Construction and Validation of an Immune-related lncRNA Prognosis Model in Thyroid Cancer

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Abstract

Background

A growing number of studies have shown that immune-related long non-coding RNAs (lncRNAs) play an important role in the development of cancer. The aim of this study was to identify immune-related lncRNAs in thyroid cancer (THCA) and to develop a prognostic model for THCA.

Methods

We downloaded immune-associated gene sets from the Gene Set Enrichment Analysis (GSEA) website and obtained THCA gene expression and clinical data from The Cancer Genome Atlas (TCGA) database. Immune-related lncRNAs were then obtained by performing a correlation analysis of the expression of lncRNAs and immune-related genes. Prognostic models for THCA immune-related lncRNAs were developed by univariate Cox regression and multiple Cox regression analysis. We confirmed the results in clinical samples using quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Results

26 immune-related lncRNAs in THCA were obtained. Then we constructed a prognosis model composed of 7 lncRNAs (LINC01614, AC017074.1, LINC01184, LINC00667, ACVR2B-AS1, AC090673.1, LINC00900). Our model could be used as an independent prognostic factor. Principal component analysis displayed that the lncRNAs in the model can distinguish between high-risk and low-risk groups. Clinical correlation analysis showed that the expression levels of AC090673.1 ($P<0.05$), LINC01184 ($P<0.001$), LINC01614 ($P<0.001$) were related to disease stage, LINC00900 ($P<0.001$), LINC01614 ($P<0.001$) were related to the T stage of THCA.

We validated this model in the cancer and paracancerous tissues from 24 THCA patients.

Conclusions

We identified seven immune-related lncRNAs as potential biomarkers for the prognosis of THCA.

1 Background

Thyroid cancer (THCA) is a malignant tumour of the thyroid epithelium. Five-year survival rate for patients with invasive THCA is less than 50%, most non-invasive patients have a good prognosis [1]. However, some patients with non-invasive THCA are prone to recur and distal metastases may occur [2]. Existing prognostic predictors for THCA do not accurately predict patients' survival and recurrence [3, 4]. Therefore, there is an urgent need to find new and reliable markers for the prognosis of patients with THCA.

Tumour immunotherapy has developed into an important treatment approach. It is gradually becoming a hot topic in oncology therapeutics and brings hope to cancer patients. In advanced follicular carcinoma and anaplastic THCA, multiple preclinical studies of cancer immunotherapy showed prospect for anticancer applications [1]. Anti-PD-1 treatment enhanced the effect of BRAF immunosuppression on tumour regression and the anti-tumour immune response in mesenchymal THCA [5]. A variety of immune cells are present in the THCA microenvironment. These cells can fight tumours or promote them. In addition, pro-inflammatory cytokines and chemokines secreted by immune cells could promote or inhibit the proliferation of tumour cells [6]. The fact that cancer can survive in this immune microenvironment illustrates the critical role of immune regulation in THCA. Studies also showed that chronic inflammation was associated with the presence and severity of THCA [7]. The expression of immune-related genes in cancer cells can regulate the abundance of infiltrating immune cells. This affects tumour diagnosis, prognosis and sensitivity to clinical treatment. These findings have prompted researchers to explore the expression of immune-related coding and non-coding genes in THCA and provide ideas for the clinical diagnosis, treatment and prognosis of THCA from an immune perspective. However, the immune-related genes associated with the prognosis of THCA patients have not been fully revealed to date. Long non-coding RNAs (lncRNAs) are important components of non-coding RNAs and play an important role in pre-transcriptional, post-transcriptional and post-translational levels as well as in tumour development. Liyanarachi S et al. found that the expression of lncRNAs *XLOC051122* and *XLOC006074* correlated significantly with lymph node metastasis and mutations in *BRAF* (V600E) in patients with papillary THCA [8]. Lei H et al. found that lncRNA TUG1 was related to tumor progression of THCA [9]. Teng H et al. showed that lncRNA *PACERR* and *CYP4A22-AS1* were associated with the aggressiveness and disease-free survival of papillary THCA [10]. The clinical relevance and prognostic importance of immune-related lncRNAs need to be further investigated in THCA.

In this study, we provided insight into the lncRNAs associated with THCA prognosis. A prognostic model was constructed and demonstrated that this model could act as an independent prognostic factor. Our study provides potential therapeutic targets for THCA.

2 Materials And Methods

2.1 Data Collection

Expression profiles and clinical information of mRNA and lncRNA of THCA patients were obtained from The Cancer Genome Atlas (TCGA) (https://cancergenome.nih.gov/). It contains 58 paracancerous tissues and 509 THCA tissues. Patients with a survival time of less than 30 days and unknown survival time were excluded.

2.2 Immune-related lncRNAs

We searched the Gene Set Enrichment Analysis (GSEA) (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp), using immune-related keywords ‘IMMUNE_RESPONSE’ and ‘IMMUNE_SYSTEM_PROCESS’, then downloaded the gene sets. The expression of immune-related genes

was extracted from TCGA and analysed by Perl. We got immune-related lncRNAs by analyzing the relevance between lncRNAs and the immune-related genes, by the limma in R. The filter criteria were: (1) the level of all genes in all samples > 0.5; (2) the level of lncRNAs in all samples were in fluctuations.

2.3 Prognostic model construction

Screening for genes significantly associated with prognosis based on univariate and multivariate COX regression analyses of the expression of lncRNAs in relation to patient survival time and status using the 'survival' package in R ($P < 0.01$). We use the function predict to calculate the risk value for each patient. The calculation formula is: (Coefficient of lncRNA1× Expression of lncRNA1) + (Coefficient of lncRNA2× Expression of lncRNA2) + … + (Coefficient of lncRNAn× Expression of lncRNAn).

2.4 Patients and Samples Collection

24 patients with TCGA admitted to Chongqing University Cancer Hospital from January to June 2017 were randomly selected. The pathological diagnosis of all patients was assessed by three pathologists simultaneously according to the WHO classification criteria. Patients who received preoperative chemotherapy, radiotherapy or any other adjuvant treatment were excluded from this study. The ethics has been approved by Chongqing University Cancer Hospital. All the participants signed informed consent forms.

2.5 RNA Extraction and Quantitative Real-Time PCR (qRT-PCR) analysis

Tissues frozen in liquid nitrogen were taken and RNA was extracted according to the miRNeasy FFPE kit (Qiagen). MiRcute Enhanced miRNA cDNA First Strand Synthesis Kit (TIANGEN) was used to reverse transcribe the RNA into complementary DNA. The qRT-PCR analysis was performed using qPCR miRcute enhanced miRNA fluorescence quantification kit (TIANGEN). Results were normalised to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression. We conducted three replicate experiments. The primers synthesized by thermofisher and were shown in Table S1.

2.6 Statistical analysis

All analyses were performed using R 3.4.3. The "survival" and "survminer" packages were used to plot survival curves for the high-risk and low-risk groups. Heat maps were drawn using the "heatmap" package. The "ggpubr" package analyzed the correlation between lncRNAs and clinical features. Principal component analysis was performed using the 'limma' and 'scatterplot3d' packages. Univariate and multivariate Cox regression analysis were used to test whether the constructed model could be used as an independent prognostic factor. SPSS 24.0 statistical software was used to analyze the experimental data. Relative expression of lncRNA was calculated using the $2^{-\Delta\Delta Ct}$ method relative to GAPDH. The difference was considered significant when $P < 0.05$.

3 Results

3.1 Acquired the immune-related lncRNAs in THCA

To make the research process clear, a flow chart was drawn to illustrate the research design and analysis methodology (Fig. 1). We obtained sequencing data and clinicopathological information from TCGA for 58 paraneoplastic and 497 cancerous tissues (Table 1). Immune-related gene sets named “Immune Response” and “Immune System Processes” were downloaded from the GSEA website. 331 immune-related genes were obtained by gene expression analysis. Next, 822 immune-related lncRNAs were obtained by correlation analysis of immune-related genes with lncRNAs (|Cor |>0.4, $P < 0.001$).

Table 1
The clinical characteristics of THCA patients in TCGA.

Characteristics	Total N (%)
Age	
< 60	387(77.9)
≥ 60	110(22.1)
Sex	
Male	127(25.6)
Female	370(74.4)
TNM stage	
I	285(57.3)
II	43(8.7)
III	112(22.5)
IV	57(11.5)
T stage	
T1	100(20.1)
T2	167(33.6)
T3	170(34.2)
T4	58(11.8)
unknown	2(0.3)

3.2 Constructed the prognostic model

To get the immune lncRNAs associated with prognosis, we analysed the relationship between the expression of immune lncRNAs and survival status. Univariate Cox regression analysis revealed that 26

immune lncRNAs were significantly associated with patients' survival ($P < 0.01$) (Table 2). Multivariate Cox regression analysis was performed on the above 26 immune-related lncRNAs to screen out 7 lncRNAs with high prognostic diagnostic value and to establish a prognostic model for immune-related lncRNAs in THCA (Table 3). The formula for calculating each patient's risk score was as follows:

Risk Score= (1.756 * *LINC01614*) + (2.649 * *AC017074*.1) + (2.323 * *LINC01184*) + (2.121 * *LINC00667*) + (3.816 * *ACVR2B-AS1*) + (-1.211 * *AC090673.1*) + (-1.486 * *LINC00900*).

Table 2
Univariate COX analysis of immune-related lncRNAs associated with THCA Prognosis.

lncRNA	p-value
QCH-AS1	0.002319745
X322234.1	0.000395310
EIPR1-IT1	0.008115204
LMO7-AS1	0.009767902
AC005083.1	0.002111020
LINC01614	0.000934414
LINC00900	0.003510985
CKMT2-AS1	0.000872865
NKILA	0.004423934
AL928654.1	0.003335810
AC106897.1	0.004553648
AC012038.2	0.003084101
AC103974.1	0.001330777
ACVR2B-AS1	0.004448331
AC007207.2	0.008541234
LINC02454	0.008238894
LINC02471	0.001869325
HCG15	0.002266351
AC017074.1	0.004580516
OXCT1-AS1	0.006377167
PSMG3-AS1	0.000741055
TRAM2-AS1	0.001763204
BX539320.1	0.005315486
LINC01184	0.009588057
AC090673.1	0.004202555
LINC00667	0.004383836

Table 3
Multivariate Cox analysis of seven immune-associated lncRNAs in THCA.

Gene symbol	Coefficients	*P* value
LINC01614	1.756	0.000
LINC00900	-1.486	0.018
ACVR2B-AS1	3.816	0.000
AC017074.1	2.649	0.003
LINC01184	2.323	0.026
AC090673.1	-1.211	0.032
LINC00667	2.121	0.034

3.3 Predicted good and poor prognosis in THCA patients

Based on the median risk score, patients were divided into a high risk group (n = 248) and a low risk group (n = 249). We ranked the risk score and marked the survival status of all patients in a scatter plot. Mortality was higher in the high-risk group than in the low-risk group, and mortality increased with the increasing risk score (Fig. 2A). The heat map revealed the relationship between the expression of lncRNAs in the model and the risk score in each patient (Fig. 2B). As the expression risk score increased, five lncRNAs had elevated expression and were defined as high risk lncRNAs (i.e. *LINC01614*, *AC017074.1*, *LINC01184*, *LINC00667*, *ACVR2B-AS1*), while *AC090673.1*, *LINC00900* had decreased expression and were defined as low risk lncRNAs. In addition, the results of the survival analysis showed a significant difference between the high and low risk groups (P = 1.41e-05) (Fig. 3).

3.4 Discriminated between high-risk and low-risk groups in different genomes

To verify the importance of lncRNAs as prognostic markers for THAC patients in our model, we did a principal components analysis to explore whether lncRNAs could distinguish between the high and low risk groups in our model. There were significant differences in the expression profiles of immune-related lncRNAs between the two groups (Fig. 4). Results indicated that the high and low risk groups can be discriminated by the lncRNAs in our model.

3.5 Evaluated the prognostic models

To further validate the accuracy and specificity of the prediction model, we performed the univariate and multivariate analyses to assess whether the model could be used as an independent prognostic factor. Univariate independent prognostic analysis revealed that the following factors were statistically significant, namely, age (P < 0.001), stage (P = 0.003), T stage (P = 0.030) and the prognostic model (P <

0.001) (Fig. 5A). Multivariate independent prognostic analysis displayed that age (P = 0.002) and the model (P = 0.025) could predict the overall survival (OS) for patients with THCA (Fig. 5B). This indicated that the model we constructed could predict the prognosis of THCA patients.

3.6 Analyzed the correlation between lncRNAs and clinical features

To investigate the correlation between the expression of lncRNAs in the model and clinical characteristics, a correlation analysis was performed. The results suggested that the level of *AC090673.1*(P< 0.05),*LINC01184*(P< 0.001)、*LINC01614*(P< 0.001) were related to the clinical stage. As the disease stage increased, the expression of *AC090673.1* and *LINC01614* went down then up, with overexpression in the late stage. In contrast, the expression of *LINC01184* was relatively high and showed a trend of increasing before decreasing (Fig. 6A). The level of *LINC00900* (P< 0.001)、*LINC01614* (P< 0.001) were associated with the T stage. As T stage increased, the expression of *LINC00900* rose slightly and then fell, while the expression of *LINC01614* decreased and then increased (Fig. 6B). These lncRNA prognostic markers could be seen to more accurately predict the clinicopathological characteristics of patients.

3.7 Detected the lncRNA expression levels in THCA patients

In order to further evaluate the reliability of the constructed model, we examined the expression levels of 7 lncRNAs in cancer and paracancer tissues of 24 THCA patients by qRT-PCR (Table 4). The results showed that *LINC01614*, *ACVR2B-AS1*, *AC017074.1*, *LINC01184* and *LINC00667* were up-regulated in THCA, while *LINC00900* and *AC090673.1* were down-regulated (Fig. 7). The experimental results were consistent with the bioinformatic analysis.

Table 4
The clinicopathological data of 24 THCA patients.

Characteristics	Total N (%)
Age	
< 60	22(91.7)
≥ 60	2(8.3)
Sex	
Male	12(50.0)
Female	12(50.0)
TNM stage	
I	11(45.8)
II	9(37.5)
III	3(12.5)
IV	1(4.2)
Tumor (T)	
T1	17(70.9)
T2	2(8.3)
T3	2(8.3)
T4	3(12.5)
Nodes (N)	
N0	12(50.0)
N1	9(37.5)
N2	2(8.3)
N3	1(4.2)
Metastasis (M)	23(95.8)
M0	1(4.2)
M1	

4 Discussion

lncRNAs play critical roles in different stages of cancer immunity, including antigen release, antigen presentation, immune activation, immune cell migration, infiltration of cancer tissue and killing of cancer cells, and immune escape of tumour cells. And lncRNAs may be key regulators in reshaping the tumour immune microenvironment [11, 12]. We analysed the correlation between lncRNAs and immune-related genes in THCA and developed a lncRNA prediction model consisting of seven lncRNAs (*LINC01614*, *AC017074.1*, *LINC01184*, *LINC00667*, *ACVR2B-AS1*, *AC090673.1*, *LINC00900*). The high-risk lncRNA *LINC01614* in this model has been studied in breast cancer (BC). Vishnubalaji R et al. found an increased expression of the lncRNA *LINC01614* in primary BC tissues and BC cell lines. It was an unfavorable prognostic marker for BC [13]. Wang Y et al. confirmed this result [14]. Sun Y et al. also found that high expression of *LINC01614* indicated a low OS rate in non-small cell lung cancer (NSCLC) patients [15]. In our model, another high-risk lncRNA *INC00667* was proved to be a tumor promoter in NSCLC. Involved in the proliferation, migration and angiogenesis of NSCLC cells, it was significantly associated with OS time of patients [16]. lncRNA *INC00667* also correlated with the prognosis of BC, ovarian and hepatocellular carcinomas [17–20]. Zhou M et al. found that *LINC01184* was overexpressed in tumor-infiltrating B lymphocytes and was involved in immunosuppression of cancer [21]. Our model suggested that *LINC01184* was a high-risk lncRNA associated with clinical stage. *ACVR2B-AS1* was also one of the high-risk lncRNAs in our model. High expression of *ACVR2B-AS1* was an independent poor prognostic factor for OS and relapse-free survival in patients with liver cancer [22]. With regard to *LINC00900*, we found it to be relevant to the clinical T stage. Wang W et al. showed that the expression of *LINC00900* increased with tumour grade [23].

In THCA, the potential role of lncRNA as an immune-related prognostic marker remains unclear. The prognostic role of lncRNAs in THCA has been investigated from different perspectives, such as methylation and competing endogenouse RNA. Li Q et al. analyzed the methylation and transcriptome of THCA, identified 483 epigenetically regulated lncRNAs (EpilncRNAs) associated with the development of THCA, and constructed a prognostic model of EpilincRNAs. The methylation-driven 5-lncRNA-based signature was found to be an independent prognostic factor and to be involved in immune and inflammation-related biological processes [24]. Li X et al. identified Lnc5m4 as an independent prognostic factor in a competing endogenouse RNA network consisting of lncRNA, miRNA and mRNA. Patients with THCA could be divided into high and low risk groups [25]. In this study, we verified the accuracy and specificity of the predictive model through univariate and multivariate Cox regression analysis. The model we constructed could be used as an independent prognostic factor.

In addition, we further analysed the correlation between the clinical characteristics of the patients and the 7 lncRNAs in the model. The results showed that *AC090673.1*, *LINC01184* and *LINC01614* were associated with clinical stage. *LINC00900* and *LINC01614* were associated with the T stage. *LINC01614* has been shown to correlate with clinical stage in patients with glioma and NSCLC [26, 27]. *LINC01184* was correlated with several clinicopathological profiles of rectal cancer, including the patient's clinical stage, tumour size, lymph nodes and distal metastases [28]. *LINC00900* was not differentially expressed in neurogliomas of different clinical stage [29]. The relationship between these lncRNAs and the clinical characteristics of THCA patients needs to be further investigated.

There are some limitations to this study and the constructed lncRNA prognostic model needs more sample validation before it can be put into clinical application. Recently, an immune-related lncRNA model was constructed based on the Gene expression omnibus database to predict the prognosis of THCA [30]. The immune-associated gene sets in our study were derived from the GSEA database and the prognostic models constructed were validated in multiple clinical samples, which made our conclusions more convincing. The prognostic model constructed in that study crossed over with our model but was not fully consistent. This may be mainly due to the different sources of data and the different sets of immune-related genes selected.

5 Conclusion

In summary, we constructed and validated a prognostic model for immune-related lncRNAs in THCA. The lncRNAs were significantly correlated with the clinical stage and T stage of THCA. Our results provide new targets for the prognosis and treatment of THCA.

Abbreviations

lncRNAs	long non-coding RNAs
THCA	thyroid cancer
GSEA	Gene Set Enrichment Analysis
TCGA	The Cancer Genome Atlas
qRT-PCR	quantitative reverse transcription polymerase chain reaction
BC	breast cance
NSCLC	non-small cell lung cancer
EpilncRNAs	epigenetically regulated lncRNAs

Declarations

Authors' contributions

WN designed the study. ZL downloaded and analysed the data. HW wrote the first draft of the article. WN and ZL revised the article. XD and LW collected clinical data from patients. JZ collected tissue samples from patients. WT and WY did the validation experiments. All authors read and approved the final manuscript.

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Ethics approval and consent to participate

The ethics has been approved by the Ethics Committee of Chongqing University Cancer Hospital. All the participants signed informed consent forms.

Consent for publication

Consent to publish the individual data was obtained in writing from the patient in this study.

Competing interests

The authors declare no conflict of interest.

Availability of data

The datasets analysed during the current study are available in the TCGA (https://cancergenome.nih.gov/) and GSEA (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp) website. The datasets supporting the conclusions of this article are included within the article and its additional files.

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References

1.Naoum GE, Morkos M, Kim B, Arafat W. Novel targeted therapies and immunotherapy for advanced thyroid cancers. *Mol Cancer*. 2018;17:51. doi: 10.1186/s12943-018-0786-0

2. Abdullah MI, Junit SM, Ng KL, Jayapalan JJ, Karikalan B, Hashim OH. Papillary Thyroid Cancer: Genetic Alterations and Molecular Biomarker Investigations. *Int J Med Sci*. 2019;16:450-460. doi: 10.7150/ijms.29935

3. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. . 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid*. 2016;26:1-133. doi: 10.1089/thy.2015.0020

4. Vuong HG, Duong UN, Altibi AM, Ngo HT, Pham TQ, Tran HM, et al. . A meta-analysis of prognostic roles of molecular markers in papillary thyroid carcinoma. *Endocr Connect*. 2017;6:R8-R17. doi: 10.1530/EC-17-0010

5. Brauner E, Gunda V, Vanden Borre P, Zurakowski D, Kim YS, Dennett KV, et al. . Combining BRAF inhibitor and anti PD-L1 antibody dramatically improves tumor regression and anti tumor immunity in an immunocompetent murine model of anaplastic thyroid cancer. *Oncotarget*. 2016;7:17194-211. doi: 10.18632/oncotarget.7839

6. Ferrari SM, Fallahi P, Galdiero MR, Ruffilli I, Elia G, Ragusa F, et al. . Immune and Inflammatory Cells in Thyroid Cancer Microenvironment. *Int J Mol Sci*. 2019;20:4413. doi: 10.3390/ijms20184413

7. Imam S, Dar P, Paparodis R, Almotah K, Al-Khudhair A, Hasan SA, et al. .Nature of coexisting thyroid autoimmune disease determines success or failure of tumor immunity in thyroid cancer. *J Immunother Cancer*. 2019;7:3. doi: 10.1186/s40425-018-0483-y

8. Liyanarachchi S, Li W, Yan P, Bundschuh R, Brock P, Senter L, et al. . Genome-Wide Expression Screening Discloses Long Noncoding RNAs Involved in Thyroid Carcinogenesis. *J Clin Endocrinol Metab*. 2016;101:4005-4013. doi: 10.1210/jc.2016-1991

9. Lei H, Gao Y, Xu X. LncRNA TUG1 influences papillary thyroid cancer cell proliferation, migration and EMT formation through targeting miR-145. *Acta Biochim Biophys Sin (Shanghai)*. 2017;49:588-597. doi: 10.1093/abbs/gmx047

10. Teng H, Mao F, Liang J, Xue M, Wei W, Li X, et al. . Transcriptomic signature associated with carcinogenesis and aggressiveness of papillary thyroid carcinoma. *Theranostics*. 2018;8:4345-4358. doi: 10.7150/thno.26862

11 Jiang MC, Ni JJ, Cui WY, Wang BY, Zhuo W. Emerging roles of lncRNA in cancer and therapeutic opportunities. *Am J Cancer Res*. 2019;9:1354-1366.

12. Yu WD, Wang H, He QF, Xu Y, Wang XC. Long noncoding RNAs in cancer-immunity cycle. *J Cell Physiol*. 2018;233:6518-6523. doi: 10.1002/jcp.26568

13. Vishnubalaji R, Shaath H, Elkord E, Alajez NM. Long non-coding RNA (lncRNA) transcriptional landscape in breast cancer identifies LINC01614 as non-favorable prognostic biomarker regulated by TGFβ and focal adhesion kinase (FAK) signaling. *Cell Death Discov*. 2019;5:109. doi: 10.1038/s41420-019-0190-6

14. Wang Y, Song B, Zhu L, Zhang X. Long non-coding RNA, LINC01614 as a potential biomarker for prognostic prediction in breast cancer. *PeerJ*. 2019;7:e7976. doi: 10.7717/peerj.7976

15. Sun Y, Ling C. Analysis of the long non-coding RNA LINC01614 in non-small cell lung cancer. *Medicine (Baltimore).* 2019;98:e16437. doi: 10.1097/MD.0000000000016437

16. Yang H, Yang W, Dai W, Ma Y, Zhang G. LINC00667 promotes the proliferation, migration, and pathological angiogenesis in non-small cell lung cancer through stabilizing VEGFA by EIF4A3. *Cell Biol Int*. 2020;44:1671-1680. doi: 10.1002/cbin.11361

17. Zhou W, Liu T, Saren G, Liao L, Fang W, Zhao H. Comprehensive analysis of differentially expressed long non-coding RNAs in non-small cell lung cancer. *Oncol Lett*. 2019;18:1145-1156. doi: 10.3892/ol.2019.10414

18. Zhu M, Lv Q, Huang H, Sun C, Pang D, Wu J. Identification of a four-long non-coding RNA signature in predicting breast cancer survival. *Oncol Lett*. 2020;19:221-228. doi: 10.3892/ol.2019.11063

19. Chen Y, Bi F, An Y, Yang Q. Identification of pathological grade and prognosis-associated lncRNA for ovarian cancer. *J Cell Biochem*. 2019;120:14444-14454. doi: 10.1002/jcb.28704

20. Gu J, Zhang X, Miao R, Ma X, Xiang X, Fu Y, et al. . A three-long non-coding RNA-expression-based risk score system can better predict both overall and recurrence-free survival in patients with small hepatocellular carcinoma. *Aging (Albany NY)*. 2018;10:1627-1639. doi: 10.18632/aging.101497

21. Zhou M, Zhang Z, Bao S, Hou P, Yan C, Su J, et al. . Computational recognition of lncRNA signature of tumor-infiltrating B lymphocytes with potential implications in prognosis and immunotherapy of bladder cancer. *Brief Bioinform*. 2021;22:bbaa047. doi: 10.1093/bib/bbaa047

22. Nie Y, Jiao Y, Li Y, Li W. Investigation of the Clinical Significance and Prognostic Value of the lncRNA *ACVR2B-As1* in Liver Cancer. *Biomed Res Int*. 2019;2019:4602371. doi: 10.1155/2019/4602371

23. Wang W, Li J, Lin F, Guo J, Zhao J. Identification of N^6-methyladenosine-related lncRNAs for patients with primary glioblastoma. *Neurosurg Rev*. 2021;44:463-470. doi: 10.1007/s10143-020-01238-x

24. Li Q, Wang P, Sun C, Wang C, Sun Y. Integrative Analysis of Methylation and Transcriptome Identified Epigenetically Regulated lncRNAs With Prognostic Relevance for Thyroid Cancer. *Front Bioeng Biotechnol*. 2020;7:439. doi: 10.3389/fbioe.2019.00439

25. Li X, Dai D, Wang H, Wu B, Wang R. Identification of prognostic signatures associated with long-term overall survival of thyroid cancer patients based on a competing endogenous RNA network. *Genomics*. 2020;112:1197-1207. doi: 10.1016/j.ygeno.2019.07.005

26. Wang H, Wu J, Guo W. SP1-Mediated Upregulation of lncRNA LINC01614 Functions a ceRNA for miR-383 to Facilitate Glioma Progression Through Regulation of ADAM12. *Onco Targets Ther*. 2020;13:4305-4318. doi: 10.2147/OTT.S242854

27. Sun Y, Ling C. Analysis of the long non-coding RNA LINC01614 in non-small cell lung cancer. *Medicine (Baltimore)*. 2019;98:e16437. doi: 10.1097/MD.0000000000016437

28. Sui YX, Zhao DL, Yu Y, Wang LC. The Role, Function, and Mechanism of Long Intergenic Noncoding RNA1184 (linc01184) in Colorectal Cancer. *Dis Markers*. 2021;2021:8897906. doi: 10.1155/2021/8897906

29. Qiu X, Tian Y, Xu J, Jiang X, Liu Z, Qi X, et al. . Development and Validation of an Immune-Related Long Non-coding RNA Prognostic Model in Glioma. *J Cancer*. 2021;12:4264-4276. doi: 10.7150/jca.53831

30. Li Z, Lin W, Zheng J, Hong W, Zou J, Zhang T, et al. . Identification of immune-related lncRNAs to improve the prognosis prediction for patients with papillary thyroid cancer. *Biosci Rep*. 2021;41:BSR20204086. doi: 10.1042/BSR20204086

Figures

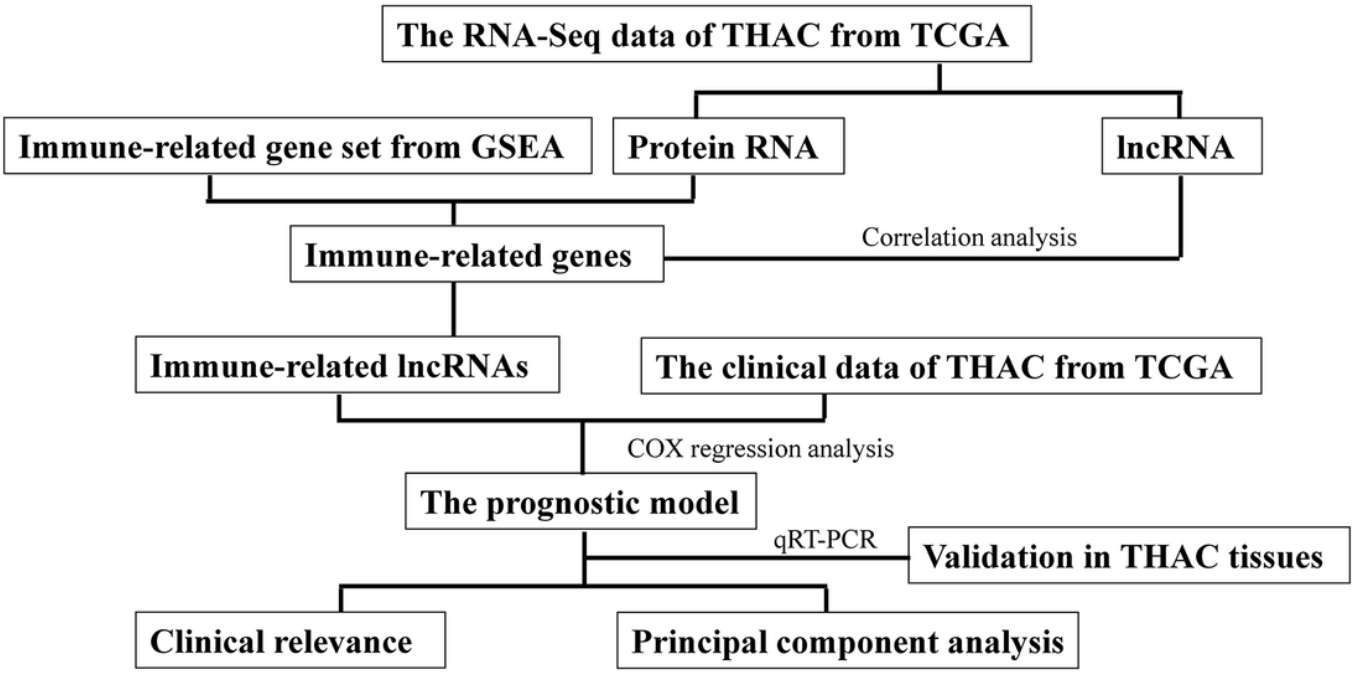

Figure 1

The process of building the prognostic model.

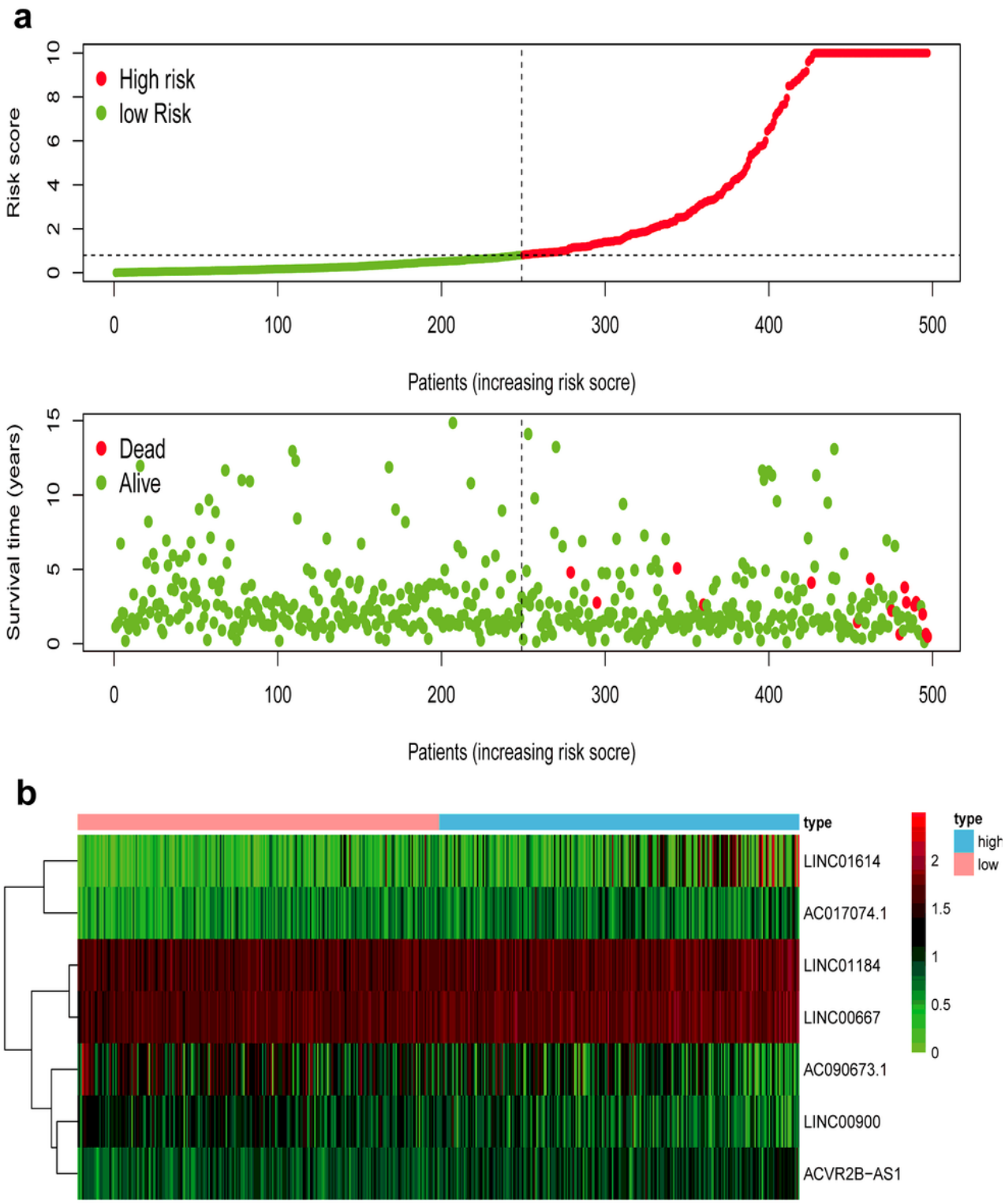

Figure 2

The prognostic model composed of 7 lncRNAs related to immunity in THCA. (A) Distribution of risk scores and survival status in the high-risk and low-risk groups of THCA patients. (B) The heat map of expression profile of the 7 immune-related lncRNAs.

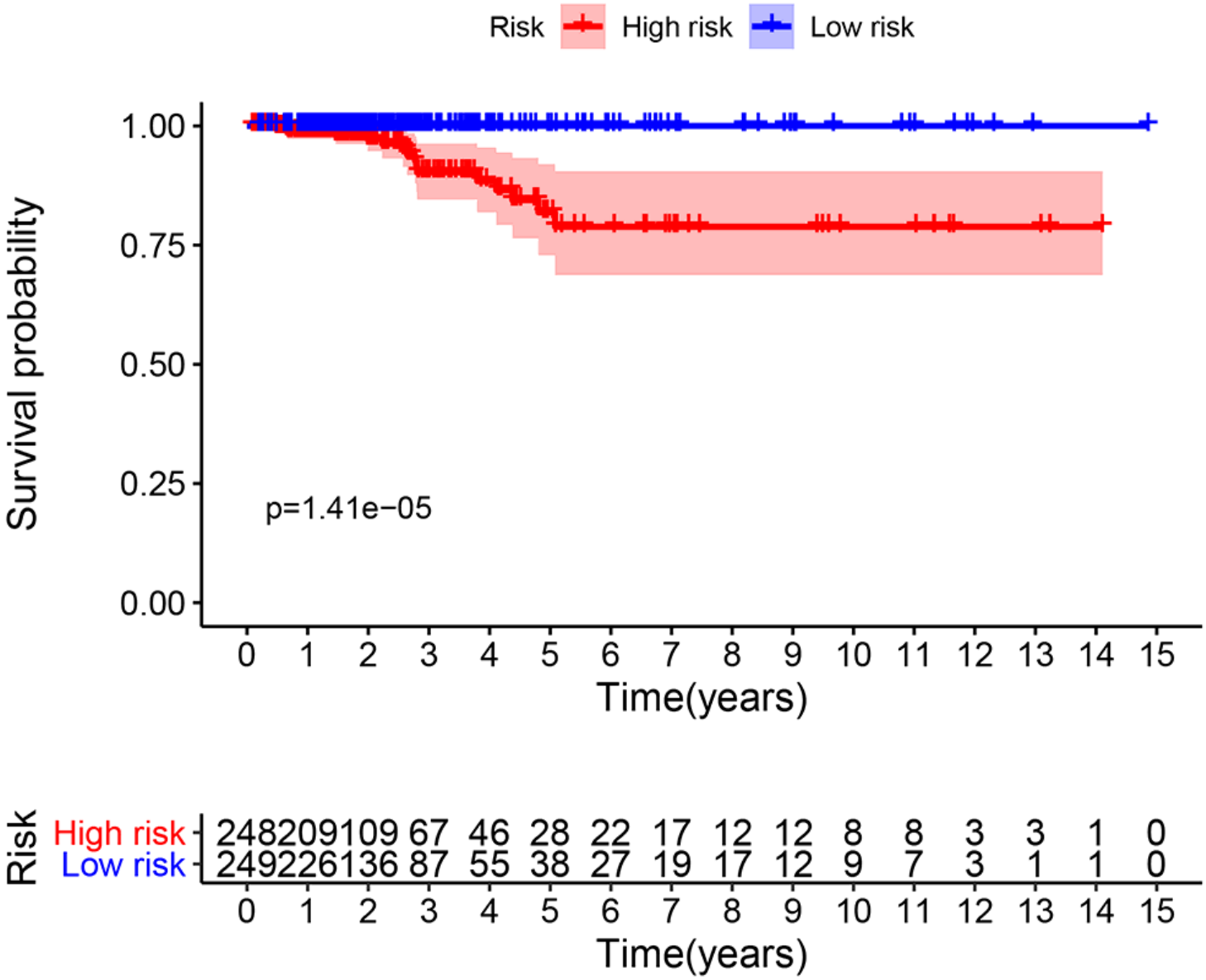

Figure 3

Overall survival analysis of THCA patients in high-risk group and low-risk group.

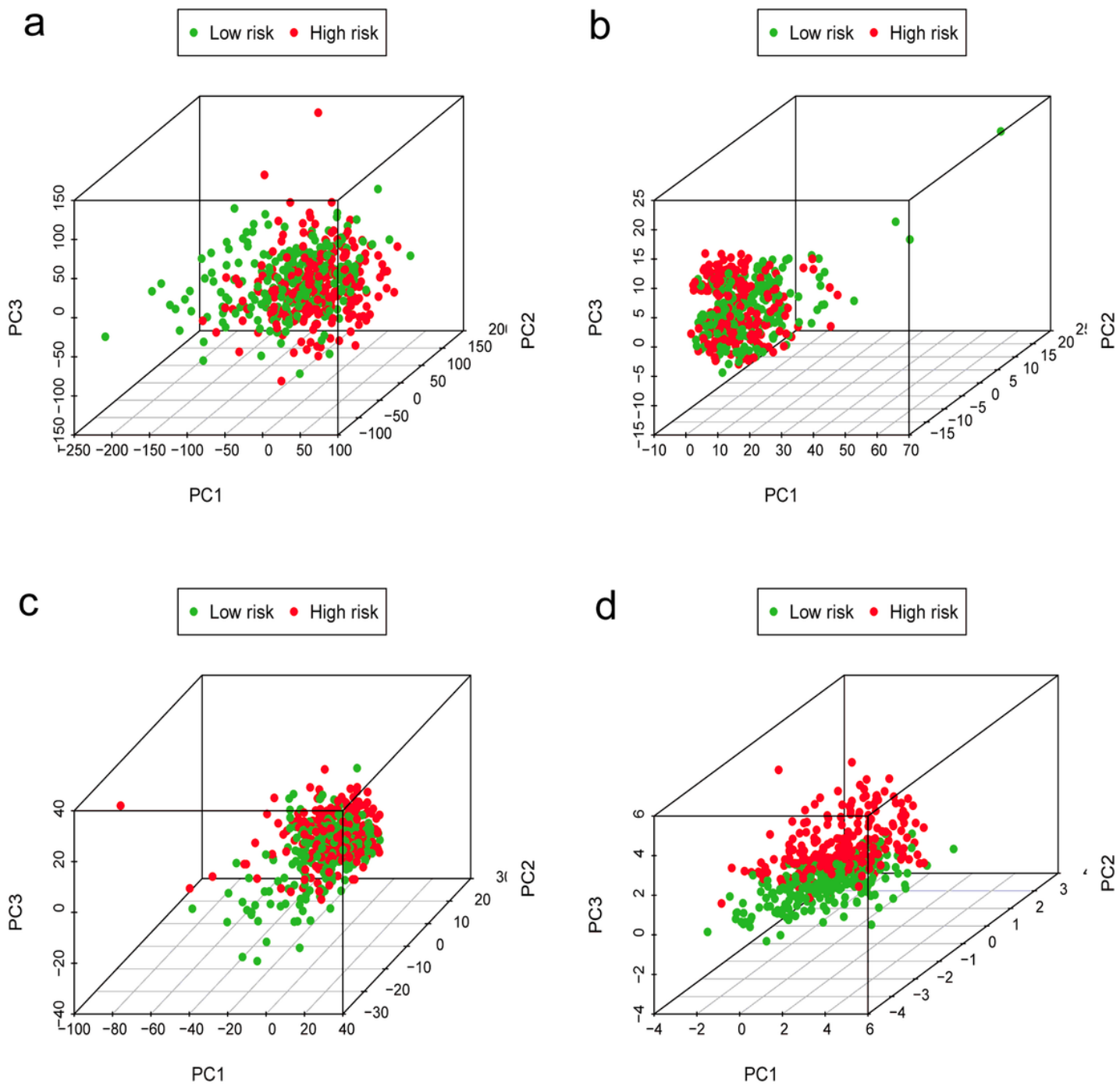

Figure 4

Different immune status between high-risk and low-risk groups. Principal component analysis between high and low risk groups in all genes (A), immune-related genes (B), immune-related lncRNAs (C), immune-related lncRNAs in the prognosis model (D).

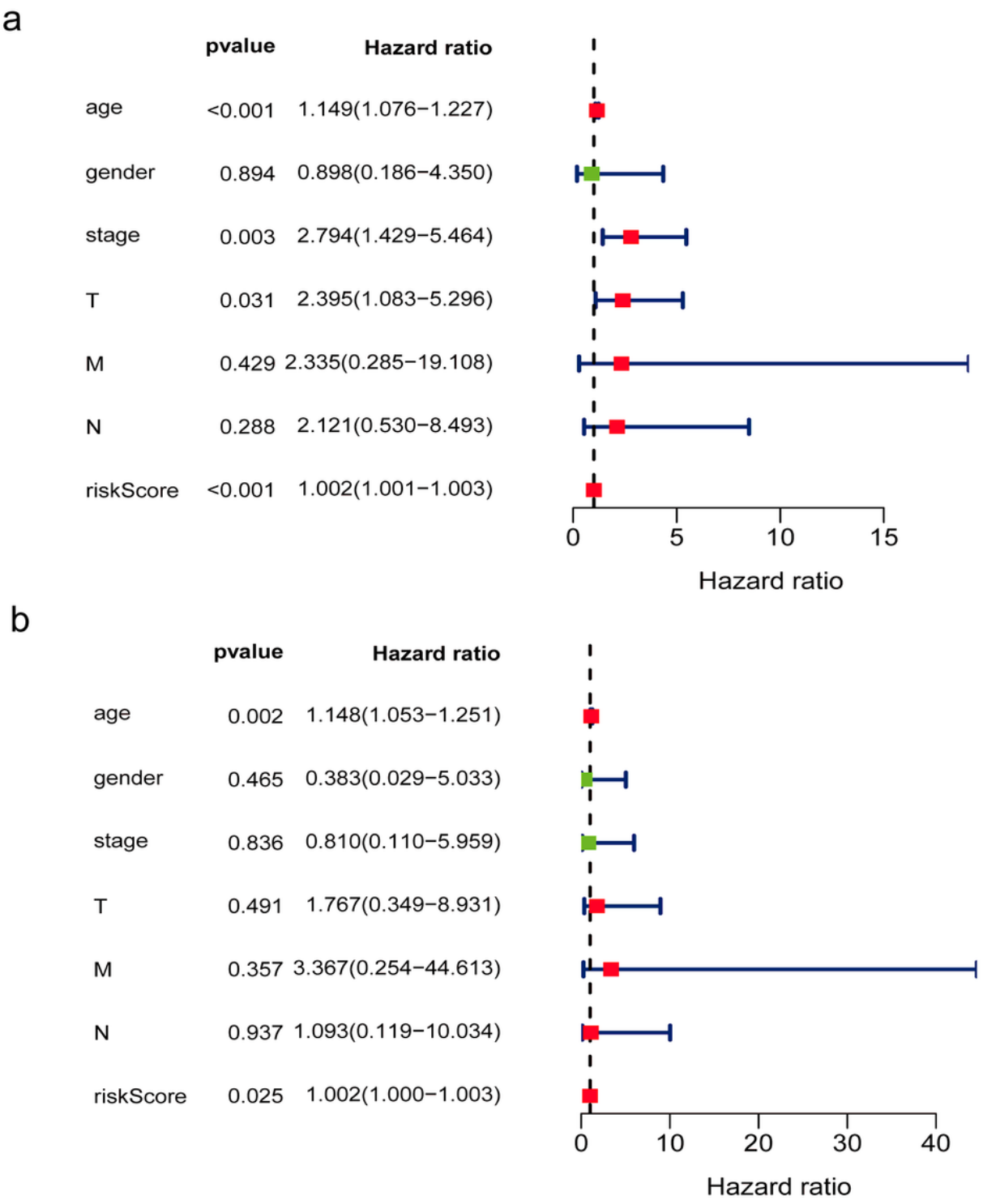

Figure 5

Immune-related lncRNA prognostic model as an independent prognostic factor. (A) Univariate Cox regression analysis to test THCA prediction tool. (B) Multiple Cox regression analysis to test THCA prediction tool.

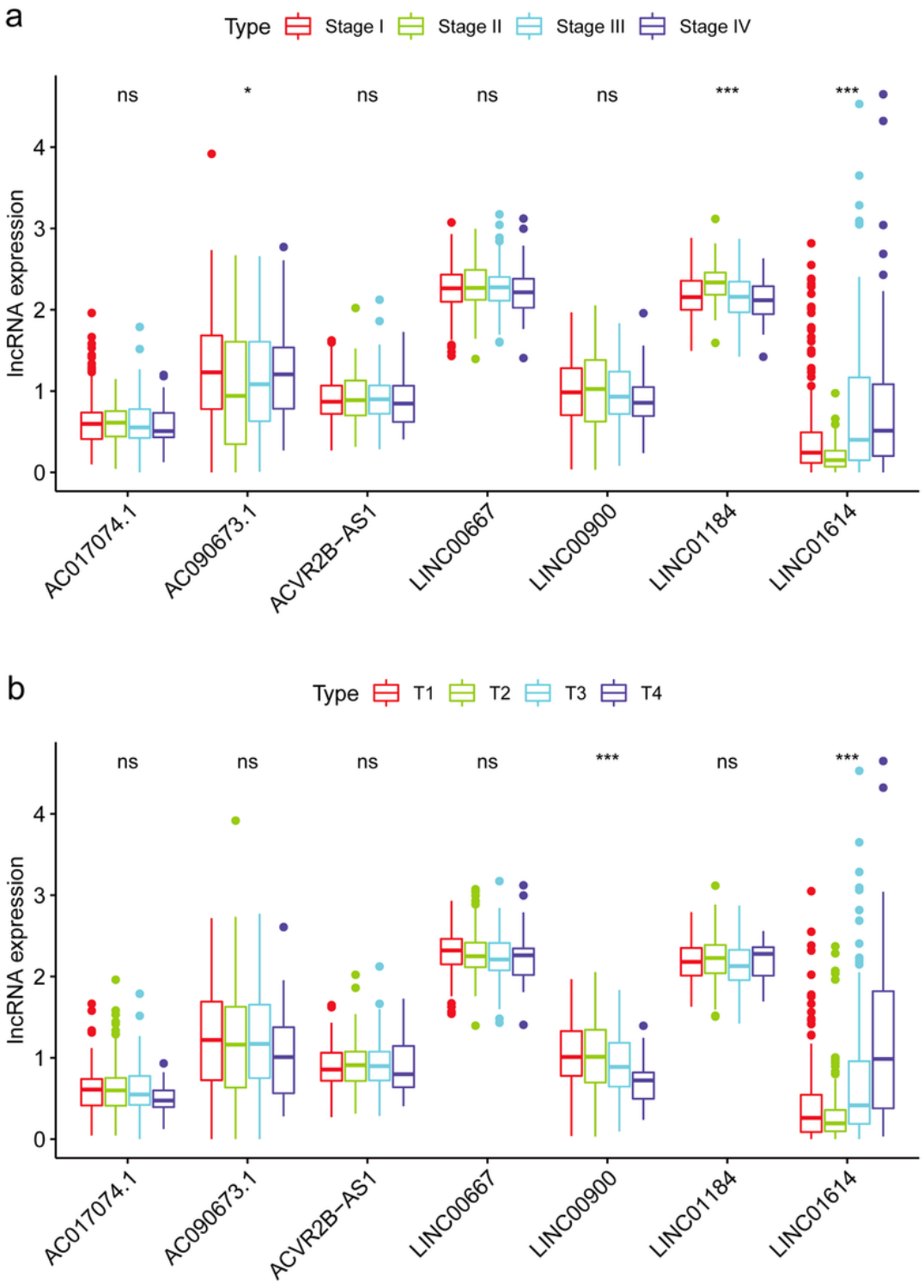

Figure 6

Relationship between seven immune-related lncRNAs and clinical characteristics of THCA patients. (A) Clinical stage. (B) T-stage.

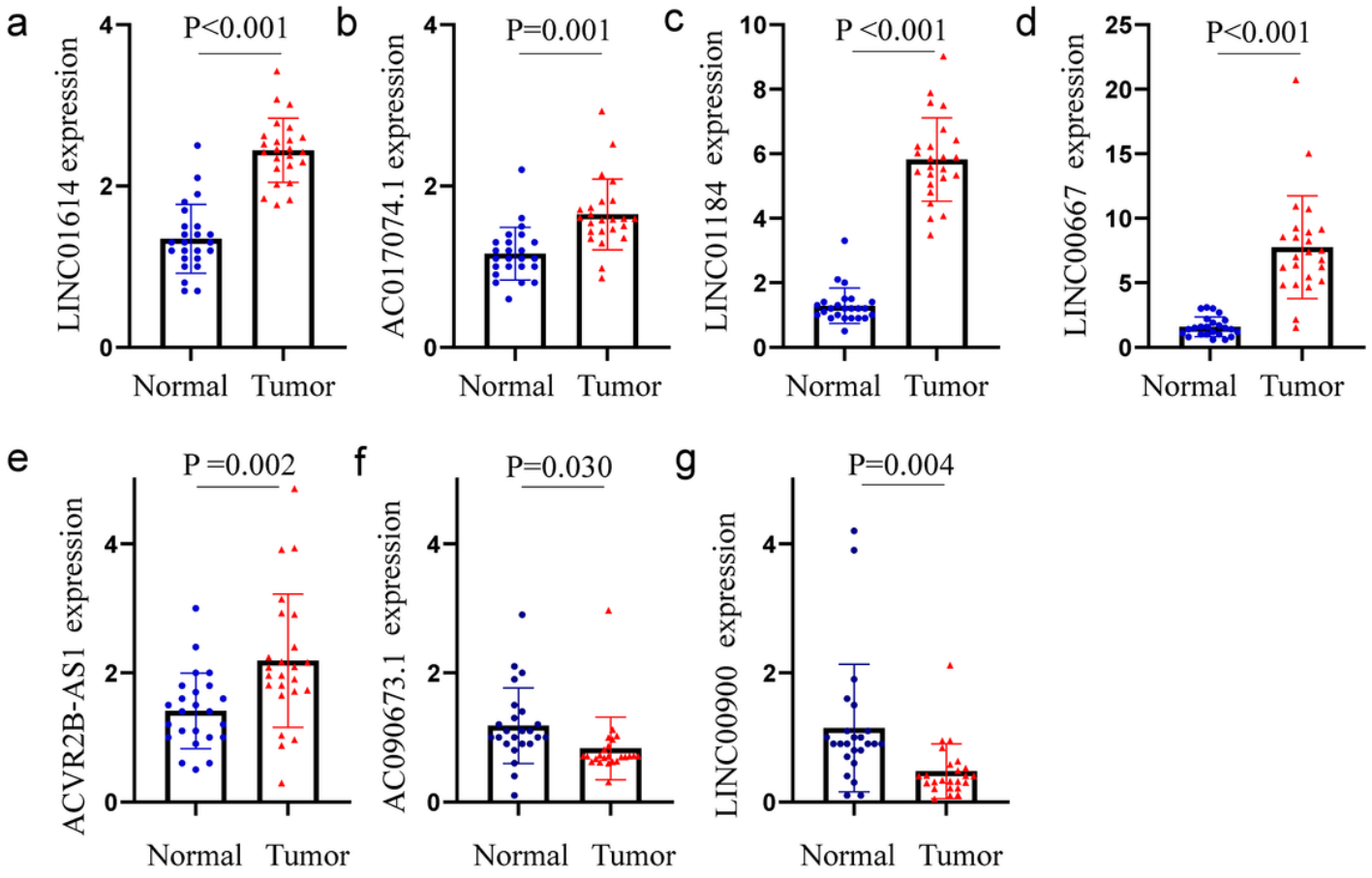

Figure 7

qRT-PCR validation of immune-related lncRNA expression in paracancerous and cancer tissues in THCA patients. The experiment was repeated three times independently.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1TableS1Theprimersequences..docx
- OriginaldataRTqPCR.xlsx
- Theclinicopathologicaldataof24THCApatients.xlsx