiver operating characteristic (ROC) curves predicted by the IALPS were drawn. The area under the curve (AUC) of the ROC curve was calculated to validate the performance of IALPS. Then, the correlation between the expression of the selected immune-associated lncRNAs and clinicopathological characteristics were analyzed using student's t-test.

Gene Set Enrichment Analysis

The Gene set enrichment analysis (GSEA 4.0.1) was performed to identify different functional enrichment of the low-risk and high-risk groups. Firstly, the mRNA expression profiles of LSCC from the TCGA dataset

were divided into the low-risk and high-risk groups, which were performed on “IMMUNE RESPONSE” and “IMMUNE SYSTEM PROCESS” gene sets.

Evaluation of Infiltrating Immune Cells

The CIBERSORT algorithm, coupled with 22 immune cell types, was applied to analysis the differences in the abundance of infiltrating immune cells of between the low-risk and high-risk groups. Student's t-test was used to compare significant immune cell types in between the low-risk and high-risk groups. to chart violin plot by boxplot in the R package.

Construction of the lncRNA–miRNA–mRNA Regulatory Network

The DIANA-LncBase v2 tool was used to explore the target miRNAs of lncRNA [13]. Three miRNA databases, including miRDB [14], miRTarBase [15], and TargetScan (http://www.targetscan.org/vert_71/) were used to predict the target mRNAs of miRNAs. The threshold of the expression correlation between lncRNAs and miRNAs was set at 0.85. Then, the lncRNA-miRNA-mRNA regulatory network was visualized using Cytoscape (version 3.6.1).

Statistical analysis

R (version 4.1.0), Perl (version 5.26.3) and software were used for all data analyses. Both univariable and multivariable Cox regression analyses were generated by the R package ‘survival’. The ROC curve was performed using the R package ‘survivalROC’. $P < 0.05$ was considered statistically significant.

Results

Identification of differentially expressed gene

A total of 110 LSCC and 12 non-tumor patients were included in this study. The detailed clinical data of included patients were summarized in **Table S1**. A total of 5504 differentially expressed genes (DEGs) were identified, including 4632 up-regulated and 872 down-regulated. Of these, there were 1016 up-regulated and 105 down-regulated lncRNAs (**Figure 1**).

Construction Immune-Associated lncRNAs signature

Seven lncRNAs were significantly associated with OS by univariate analyses, and two were risk factors and five were protective factors (**Table 1**). We constructed a prognostic signature according to the results of multivariate Cox regression analysis(**Table 2**). LSCC patients were divided into low-risk and high-risk groups with different clinical outcomes of OS (**Figure 2**). The formula was as follows: $[LINC02154 \text{ Expression level} * (0.646447)] + [SNHG12 \text{ Expression level} * (-0.712531)] + [CHKB-DT \text{ Expression level} * (1.261561)] + [AL158166.1 \text{ Expression level} * (0.746432)] + [AC027307.2 \text{ Expression level} * (-0.815512)] + [AL121899.1 \text{ Expression level} * (0.879183)]$.

Validation of the Constructed Immune-Associated lncRNA Signature

According to discrete clinical outcomes, the IALPS may be a significant tool for differentiating LSCC patients (**Figure 3a**). The area under the curve (AUC) are 0.817 (one-year), 0.847 (three-year) and 0.895 (five-year), suggesting that IALs-based signature was accurate in survival predicting (**Figure 3b**). After adjusting for age, gender, tumor grade, tumor stage and other parameters, multivariate cox regression analysis indicated that IALPS can be an independent predictor (**Figure 3c, 3d**). Further analysis, CHKB-DT and AL121899.1 were significantly related to tumor grade, and LINC02154 and AL158166.1 were correlated with N stage (**Table 3**).

GSEA analysis and evaluation of infiltrating Immune Cells

GSEA analysis of the two risk groups were performed using the GSEA software. The results revealed that immune-associated responses were further enriched in low-risk groups compared to high-risk groups (**Figure 4a, b**). Naïve B cell, CD8 T cell and T cells follicular helper of LSCC patients in the low-risk group were significantly higher than that in the high-risk group, while macrophages and dendritic cells were highly infiltrated in high-risk group (**Figure 4c**). These results indicate that patients with high and low risk scores may be in different immune states.

The lncRNA-miRNA-mRNA Regulatory Network

The lncRNA-miRNA-mRNA regulatory network has been extensively studied, showing that lncRNAs competitively sponge miRNAs at the post-transcriptional level, thus leading to depression of targeted genes. To explore the regulation of IALPS based on six lncRNAs, we constructed a regulatory network consisting of six lncRNAs, 75 miRNAs, and 156 differentially expressed mRNAs (**Figure 5**).

Discussion

LSCC is one of the most common cancers of the upper respiratory with high incidence and low 5-year survival rate [16]. The occurrence and development of LSCC is a complicated process controlled involving multiple genetic and epigenetic changes [17]. Among these genes, lncRNA has a crucial role in LSCC progression. For instance, lncRNA FOXD2-AS1 was shown to enhance chemotherapy resistance in LSCC by activating STAT3 [18], and lncRNA LINC00152 can promote laryngeal cancer progression by sponging MiR-613 [19]. However, due to the limited number of screened IALs, many potential and valuable IALs still need to be identified to improve the predictive accuracy for LSCC patients. This study comprehensively analyzed the IALs expression, found potential target genes and established six IALs (LINC02154, SNHG12, CHKB-DT, AL158166.1, AC027307.2 and AL121899.1) based prognostic signature. This

prognostic signature could be used to evaluate the immune status and predict LSCC patients' clinical outcomes. These IALs may serve as potential clinical biomarkers.

Small Nucleolar RNA Host Gene 12 (SNHG12) functions as an oncogene in many cancers including renal cell carcinoma [20], oral squamous cell carcinoma [21]. SNHG12 also acts as a competitive endogenous RNA (ceRNA) to regulate their downstream targets by sponging those miRNAs. For example, SNHG12 has been reported to interact with multiple miRNAs including miR-326[21] and miR-129-5p [22, 23]. Therefore, SNHG12 might also be implicated in affecting the tumorigenesis and progression in LSCC through a similar mechanism. Additionally, SNHG12 can promote LSCC cells progression via binding miR-129-5p, which targets WWP1 [23]. In accordance with our results, SNHG12 was a high-risk immune-associated lncRNA, both with prognostic value in LSCC patients. AC027307.2 was first reported in colon adenocarcinoma, and it was significantly associated with stage and OS in colon adenocarcinoma [24]. Further analysis, LINC02154 and AL158166.1 were correlated with N stage, and CHKB-DT and AL121899.1 level were significantly associated to tumor grade. Studies have shown that LINC02154 is significantly upregulated in renal cell carcinoma and its high expression is one of the risk factors for poor prognosis [25]. In addition, CHKB-DT was a tumor grade related lncRNA for Esophagus Squamous Cell Carcinoma [26]. Moreover, AL121899.1 was a tumor suppressor lncRNA in esophageal squamous cell [27]. For one remaining immune-associated lncRNA (AL158166.1), there were no studies to report their prognostic roles in cancer. Thus, more the functional experiments should be conducted to explore how these lncRNAs affect the prognosis of patients with LSCC through immune exactly.

The imbalance of the immune response in the tumor microenvironment plays crucial role in cancer tumorigenesis, progression and response to therapy. This study revealed that Naïve B cell, CD8 T cell and T follicular helper (Tfh) cells were significantly higher in low-risk group, while macrophages and dendritic cells were highly infiltrated in high-risk group. It suggested that, in low-risk patients, the infiltration level of B cell and T cell may be increased and immune cells are important for LSCC. High density CD8 + T cells were associated with a favorable prognosis of LSCC [28, 29]. Tfh cells are a subset of CD4 + T cells, have been described as helping B cells in the germinal center [30]. Previous studies have considered the significant role of dysfunctional infiltrating Tfh cells in multiple types of cancers, including breast cancer [31], lung cancer [32] and ovarian cancer [33]. In contrast to T cells, the role of B cells in tumor immunity and progression is controversial [34]. B cells have been shown to suppress T cells and increase tumor growth in pancreas cancer [35]. On the other hand, B cells were associated with better overall survival in HPV-associated HNSCC [36]. Tumor-associated macrophages (TAM), known as multifunctional antigen-presenting cells, can promote cancer immunosuppression [37]. In addition, TAMs can be used to predict metastasis and prognosis in laryngeal carcinoma [38].

Taken together, this study confirmed the IALs have prognostic value in LSCC and could reflect the infiltration of immune cells. Although the gold standard used to guide tumor management is the TNM staging system, but it does not take into account genetic characteristics. Combining the TNM staging system and analysis of IALs may thus represent a more accurate method for the identification of patients with high-risk NPC, which will help treatment decision-making and improve the survival rate.

Several limitations existed in this study. First, these results need to be verified through prospective studies. Second, other researches may draw different results due to different experimental variations and statistical methods. Third, the functional experiments should be conducted to further indicate the potential molecular mechanisms for predicting the effect of immune-associated lncRNAs. Beyond these limitations, we hope this study could inspire scientists on LSCC immunotherapy.

Conclusions

A novel prognostic signature based on IALS (LINC02154, SNHG12, CHKB-DT, AL158166.1, AC027307.2 and AL121899.1) was identified as an effective way to predict the prognosis of LSCC.

Abbreviations

LSCC: laryngeal squamous cell carcinoma, IALS: immune associated lncRNAs, AUC: Area under the curve, SROC: summary receiver operating characteristic, OS: overall survival.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

No identifying patient details are contained within this manuscript.

Availability of data and material

The datasets supporting the current study are available from the TCGA.

Competing interests

The authors declare that they have no conflict of interests.

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Authors' contributions

(i) Conception and design: Weixing Liu, Pei Li, Yue Liu, Jin Ye, (ii) Administrative support: Jin Ye, (iii) Provision of study materials or patients: Weixing Liu, Pei Li, Yue Liu, (iv) Collection and assembly of data: Weixing Liu, Pei Li, Zhiyuan Wang (v) Data analysis and interpretation: Weixing Liu, Pei Li, Yue Liu,

Zhiyuan Wang, Jiamin Liu, (vi) Manuscript writing: All authors, (vii) Final approval of manuscript: All authors.

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Tables

Table 1. General characteristics of LSCC survival-associated IALs.

Gene	LogFC	FDR	HR	P-value
LINC02154	6.52838	1.36E-05	1.8838	0.0002
SNHG12	1.55000	0.00187	0.5469	0.0049
LINC01063	2.35923	0.00035	2.1622	0.0050
CHKB-DT	1.21624	0.00034	2.4844	0.0048
AL158166.1	3.60835	3.08E-05	2.0092	0.0014
AC027307.2	1.14922	7.42E-05	0.4375	0.0087
AL121899.1	-1.39362	0.00121	2.2739	0.0030

Abbreviations: HR, hazard ratio.

Table 2. Multivariate Cox regression analysis results.

IncRNA	coef	HR	HR.95L	HR.95H	pvalue
LINC02154	0.646447	1.908747	1.320517	2.759005	0.000584
SNHG12	-0.712531	0.490401	0.322784	0.745059	0.000841
CHKB-DT	1.261561	3.530927	1.845915	6.754075	0.000138
AL158166.1	0.746432	2.109461	1.218646	3.651450	0.007669
AC027307.2	-0.815512	0.442413	0.225209	0.869099	0.017923
AL121899.1	0.879183	2.408931	1.298192	4.470020	0.005314

Table 3. Relationships between the expressions of the IALs and the clinicopathological factors in LSCC.

lncRNA	Age (≥ 60 / <60)		Grade (3-4/1-2)		Pathological stage (IV-III/ I-II)		T stage (T3-T4/ T1- T2)		N stage (N1-3/ N0)	
	t	p	t	p	t	p	t	p	t	p
LINC02154	-0.78	0.43	0.94	0.35	0.87	0.39	0.09	0.93	2.48	0.02
SNHG12	-1.28	0.20	-1.68	0.10	-0.51	0.61	0.67	0.51	0.05	0.96
CHKB-DT	-0.14	0.89	3.27	<0.01	-0.34	0.73	-0.62	0.53	0.95	0.34
AL158166.1	0.23	0.82	0.96	0.34	-0.20	0.84	1.33	0.20	-2.49	0.02
AC027307.2	-1.19	0.24	-0.81	0.42	0.41	0.68	0.54	0.59	-0.52	0.61
AL121899.1	-1.18	0.24	4.47	<0.01	0.17	0.86	0.29	0.77	-0.61	0.54

Figures

Figure 1

Differentially expressed lncRNAs. Heatmap (a) and volcano plot (b) demonstrating differentially expressed lncRNAs between LSCC and non-tumor tissues. TCGA database included 110 LSCC(T) and 12 non-tumor tissues(N). The rows represent differentially expressed lncRNAs, whereas the columns represent samples. Red dots represent signifies highly expressed lncRNAs, blue dots represent signifies low expressed lncRNAs and black dots represent no differentially expressed lncRNAs.

Figure 2

Development of the prognostic signature based on immune-associated lncRNAs. (a) Heatmap of expression profiles of included genes. The rows represent differentially expressed included genes, whereas the columns represent high risk and low risk group. (b) Rank of prognostic index and distribution of groups. (c) Survival status of patients in different groups.

Figure 3

The prognostic value of prognostic signature in LSCC. (a) KM survival curves of high and low risk group in laryngeal cancer. P value was calculated using the log-rank test. Patient in high-risk group suffered shorter progression-free intervals ($P < 0.01$). (b) Survival-dependent receiver operating characteristic (ROC) curve validation of prognostic value of the prognostic index. (c) Univariate regression analysis of LSCC. (d) multiple regression analysis of LSCC. Blue represent Hazard ratio < 1 and red represent Hazard ratio ≥ 1 .

Figure 4

GSEA analysis and the different abundance of infiltrating immune cells of between the low-risk and high-risk groups. (a) GSEA analysis on “IMMUNE RESPONSE”, (b) GSEA analysis on “IMMUNE SYSTEM PROCESS” gene sets, (c) The different abundance of infiltrating immune cells.