Construction of a 10-gene prognostic score model of predicting recurrence for laryngeal cancer

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Abstract
We constructed a prognostic score (PS) model to predict the recurrence risk in patients previously diagnosed with laryngeal cancer (LC). Here the training dataset, consisting of 82 LC samples, was downloaded from The Cancer Genome Atlas (TCGA). The PS model then divided the LC samples into high- and low-risk groups, which predicted well the survival time of LC in three datasets (TCGA dataset: AUC = 0.899; GSE27020: AUC = 0.719; and GSE25727: AUC = 0.662). Therefore, the PS model based on the 10 genes and its nomogram is proposed to help predict the recurrence risk in patients with LC.

Keywords: Laryngeal cancer, Recurrence, Prognosis

Introduction
Laryngeal cancer (LC) has been identified as the one of the most common types of head and neck cancers, which resulted in approximately 11,150 new cases in the United States in 2018 [1]. During the past decades, various treatment strategies have been devised for treating LC. However, the 5-year overall survival (OS) of patients with LC remains unsatisfactory [2]. According to the SEER database from 2006 to 2012, the 5-year OS of LC remained as low as 60.7%, which has not increased significantly in the last few decades [3]. Furthermore, the local recurrence of LC is common among patients, such as those with moderately or poorly differentiated squamous cell carcinoma, in addition to the thyroid cartilage plate invasion. Hence, comprehensive treatment and closer follow-up should be given to these patients [4]. Nevertheless, the identification of novel prognostic gene markers that can help distinguish the recurrence risk in patients with LC is vital for improving the OS of patients with LC.

In recent decades, the occurrence of next-sequencing technologies has made rapid disease and recurrence detection possible. Notably, existing evidence has indicated that many gene biomarkers have predictive values for LC. Likewise, Zhang et al. [5] indicated that five genes (EMP1, HOXB9, DPY19L2P1, MMP1, and KLHDC7B) had the potential function to predict LC recurrence. Cury et al. [6] also argued that DSG2 overexpression was associated with shorter OS. And, it is also indicated that high plasma protein levels of DSG2 indicated its detection in liquid biopsy, which is proposed to be applied as a recurring biomarker for LC. Pedro et al. [7] have also reported that ALCAM overexpression was an independent biomarker for predicting recurrence of laryngeal squamous cell carcinoma in patients. Nevertheless, although previous studies have identified numerous gene targets that account for the LC recurrence, further investigations are needed to explore the effect of these featured genes on the recurrence risk in patients with LC.

Therefore, according to the multiple bioinformatics data, we screened the genes significantly correlated with
recurring LC using meta-analysis and L1-penalized optimization algorithm. Then, we constructed the risk model for predicting recurrence risk in patients with LC.

**Method**

**Data source**

The mRNA sequencing data of head and neck samples (including 604 samples) were obtained from The Cancer Genome Atlas (TCGA) database (https://tcga-data.nci.nih.gov/docs/publications/tcga/) based on the Illumina HiSeq 2000 RNA Sequencing platform. The positions of the 604 samples were in the alveolar ridges (n = 18), tongue roots (n = 30), buccal mucosa (n = 22), floor of the mouth (n = 67), hard palates (n = 8), hypopharynx (n = 9), larynx (n = 138), lips (n = 3), mouth (n = 38), tongue (n = 158), oropharynx (n = 10), and tonsils (n = 45). The rest of the samples were from uncertain tumor locations. Among the 138 throat samples, we screened 82 LC samples with recurrence and prognosis information (28 and 54 samples with and without recurrence, respectively) in our study.

Additionally, we searched for validation dataset using the keyword “larynx cancer” from the National Center for Biotechnology Information Gene Expression Omnibus database (http://www.ncbi.nlm.nih.gov/geo/). The screening standards were as follows: (1) gene expression profile data, (2) the samples were from the tumor tissue specimen of patients, (3) human expression profile data, and (4) the samples with information of recurrent or non-recurrent prognosis. Two validation datasets were obtained. One was GSE27020 that composed of 109 LC tissue samples (34 and 75 samples with and without recurrence, respectively) based on the Affymetrix Human Genome U133A, the Array platform, and the other one was GSE25727 that included 56 LC tissue samples (17 and 39 samples with and without recurrence, respectively) based on the Illumina HumanRef-8 WG-DASlv3.0 platform.

**Screening of differentially expressed genes**

A meta-analysis on TCGA dataset, GSE27020 and GSE25727, was conducted using an ES function of MetaDE [8] (version: 1.0.5, https://cran.r-project.org/web/packages/MetaDE/) in R3.4.1 to screen the differentially expressed genes (DEGs) [9]. Subsequently, we screened for DEGs [9] that showed consistent expression in these two datasets between samples with recurrence and those without recurrence by calculating the tau², Q, and Qpval values (criterion for judgment; tau² = 0 indicates that each research object is homogeneous and unbiased; the statistic Q obeys the Chi-square test with a degree of freedom of k-1, whereas Qpval > 0.05 indicates that each research object is homogeneous and unbiased). Then, the false discovery rate (FDR) value was obtained using multiple test corrections. FDR < 0.05 showed that the difference was significant. Additionally, each dataset was calculated to express the fold change, after which several parameters were selected, and the threshold value was set. The set parameters were as follows: (1) To ensure that the source of each selected characteristic gene was homogeneous and unbiased (that the expression of each featured gene in each data set was consistent), Tau² = 0 and Qpval > 0.05 were selected as homogeneity test parameters. (2) FDR < 0.05 was selected as the significant threshold of expression difference between the genomes. (3) After screening with log2 FC, the genes having similar direction of difference (with the same symbol of log2 FC) were retained. After combining multiple screening parameters, we set the selection of threshold parameters:

I. We ensure that the source of each selected characteristic gene is homogeneous and unbiased, that is, the expression in each data set is consistent, so tau² = 0 and Qpval > 0.05 are selected as homogeneity test parameters;

II. FDR < 0.05 was considered as the threshold of significant difference in expression between gene groups;

III. Combined with Log2FC for screening, we retained genes with the same direction of difference (consistent Log2FC symbols) in the three datasets.

The threshold was set to a false discovery rate < 0.05. Then, the Gene Ontology Biology Process (GO-BP) annotation analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were conducted for these DEGs with consistency.

**Establishment and verification of a risk assessment model**

On the basis of the DEGs, we conducted univariate Cox regression analysis in the survival package [10] (version 2.4, http://bioconductor.org/packages/survival/) to screen DEGs significantly related to the prognosis in TCGA dataset. The multivariate Cox regression analysis was then used to screen DEGs that can be used as independent prognostic factors. The log-rank P < 0.05 was also regarded as the threshold of significant correlation.

Furthermore, the Cox proportional hazard model [11] based on the L1-penalized (Lasso) in the penalized package (version 0.950; http://bioconductor.org/packages/penalized/) [12] of the R3.4.1 language was used to screen out the optimized prognostic-associated signature DEG combinations based on the aforementioned DEGs related to the prognosis [13]. Then, on the basis of the prognostic coefficient of prognosis-related DEGs, the prognostic score (PS) prediction model was established in the training dataset using the following formula:
ExpDEGs, time \[17\]. It was calculated using the survcomp package constructed using the RMS software package (version sis, the nomogram with 3- and 5-year survival rates was \( < 0.05 \). Next, to further explore the \( P \) set to log-rank independent prognostic clinical factors. The threshold was used to measure the association between the risk model and prognosis. Simultaneously, we screened these optimized DEGs from the validation dataset (GSE25727 and GSE27020). Then, the PS score of each sample was obtained using the PS calculation method. The validation dataset samples were also separated into high- and low-risk sample groups in the same manner as in TCGA dataset samples. Thereafter, the KM curve method of the survival package (version 2.41–1) \[10\] in the R3.4.1 language was used to evaluate the relationship between the high- and low-risk groups, compared with the actual survival prognosis information from the validation dataset samples.

**Screening of independent prognostic clinical factors for performance evaluation**

Combining the clinical factors including recurrence, age, gender, pathologic (M, N, and T), pathologic stage, grade, alcohol history, angiolymphatic invasion, and perineural invasion in TCGA (Additional file 1: Table S1), we used univariate and multivariate Cox regression analysis methods in the R3.4.1 language survival package (version 2.41–1) \[10\] to screen the independent prognostic clinical factors. The threshold was set to log-rank \( P<0.05 \). Next, to further explore the correlation between independent factors and prognosis, the nomogram with 3- and 5-year survival rates was constructed using the RMS software package (version 5.1.2; https://cran.r-project.org/web/packages/rms/index.html) in R3.4.1 \[9, 15\].

Next, the PS and risk models were compared using the area under the receiver-operating characteristic curve (AUROC) \[14\] and the concordance index (C-index). Additionally, the AUROC is a quantitative indicator of the receiver-operating characteristic (ROC) curve, which was calculated using the pROC in the R3.4.1 language (version 1.14.0, https://cran.r-project.org/web/packages/pROC/index.html). In contrast, the C-index is referred to as the scores of all individual pairs correctly sorted on the basis of the Harrell C statistics \[16\] to predict the survival time \[17\]. It was calculated using the survcomp package (http://www.bioconductor.org/packages/release/bioc/html/survcomp.html) in the R3.4.1 language.

**Results**

**Identification of DEGs**

A total of 981 DEGs were detected among TCGA datasets, GSE25727 and GSE27020, which contained 347 down-regulated genes and 634 up-regulated genes (Fig. 1 and Additional file 2: Table S2). The DEGs were significantly different among the various types of samples from the three datasets. This result indicated that the DEGs expressed significant difference among the three datasets.

The GO results suggested that these genes are involved in 41 GO-BP terms, such as regulation of cell migration \( (P=2.26E−04) \) and regulation of locomotion \( (P=2.48E−04) \). Simultaneously, these DEGs were enriched in 10 KEGG pathways, including the Jak–STAT signaling pathway \( (P=9.44E−03) \) (Fig. 2 and Table 1).

**Constructing the prognosis prediction model**

A total of 206 prognosis-related DEGs were screened using univariate Cox regression analysis with a threshold of \( P<0.05 \) (Additional file 3: Table S3). On the basis of the aforementioned DEGs, we obtained 96 DEGs via the multivariate Cox regression analysis. Subsequently, 10 optimized DEGs \( (CD38, ZNF212, POR, CC2D1A, GRAMD4, FH, SLC24A3, GATA2, FOXD1, \text{and} \ MMP10) \) were selected using the L1-penalized algorithm (Table 2).

**Evaluation and comparison of the prognostic risk prediction model’s effectiveness**

As shown in Fig. 3, the PS value based on the 10 optimized DEGs could distinctly divide 82 patients with LC into high- and low-risk groups in TCGA training dataset, which indicated that the patients in the high-risk group were related to shorter OS in TCGA dataset \( (P=3.853e−12) \). Meanwhile, we obtained the similar results from the validation datasets, which included GSE27020 \( (P=4.259e−06) \) and GSE25727 \( (P=0.0045) \).

Furthermore, the ROC curves based on the PS prediction model indicated that this PS model accurately predicted the patient survival time in both TCGA dataset \( (AUC=0.899) \) and validation dataset \( (AUC=0.719; \text{GSE25727:} \ AUC=0.662) \).

**Screening of independent prognostic clinical factors**

As expressed in Table 3, the PS model was substantially correlated with the LC clinical condition, which was an independent prognostic parameter. Subsequently, the PS model status was included in the nomogram model to
predict the 3- and 5-year OS in patients with LC. After that, the score of each index was observed on the point table at the upper apex of the nomogram. Next, the scores of each index were added to estimate the 3- and 5-year survival probability (Fig. 4). These results indicated that the nomogram on the basis of the PS model status had high prediction accuracy for the survival and prognosis of patients with LC.

Discussion
As shown by the previous reports, it is important to detect several crucial gene biology markers associated with the LC survival prognosis, as this could provide a vital theoretical reference for treating patients with LC. Therefore, in our study, a PS model was established on the basis of 10 independent prognostic genes (CD38, ZNF212, POR, CC2D1A, GRAMD4, FH, SLC24A3, ...
Fig. 2 GO-BPs (A) and KEGG pathways (B) involved in DEGs
Multi-variate Cox regression analysis LASSO coefficient showed that GATA2 was determined to be an independent recurrence risk factor for LC recurrence, helping in the clinical decision-making. In our study, among the 206 DEGs related to LC recurrence, 96 independent prognosis-related DEGs were screened using multivariate Cox regression analysis. We identified 10 metabolic genes associated with prognosis and were further revealed by LASSO-based Cox proportional hazard model analysis to construct the RS survival prediction model, including CD38, GATA2, POR, ZNF212, CC2D1A, GRAMD4, FH, SLC24A3, MIMP10. The KM curves showed that the patients with LC in the low-risk group had remarkably better survival than the low-risk group for TCGA dataset (P = 3.853e−12). Meanwhile, we observed the similar findings in the validation datasets including GSE27020 (P = 4.259e−06) and GSE25727 (P = 0.045). A study from Xiang et al. [18] showed a PS model was constructed to predict the recurrence in patients with LC. The PS value demonstrated good accuracy in predicting the relapse with an AUC of 0.859 was at 1 year, 0.822 at 3 years, and 0.815 at 5 years survival predictive accuracy. Besides, Zhang et al. [19] constructed a four-gene signature that could be used to predict the prognosis of patients with LC. It is hypothesized that the four-gene model might affect the prognosis of patients with LC via mechanisms involved in the immune response and negative regulation of the Wnt signaling pathway. Moreover, Fan

### Table 1 GO biological process and KEGG pathway significantly related to target genes

| Category           | Term                                           | Count | P value | Genes                  |
|--------------------|------------------------------------------------|-------|---------|------------------------|
| Biology Process    | GO:0030334 – regulation of cell migration     | 24    | 2.26E−04| DLC1, PARD6B, IRS2, FLT1 |
|                    | GO:0040101 – regulation of locomotion         | 26    | 2.48E−04| DLC1, PDGFB, ENPP2, TAC1, |
|                    | GO:0015015 – skeletal system development      | 36    | 4.90E−04| TF1, GNA1, HEXA, HOXD12 |
|                    | GO:0051270 – regulation of cell motion        | 25    | 6.45E−04| SORT1, PDGFB, PBX1, IGBP3 |
|                    | GO:0030434 – regulation of organelle organization | 27   | 6.92E−04| DLC1, SHROOM2, CAPZ1, TAC1 |
|                    | GO:0007242 – intracellular signaling cascade  | 103   | 1.24E−03| RAB9A, ADCY7, FLEKH1M, GNA11 |
|                    | GO:0030029 – actin filament-based process      | 28    | 1.53E−03| CHEK1, ARFS, TS35TS, P2RY1 |
|                    | GO:0007517 – muscle organ development         | 25    | 2.22E−03| NMUR1, RHOF, CHUK, RAP2B |
|                    | GO:0030036 – actin cytoskeleton organization   | 26    | 2.66E−03| GDI1, FLTM, MCF2, IGF1, |
|                    | GO:0045184 – establishment of protein localization | 66   | 3.89E−03| RAB9A, APOBEC1, AP1G1, SLC1A5A2 |
| KEGG pathway       | hsa01040: Biosynthesis of unsaturated fatty acids | 6     | 1.49E−02| BAAT, ELOVL5, HSD17B12, ELOVL2, |
|                    | hsa04800: Glutathione metabolism              | 9     | 1.95E−02| GGT5, GASTA4, G6PD, RRM2 |
|                    | hsa04960: Aldosterone-regulated sodium reabsorption | 8   | 2.03E−02| MAPK1, IRS2, MAPK3, IGF1, |
|                    | hsa00670: One carbon pool by folate           | 5     | 2.07E−02| MTHFD2, MTHFR, SHMT2, MTR |
|                    | hsa04310: TGF-beta signaling pathway          | 12    | 3.51E−02| INHHB, MAPK1, SP1, ROCK2 |
|                    | hsa00510: N-Glycan biosynthesis               | 8     | 3.60E−02| MAN2A1, B4GALT3, GANAB, MAN1B1 |
|                    | hsa04314: Cell adhesion molecules (CAMs)      | 16    | 3.65E−02| CLDN8, CLDN7, CLDN17, MFZL1, |
|                    | hsa04540: Gap junction                        | 12    | 4.05E−02| MAPK1, PLCB4, GNA13, ADCY7 |
|                    | hsa04722: Neurotrophin signaling pathway      | 15    | 4.44E−02| PDK1, IRS2, CAMK2G, IRS1 |

### Table 2 Information of optimizing DEGs combination

| Symbol    | Multi-variate Cox regression analysis | LASSO coef |
|-----------|---------------------------------------|------------|
|           | HR                                    | 95% Cl     | P value   |
| CD38      | 0.4053                                | 0.217-0.758| 0.0046    |
| ZNF212    | 0.0902                                | 0.022-0.370| 8.38E−04  |
| POR       | 0.0896                                | 0.024-0.331| 3.0E−04   |
| CC2D1A    | 0.1025                                | 0.020-0.527| 6.38E−03  |
| GRAMD4    | 0.0867                                | 0.018-0.248| 5.27E−05  |
| FH        | 0.7113                                | 9.1E−03-0.565| 0.0123    |
| SLC24A3   | 2.2113                                | 1.160-4.215| 0.0159    |
| GATA2     | 5.7842                                | 1.536-21.789| 9.49E−03  |
| FOXD1     | 3.0618                                | 1.283-7.308| 0.0117    |
| MMP10     | 2.0820                                | 1.415-3.064| 2.0E−04   |

DEG: differentially expressed genes, HR: hazard ratio, CI: confidence interval.
Liu et al. [20] indicated that the constructed nomogram of the LC survival risk was good for predicting accuracy, which is helpful for doctors to make a more accurate prognosis evaluation of patients with LC, and can be used to guide and optimize the treatment of patients with LC. Likewise, the 10 independent prognostic genes were used to construct the PS model might be novel biomarkers for risk recurrence of patients with LC. Furthermore, we also constructed a nomogram with C-index of 0.822 using the PS model, which indicated that the nomogram performance has a good concordance with the prediction of 1-, 3-, and 5-year OS. Therefore, the PS model based on the 10 DEGs has the potential ability in the area of prognostic prediction.

In our study, some limitations exist. First, we found that the PS model based on 10 genes had a good...
Table 3  Information of clinical factors

| Clinical characteristics | TCGA (N = 82) | Uni-variables Cox | Multi-variables Cox |
|--------------------------|---------------|-------------------|--------------------|
|                          | HR            | 95%CI             | P                  |
|                          | HR            | 95%CI             | P                  |
| Age (years,mean ± sd)    | 60.80±8.29    | 0.973             | 0.933–1.015        | 0.201               | 0.961             | 0.875–1.054        | 0.395               |
| Gender (female/male)     | 11/71         | 0.375             | 0.161–0.872        | 0.0227              | 1.008             | 0.060–16.918       | 0.996               |
| Pathologic M(M0/M1/–)    | 78/0/4        | –                 | –                  | –                   | –                 | –                 | –                   |
| Pathologic N(N0/N1/N2/N3/–) | 38/17/20/2/5 | 0.345             | 0.935–1.935        | 0.111               | 1.568             | 0.753–3.262        | 0.229               |
| Pathologic T(T1/T2/T3/T4/–) | 2/10/24/0/3 | 0.770             | 0.511–1.159        | 0.211               | 4.321             | 0.290–64.345       | 0.288               |
| Pathologic stage (I/II/III/IV/–) | 2/5/18/54/3 | 0.862             | 0.543–1.367        | 0.527               | 0.139             | 0.006–2.992        | 0.207               |
| Neoplasm grade(1/2/3)    | 7/48/26/1    | 0.923             | 0.561–1.516        | 0.75                | 1.442             | 0.325–6.407        | 0.630               |
| Alcohol history(yes/no/–) | 57/23/2      | 0.861             | 0.432–1.714        | 0.67                | 2.703             | 0.437–16.736       | 0.285               |
| Angiolymphatic invasion(yes/no/–) | 24/35/23        | 1.184             | 0.225–2.669        | 0.684               | 3.128             | 0.405–24.172       | 0.274               |
| Perineural invasion(yes/no/–) | 16/41/25      | 1.037             | 0.421–2.555        | 0.938               | 0.235             | 0.020–28.18        | 0.253               |
| PS model status(high/low) | 41/41         | 8.967             | 4.794–16.77        | 6.61E-12            | 86.677            | 13.996–536.769     | 1.62E-06            |
| Recurrence (dead/alive)  | 28/54         | 1.386             | 0.740–2.594        | 0.308               | 0.807             | 0.158–4.132        | 0.797               |
| Recurrence free survival time (months, mean ± sd) | 36.91 ± 30.17 | –                 | –                  | –                   | –                 | –                 | –                   |

N number, TCGA The Cancer Genome Atlas, HR hazard ratio; CI confidence

Fig. 4  Construction of nomogram to predict the prognostic ability for patients with LC. A  A nomogram was constructed using the PS model to predict the prognosis for patients with LC. The calibration plots for 1-year (B), 3-year (C), and 5-year (D) survival time.
predictive ability to predict LC recurrence. However, we failed to determine their detailed mechanisms. Then, only the PS model was screened through the multivariate Cox regression analysis with a threshold of $P<0.05$. Therefore, we could not analyze other models based on other risk factors. Additionally, our study required large samples and clinical data to confirm whether the model we constructed would accurately distinguish high- and low-risk patients with recurrent LC. Finally, corresponding experimental studies should be conducted to verify the functions of these ten key genes.

**Conclusion**

A 10-gene PS model and nomogram are proposed to help predict the recurrence risk in patients with LC.

**Supplementary Information**

The online version contains supplementary material available at https://doi.org/10.1186/s40001-022-00829-2.

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**Author contributions**

YNL and ZGG participated in the design of this study, and they both performed the statistical analysis. CP carried out the study and collected important background information. XLJ drafted the manuscript. All authors read and approved the final manuscript.

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**Availability of data and materials**

The raw data were collected and analyzed by the authors, and are not ready to share their data because the data have not been published.

**Declarations**

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competition of interests**

The authors declare no conflict of interest.

**Competing interests**

The authors declare no conflict of interest.

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