Identification of Robust Immune-Associated lncRNAs Signature for Immune Checkpoint Blockade and Prognosis in Pancreatic Ductal Adenocarcinoma

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Research

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Abstract

Background: An increasing body of evidence has suggested that long non-coding RNAs (lncRNAs) can serve as essential regulators in cancer immunity. We aimed to establish a robust immune-associated lncRNA signature for pancreatic ductal adenocarcinoma (PDAC) outcome prediction to enhance prognostic accuracy.

Methods: Pancreatic cancer samples were obtained from the The Cancer Genome Atlas (TCGA) project. Immune-related lncRNAs displaying significant association with overall survival (OS) were screened through univariate Cox regression analysis and the least absolute shrinkage and selection operator (LASSO) algorithm. The prediction reliability was further estimated in the internal validation set and combination set. Gene set enrichment analysis (GSEA) of the lncRNA model risk score was performed for functional annotation. The correlation between immune checkpoint inhibitors and this signature was examined.

Results: 5 immune-related lncRNAs were confirmed to establish five-immune-related lncRNAs prognostic signature. The constructed risk model showed significant correlation with PDAC OS. The area under the curve (AUC) for this lncRNA model was up to 0.747. This immune-related lncRNA signature was correlated with disease progression and worse survival and was an independent prognostic biomarker. Our signature mediated chondrocyte development, laminin binding and so forth. This risk score model was associated with immune cell infiltration (i.e., CD4 T cells, etc.,) and immune checkpoint blockade (ICB) immunotherapy-related molecules (i.e., PDCD1 and CTLA4).

Conclusion: The immune-related lncRNA signature we established possesses latent prognostic value for patients with PDAC and may have the potential to measure the response to ICB immunotherapy, which could guide for immunological treatment in PDAC.

1. Background

Globally, pancreatic ductal adenocarcinoma (PDAC) as one of the most frequently diagnosed malignant tumors ranks the seventh leading cause of tumor correlated death in both females and males (1, 2). On the basis of the latest global cancer statistics, there was almost 459,000 newly diagnosed PDAC cases and an estimated 432,000 associated deaths in 2018 worldwide(1). Mainly due to difficult early diagnosis and rapid progress, the harsh reality is that a majority of PDAC patients have advanced disease or metastatic lesion at diagnosis(2). Over the years, the current conventional therapy for most advanced PDAC patients has been gemcitabine-based regimens(3), or positive chemotherapy to de-bulk the tumor and increase probability for surgical resection(4). Immune checkpoint inhibitors have dramatically yielded benefit in a great body of malignancies, however, patients with PDAC has not yet obtained this benefit(5), mainly owing to its extremely immunosuppressive tumor stroma. There are numerous of T cells, myeloid-derived suppressor cells, macrophages, and tumor correlated fibroblasts in its microenvironment, all of which hindered efficient immune therapy and boosted cancer cell proliferation (6). Encouragingly, a small

subset of patients with PDAC exhibited high effector T-cell infiltration and have better prognosis(7–9), suggesting the potentiality for effective management of PDAC with immunotherapy. Besides, the genetic heterogeneity of PDACs is marked, which reduces the efficacy of clinical management and makes it incredibly tough to accurately predict clinical outcomes in patients with PDAC(10). Though we have made great achievement in the identification and validation of prognostic and predictive bimolecular indicators for PDAC, robust predictive biomarkers for ICB therapy outcome have not been exploited in PDAC. As such, the most feasible strategy for precise prognostic determinations of how a given malignance will progress or respond to ICB treatment may be one based on molecular risk allocation, stratifying patients on line with particular molecular signatures correlated with prognosis, improving efficacy accordingly.

Long noncoding RNA (lncRNA) whose RNA transcripts length longer than 200 bp is not able to code protein (11). To this day, more and more studies have arrived at an agreement that lncRNAs serve as a crucial regulator in cancer immunity, including antigen release, and immune activation (12, 13). An independent research found that lncRNA GAS5 expression level was downregulated in HCC patients and lncRNA GAS5 interference promoted tumor cell migration and dampen NK cell cytotoxicity (14). Analogously, lncRNA TCONS_00019715 may function to facilitate tumoricidal activities through regulating macrophage polarization to the M1 phenotype(15). Several researches have suggested the latent role of the lncRNAs risk model for prognostic prediction for patients with PDAC(16, 17). Nevertheless, the existing immune-related lncRNA risk score signature for PDAC patients' responsiveness to ICB is lacking.

Here, we employed a systematic analyze to recognize and confirm a robust and novel biomolecular risk model based on the immune-associated lncRNAs classifier for PDAC prognosis. Then, we explored the latent role of this immune-correlated lncRNAs risk score model in immune checkpoint inhibitor treatment. Our findings established an immune-correlated lncRNAs signature on account of lncRNA expression level which could precisely forecast PDAC survival through comprehensive analysis of genomic files and thus put promising insights into the approaches of predicting prognosis and responsiveness to ICB in PDAC patients.

2. Methods

2.1 Patients and Datasets

We downloaded pancreatic ductal adenocarcinoma cases from The Cancer Genome Atlas (TCGA) portal (http://cancergenome.nih.gov). Patients without intact genomics or clinical data were excluded (n = 8), leaving 177 PDAC samples in the final cohort. The analysis process flow chart was presented in Fig. 1. Altogether 177 PDAC cases were stochastically assigned into the training and validation set at the rate of 1:1 for systematic analysis employing R project "caret" package. Both training and validation set need to comply with the following requirements: (1) cases were stochastically classifier as train and test group; (2) samples in two sets had similar clinicopathological characteristics. The testing cohort with 88

patients and the combination group were further employed to confirm results derived from the training group. There was no necessity to obtain Ethics Committee approval, owing to all information were publicly available and open-access.

2.2 Immune-Related lncRNAs

The lncRNA profile was determined applying a constructed mining method refer to published articles(18). Briefly, genes were recognized as protein-coding genes or non-coding genes based on their Ensembl IDs or Refseq IDs, and only the long non-coding genes in NetAffx Annotation files were retained. We downloaded the immune gene data from the ImmPort data project, and 2,483 immune-associated genes were gained (19, 20). We employed the Pearson correlation to analyze the correlation between immune-related genes and lncRNAs. The square of correlation coefficient $P < 0.005$ and $|R| > 0.4$ was set for immune-associated lncRNAs. To visualize coexpression networks, we employed Cytoscape software 3.7.2.

2.3 Identification of Predictive Immune -Correlated lncRNAs

To evaluate the prognosis of immune-correlated lncRNAs, we employed this lncRNAs signature to assemble a unitive risk score model in PDAC. Firstly, we employed univariate Cox regression analysis for 1394 immune-correlated lncRNAs in the training group. The results with $p < 0.01$ was considered to be statistical significance. And 11 immune-related lncRNAs were filtered out. Secondly, these recognized lncRNAs were further screened and confirmed via the least absolute shrinkage and selection operator (LASSO) algorithm using R project “glmnet” package. Then, a multivariate Cox regression model was analyzed. Finally, we identified 5 immune-correlated lncRNAs and calculated their corresponding coefficients to construct the prognostic lncRNAs risk score signature in PDAC. Finally, this immune-related lncRNA prognosis risk model was established based on linearly combining the formula below with the expression level multiplied regression analysis (β). Risk score = βlncRNA1 × lncRNA1 expression + βlncRNA2 × lncRNA2 expression + · ···· +βlncRNA n × lncRNA n expression. Here, β was the regression coefficient of the multivariate Cox regression analysis(21). Besides, we compared these 5 lncRNAs expression levels in PDAC and normal tissue specimens utilizing The Genotype-Tissue Expression (GTEx) and TCGA transcriptomic profiles.

2.4 Validation of the immune-correlated lncRNAs risk model

Based on their respective risk score, patients together with their clinical data were allocated. We employed the median risk score to assign cases into high-risk and low-risk sets for further research. Kaplan–Meier survival curves were analyzed in both sets. Then, the time-dependent receiver operating characteristic (ROC) curves were plotted to evaluate the predictive survival performance. Moreover, multivariate Cox regression analysis was performed to validate whether the signature could be used as an independent biomolecular indicator to predict survival. The predictive precision of this immune-related lncRNA risk score model was further confirmed in the testing set (n = 88) and combination cohort. $P < 0.05$ was deemed statistically significant, and each test was two-sided.

2.5 Development of nomogram

To estimate the prognostic capability of risk score, stage, gender, age and WHO grade for 1/3/5-year OS, receiver operating characteristic (ROC) curves was carried out to assess the area under the curve (AUC) values (22). To open up a quantitative method to forecast the survival of PDAC patients, we constructed and plotted a nomogram that including this seven-lncRNA risk score and other clinical characteristics to assess 1-, 3-and 5-year OS possibility.

2.6 Function of immune-correlated lncRNA signature on PDAC

We carried out Gene set enrichment analysis to investigate underlying mechanisms significantly correlated with our 5 immune-correlated lncRNA risk model. We analyzed the gene sets of "c5.go.v7.2.symbols.gmt[gene ontology]" from the Molecular Signatures Database through GSEA(23). To achieve a normalized enrichment score for each analysis, gene set permutations with 1,000 times were carried out. A nominal $p < 0.05$ were deemed significant results.

2.7 Association with Tumor-infiltrating immune cells

CIBERSORT consisted of 22 TIIC subsets utilizes a deconvolution strategy to reveal the abundance of tumor-infiltrating immune cells (TIICs) (24). The correlation between 22 TIIC subsets with the immune-related lncRNA risk model was carried out to investigate whether our as-constructed immune-correlated lncRNAs signature serve as key roles in immune infiltration of PDAC.

2.8 Association with Immune checkpoint inhibitors related genes

According to previous research, immune checkpoint blockade therapy-correlated crucial genes expression might be associated with responsiveness to immune checkpoint blockade treatment(25). We employed six key genes of immune checkpoint inhibitors therapy: programmed death 1 (PD-1, also known as PDCD1), programmed death ligand 1 (PD-L1, also known as CD274), programmed death ligand 2 (PD-L2, also known as PDCD1LG2), T-cell immunoglobulin domain and mucin domain-containing molecule-3 (TIM-3, also known as HAVCR2), cytotoxic T-lymphocyte antigen 4 (CTLA-4), and indoleamine 2,3-dioxygenase 1 (IDO1) in PDAC(26–28). To explore the latent role of our as-constructed lncRNAs risk model in ICB treatment in patients with PDAC, we analyzed the association between our risk score model and these six immune checkpoint blockade key genes expression.

2.9 Statistical analysis

Statistical significance was set at a threshold of a two-tailed $P < 0.05$. Construction of the immune–lncRNA coexpression network was completed using CYTOSCAPE software (29) (version 3.4.0; The Cytoscape Consortium, San Diego, CA, USA). R software (version 4.0.2; R Foundation) was adopted for all analyses. GSEA (http://www.broadinstitute.org/gsea/index.\jsp) was utilized to distinguish between two sets of functional annotations.

3. Result

3.1 Establish of a Coexpression Network

We obtained 14,142 lncRNAs from TCGA- PAAD. Altogether 2,483 immune-correlated genes were downloaded in the ImmPort portal(https://www.immport.org). The lncRNA and immune-related gene coexpression network was assembled to visualize these immune-correlated lncRNAs. Ultimately, 1394 immune-correlated lncRNAs were screened ($P < 0.005$ and $|R| > 0.4$) in our research (Table S1).

3.2 Identification of Immune-Correlated lncRNA Signature.

Cases from TCGA- PAAD cohort were randomly assign into training set and internal testing group at the ratio of 1:1 (Tables S2, S3). On the basis of the results of univariate Cox model, we found 11 immune-related lncRNAs possess valuable prognostic performance in PDAC patients ($P < 0.05$, Table S4). Next, we applied LASSO Cox regression algorithm to validate further variables and 7 immune-related lncRNAs were recognized (Figs. 2A,2B and Table S5). Multivariate proportional hazards model was carried out, then 5 immune-related lncRNAs finally recognized as the biomolecular predictors of prognosis in PDAC patients. The forest plot of each immune-related lncRNAs together with survival was shown in Fig. 2C. Table 1 presented that LINC02257 and LINC01426 were unfavorable lncRNAs, whereas AC006504.8, FLVCR1 – DT and AC068282.1 were protective lncRNAs. Comparison of genomics files in GTEx as well as TCGA presented that the expression level of most lncRNAs was notably differentially expressed in PDACs (Figs. 2D and 2E). We further assembled immune lncRNAs co-expression networks (Figures S2A, S2B). Subsequently, five immune-correlated lncRNA signature was developed utilizing a risk score method(30–32). Subsequently, the risk score was calculated as follows: (0.3622 × LINC02257 expression) + (-0.6891 × AC006504.8 expression) + (0.4879 × LINC01426 expression) + (-0.7337 × FLVCR1-DT expression) + (-0.7348 × AC068282.1 expression).

Table 1
Multivariate Cox results of lncRNAs based on TCGA PAAD data.

id	coef	HR	HR.95L	HR.95H	pvalue
LINC02257	0.362284	1.436607	1.097942	1.879734	0.008262
AC006504.8	-0.6892	0.501979	0.239843	1.050616	0.06741
LINC01426	0.487992	1.629043	1.188965	2.232008	0.002388
FLVCR1-DT	-0.73376	0.480101	0.222018	1.038192	0.062222
AC068282.1	-0.73489	0.47956	0.203948	1.127632	0.092064

3.3 Confirmation of Immune-Correlated lncRNA Signature

Every case gained a risk score in the group of PDAC by adopting this 5 immune-related lncRNA model, and all patients were randomized into high-risk or low-risk set based on the median threshold. Figure 3A presented the allocations of 5 lncRNAs expression levels together with cases and groups. The distributions of risk score as well as survival time in the training group, which indicated that cases in high-risk set commonly had poorer prognosis in PDAC(Figs. 3B, 3C). Besides, Kaplan–Meier curves displayed that cases with low risk presented significant better prognosis compared with high-risk cases ($P = 6.821e - 07$) in the training set (Fig. 3D). To further estimate the value of this immune-related lncRNAs classifier in forecasting survival of PDAC, we analyzed ROC curves. Figure 3E shown that the value of the area under the curve (AUC) for this immune-related lncRNAs signature reached 0.747. Furthermore, the hazard ratio (HR) for risk score in univariate proportional hazards model analysis was 1.213 (95% CI: 1.119 − 1.314; Fig. 3F). And we obtained accordant results employing multivariate Cox model (HR = 1.254, 95% CI: 1.133 − 1.387; Fig. 3G).

3.4 Validation of the lncRNA Model

Next, we further validated these findings in the internal validation group and the whole set to confirm the prognostic value of this lncRNA risk model among distinct populations. The allocations of lncRNA expression level, survival time, and risk score in the internal testing set and the whole cohort were presented Figs. 4A-C and Figures S2A-C. Moreover, we analyzed Kaplan–Meier curves and found that the PDAC cases in low-risk set presented a notably better prognosis than the high-risk score cases in the internal validation cohort and the combination set (Fig. 4D and Figure S2D; all $P < 0.05$). The values of area under the ROC (AUC) reached both up to 0.71 or more in the internal validation group and the whole cohort (Fig. 4E; Figure S2E), indicating an outstanding performance of this immune-related lncRNA model to predict survival in PDAC. In line with the findings obtained in the training group, this immune-related lncRNA risk score was an independent predictive bimolecular indicator in both univariable as well as multivariable regression analysis of the test group and the whole set (Figs. 4F-G and Figures S2 F-G).

3.5 Validation of predictive value of this lncRNAs signature

To confirm whether the survival prediction was best by this lncRNA signature among all clinical parameters, age, gender, stage and grade were collected as the candidate predictive biomolecular indicators. We analyzed the AUC curve for 1-, 3-, and 5-year prognosis and found that our risk model possesses the highest AUC among these factors(Figs. 5A, B, and C). We assembled a nomogram on the basis of risk signature and age to forecast prognosis of PDAC cases (Fig. 5D). Stage, gender and grade were excluded from the nomogram due to their AUCs were lower than 0.6. By and large, the risk score model we established can provide the most helpful and precise guide to predicting survival among these prognostic indicators.

3.6 Functional Annotation of Immune-related lncRNA Signature

To explore the biological implications of this immune-associated lncRNAs risk model mediated in PDAC occurrence and progression, we employed out GSEA in both the low-risk and high-risk sets. Our findings

displayed that the high-risk score of lncRNAs signature presented marked enrichment in pathways, such as chondrocyte development, keratinocyte proliferation, laminin binding and so on (Fig. 6).

3.7 Association of the Prognostic lncRNAs risk model with ICB treatment key gene and immune cell infiltration

We further explored whether this immune-related risk score model was correlated with TIICs. We found the as-constructed signature exhibited the marked negative association with CD4 + T cells infiltration (r = − 0.184; p = 0.014, Figs. 7A). These results strongly provide evidence to support that our prognostic lncRNA risk score model was significantly correlated with immune cell infiltration in PDAC.

Then, we explored six key immune checkpoint inhibitors-related genes: PDCD1, CD274, PDCD1LG2, CTLA-4, HAVCR2, and IDO1(26–28). We analyzed the correlation between the ICB therapy key targets and immune-related lncRNA signature to investigate the latent role of this lncRNAs risk score model in the ICB immunotherapy in patients with PDAC (Fig. 7B). The findings presented that this immune-related lncRNA risk score model was markedly negative correlated to PDCD1 (r = − 0.184; p = 0.014) and CTLA4 (r = − 0.168; p = 0.025; Figs. 7C-D), indicating that this immune-related lncRNAs risk model might be a key part in the assessment of responsiveness to ICB immunotherapy in PDAC.

4. Discussion

Pancreatic ductal adenocarcinoma (PDAC) is regarded as a devastating malignant tumor and will rank the second leading cause of cancer deaths by 2030(33). The 5-year prognosis remains very poor, as up to 85% of cases present with either distant metastatic lesion or unresectable disease(34). Moreover, PDACs characteristic with a high intra- and inter-tumor heterogeneity, resulting in patients with the same clinical features exhibit distinct clinical outcomes and response to treatments(35). As the only predictive indicator utilized routinely in clinical application to forecast the survival and guide clinical management, the AJCC TNM staging fails to forecast the clinical outcome for patients with the same clinical stage (36). Thus, there is urgent need to exploit the novel and effective biomarkers for early diagnosis, treatment option, and survival evaluation to improve the prognosis for PDAC. In the past decades, more and more studies provided evidences to support that lncRNAs might play key roles in tumorigenesis, progression and invasion in PDAC(37–39).

According to previous researches, lncRNAs were reported as crucial regulators in regulating cancer immunity(13). A recent research from Mineo Marco et al reported that INCR1 knockdown can improve CAR T cell therapy via sensitizing tumor cells to cytotoxic T cell-mediated clearing (40). Another research showed encouraging potentiality for new clinical management decisions on the basis of epigenetic regulation targeting LncMALAT1, which can coordinate with the immune system(41). More and more evidences has strongly supported that immune-related lncRNAs may be novel disease biomolecules for cancer clinic treatment and possess valuable prognostic significance for survival(42, 43). Several

immune-related lncRNA signatures have been explored in some tumors, such as bladder cancer, breast cancer, and colon cancer(44–46). Nevertheless, the latent role of immune-correlated lncRNAs risk score model as a helpful predictive indicator needs further validated in cancer immune checkpoint therapy, especially in PDAC.

Here, our study assembled an immune-associated lncRNA signature and explored its predictive performance, as well as its role in immune cell infiltration and the assessment of responsiveness to immune checkpoint blockade treatment for PDAC patients. In our research, immune-associated lncRNAs were systematically identified through employing univariate Cox regression model as well as the biostatistics method. Subsequently, we employed LASSO algorithm analysis in lncRNA files derived from TCGA database, and final 5 significant immune-related lncRNAs were recognized. These five lncRNAs were included into developing the predictive risk score model. Subsequently, Kaplan–Meier curves, the timedependent ROC curves, and Cox regression analysis were employed to confirm the predictive performance of this immune-correlated lncRNAs risk score model, which can serve as an independent biomolecular indicator to forecast PDAC survival. Further validation was analyzed in both the internal testing group and combined cohort.

Subsequently, our pathway enrichment results suggested the latent impact of our immune-related lncRNA risk model on PDAC tumorigenesis and progression via chondrocyte development, keratinocyte proliferation, laminin binding and so on. Our results provide new evidence for strongly supporting that lncRNAs whose functions was still unclear may be novel biomarkers for predicting clinical outcomes in PDAC. Nonetheless, these findings require to be confirmed in further researches.

With the rise of immunotherapy, immune checkpoint blockade (ICB) treatment has markedly transformed anti-tumor immunopathological treatment (47–49). Preclinical research of immunotherapy for pancreatic cancer showed some promise, making it in the limelight(50). Its efficacy in treating patients with PDAC, however, is limited by its immunosuppressive microenvironment (51). Such biomolecules as immune checkpoint gene and tumor mutational burden cannot accurately forecast clinical outcomes from ICB treatment. Thus, exploiting biomolecular markers that can precisely forecast responsiveness to ICB is crucial to further advance precision immunotherapy(27, 52). Several studies reported that lncRNAs associated with immune reaction could forecast responsiveness to clinical treatment (53, 54). In this study, the association analyses shown that PDCD1, CD274, PDCD1LG2, CTLA-4, HAVCR2, and IDO1 were coexpressed. Besides, this immune-correlated lncRNA risk score was significantly correlated with the ICB treatment target genes (i.e., PDCD1 and CTLA4). These findings indicated that this immune-related lncRNA risk score model may serve as a key part to measure the responsiveness to ICB treatment of PDAC patients. Recently, accumulating evidences have supported that numerous lncRNAs possess key roles in regulating immunity, such as immune cell infiltration, antigen presentation and so on(12, 13). Here, our results shown that this immune-correlated lncRNAs risk model was markedly correlated with CD4 + T cells infiltration in PDAC, which indicated that as-constructed immune-correlated lncRNA risk score model might serve as a key role in immune cell infiltration in PDAC.

Compared with published researches that investigated the lncRNA prognostic performance in PDAC, some superiorities of our research are as follows. First of all, our study is the first to explore the correlation between immune-related lncRNA signature and immune infiltration in PDAC. Besides, as far as we know, this research is the first to exploit immune-related lncRNAs signature which may precisely predict responsiveness to ICB in PDAC.

5. Conclusions

By and large, these five immune-related lncRNAs risk scores model was been found significant correlated with PDAC prognosis, and it could serve as an independent prognostic biomolecular indicator to predict PDAC prognosis. Moreover, as-constructed immune-associated lncRNA signature was be observed to be significantly correlated to immune cell infiltration as well as responsiveness to ICB treatment in PDAC. Conclusively, this research provided a promising avenue to facilitate the individualized survival prediction and gauge responsiveness to ICB antitumor immunotherapy in PDAC, which may present valuable clinical applications in PDAC ICB immunotherapy. Nevertheless, our findings should be confirmed in future researches which explore PDAC occurrence and development mechanisms and the implication of these 5 immune-related lncRNAs.

Abbreviations

AUC: area under the curve

PDAC: pancreatic ductal adenocarcinoma

CTLA-4: cytotoxic T-lymphocyte antigen 4

CI: confidence interval

CD274: Also known as PD-L1

GSEA: Gene set enrichment analysis

HR: hazard ratio

HAVCR2: Also known as TIM3

IDO1: indoleamine 2,3-dioxygenase 1

ICB: immune checkpoint blockade

LncRNAs: long non-coding RNAs

LASSO: least absolute shrinkage and selection operator

OS: overall survival

PD-1: Programmed Cell Death 1

PD-L1: Programmed Cell Death-Ligand 1

PD-L2: Programmed Cell Death-Ligand 2

PDCD1: Also known as PD-1

PDCD1LG2: Also known as PD-L2

ROC: receiver operating characteristic

TCGA: The Cancer Genome Atlas

TIICs: tumor-infiltrating immune cells

TIM-3: T-cell immunoglobulin domain and mucin domain-containing molecule-3

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets generated for this study can be found in the https://portal.gdc.cancer.gov.

Competing interests

The authors declare no conflicts of interest.

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Authors' contributions

Wen Huang conceived and designed the experiments. Qianhui Xu, and Yuxin Wang analyzed the data. Qianhui Xu wrote the manuscript. All authors read and approved the final manuscript.

*Qianhui Xu, and Yuxin Wang contributed equally to this paper.

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Figures

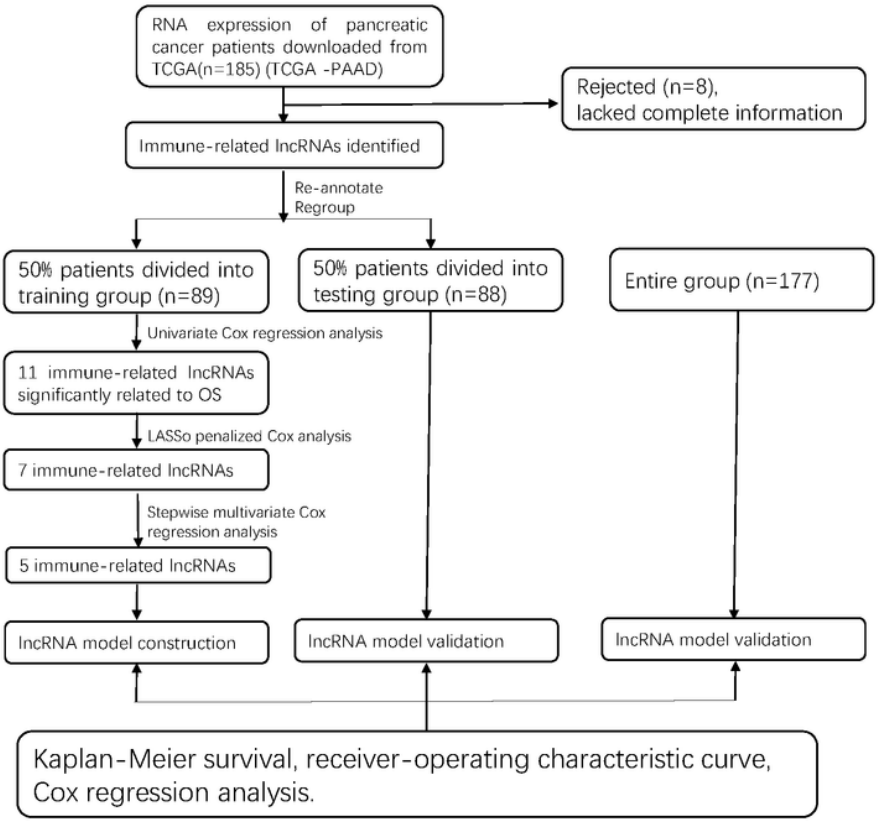
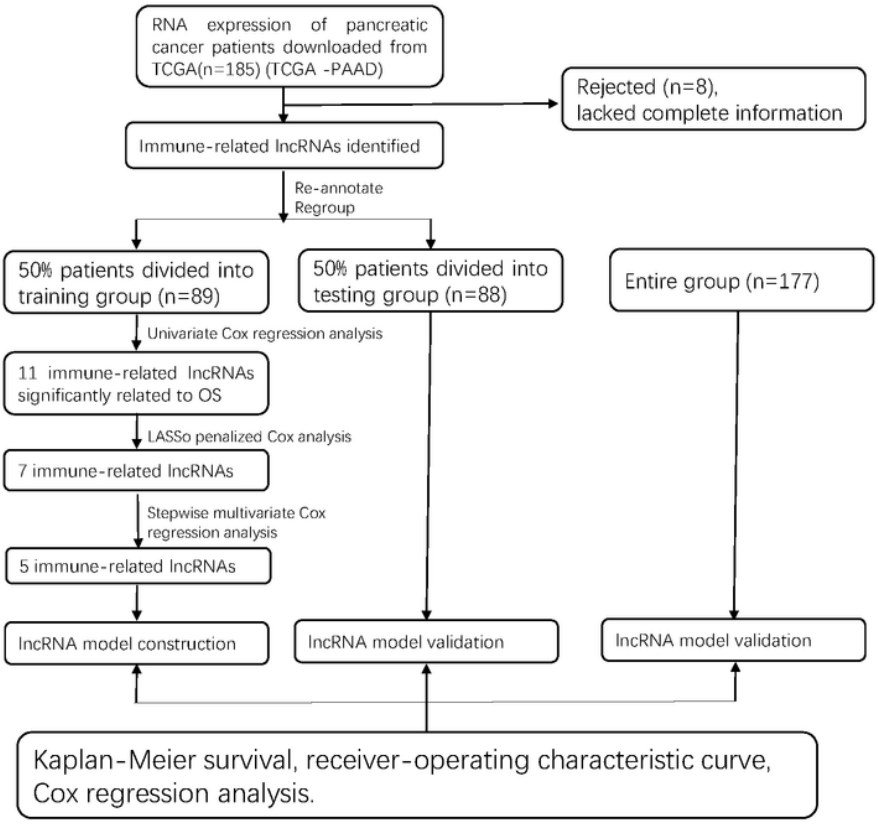


Figure 1

Overall research design. Flow-process diagram presenting the process utilized to identify immune-lncRNAs.



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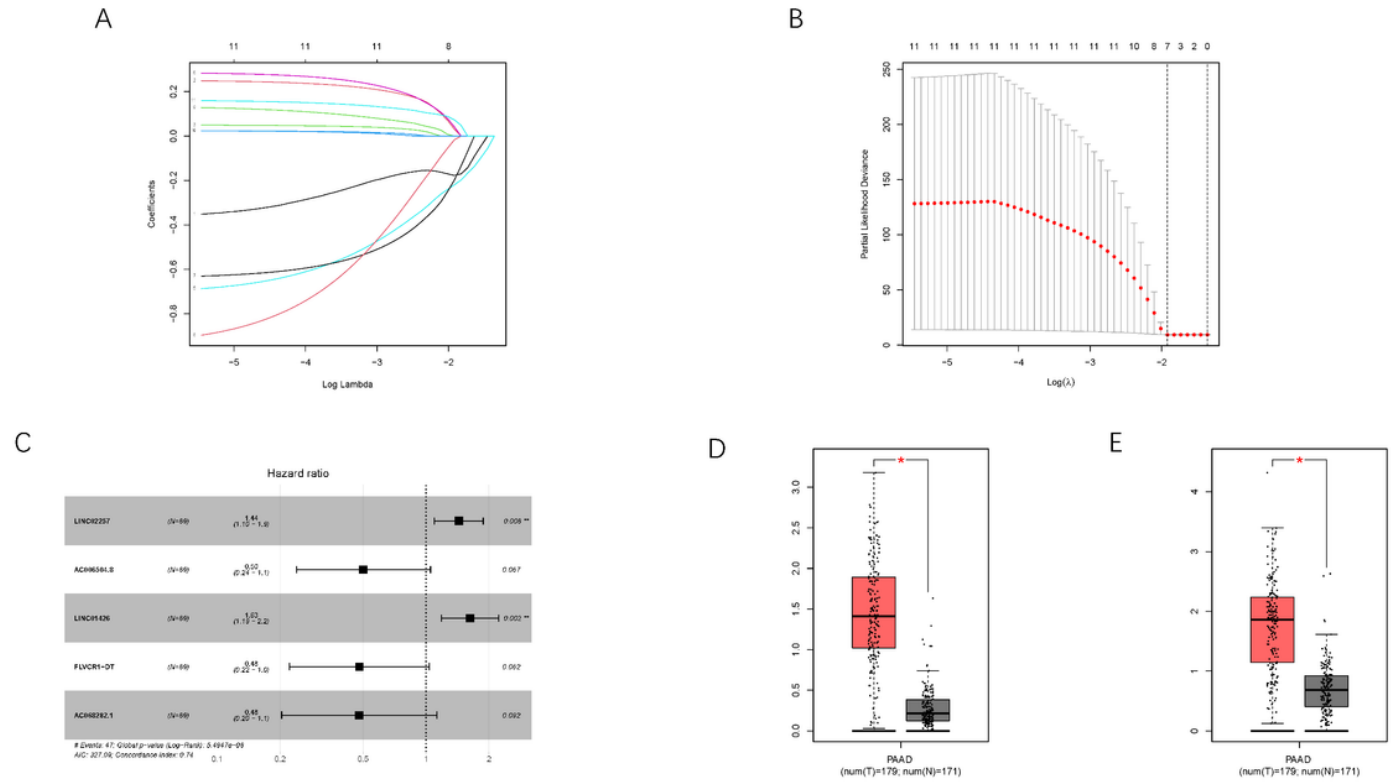
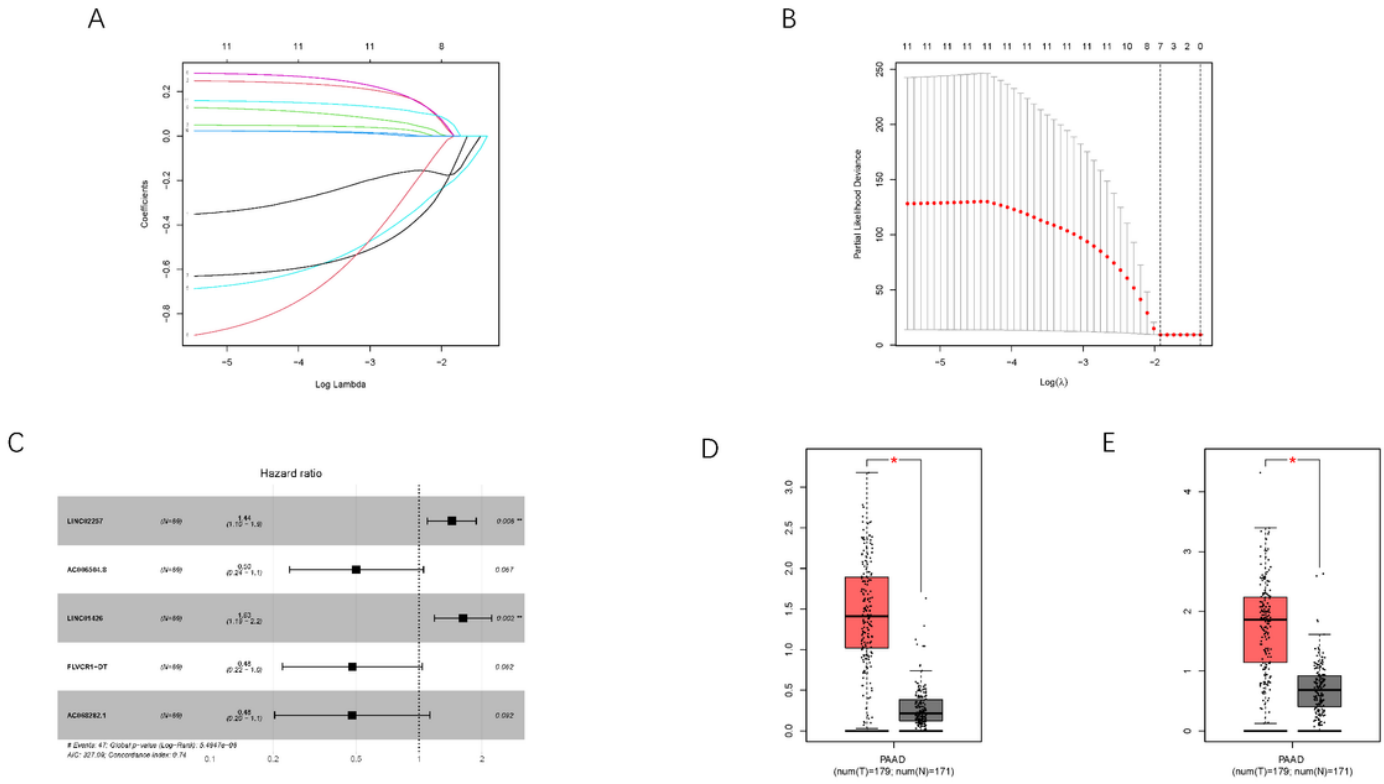


Figure 2

Regression coefficient diagram based on LASSO algorithm. A) LASSO coefficient profiles of 11 immune-lncRNAs. A vertical line is drawn at the value chosen by 10-fold cross-validation. B) Ten-time cross-validation for tuning parameter selection in the lasso regression. The vertical lines are plotted based on the optimal data according to the minimum criteria and 1-standard error criterion. The left vertical line represents the 7 lncRNAs finally identified. C) Forest plots showing the relationships of each lncRNA subsets with OS in training group. The unadjusted HRs are presented with 95% CIs. D) LINC01426 expression level of lncRNA in TCGA cohort. (P<0.05) E) FLVCR1-DT expression level of lncRNA in TCGA cohort. (P<0.05)



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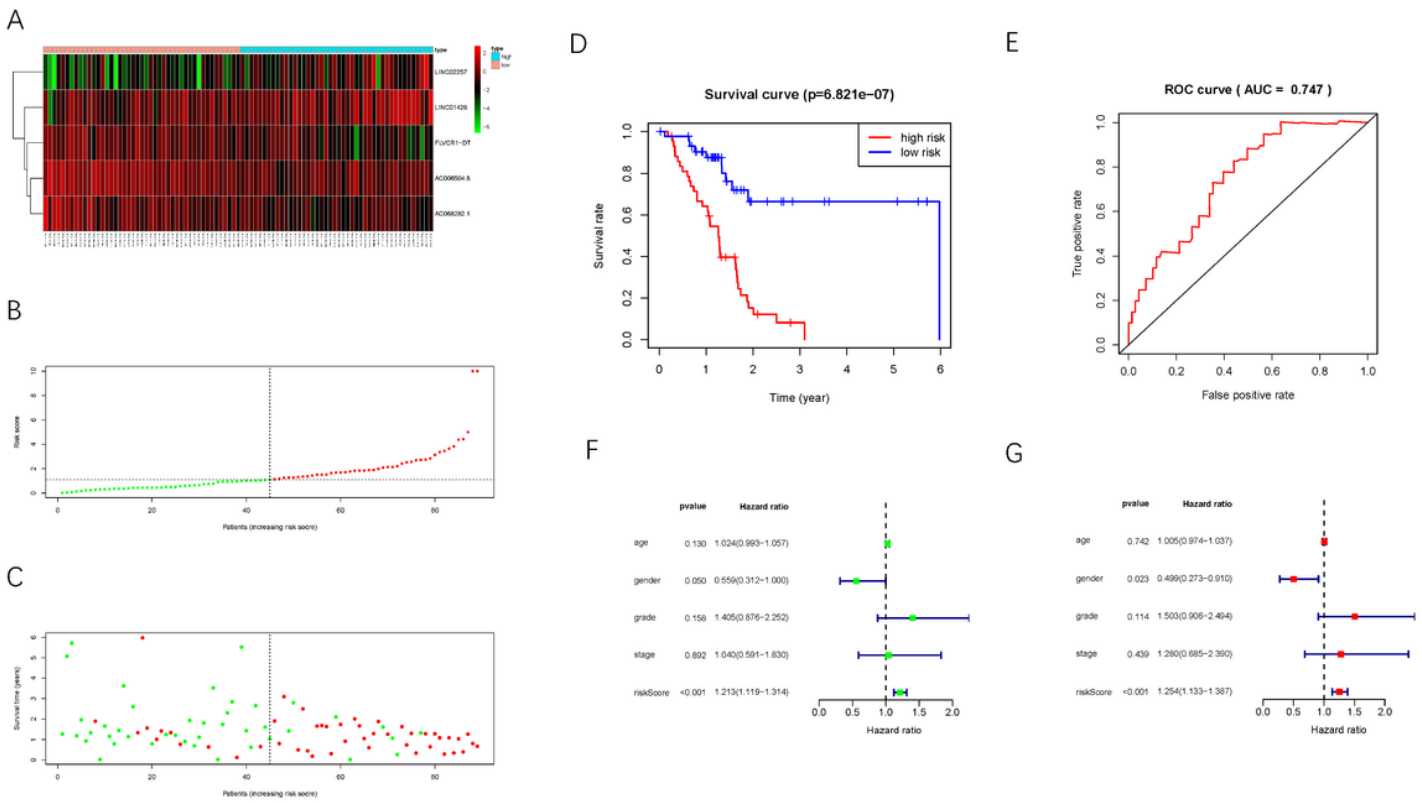
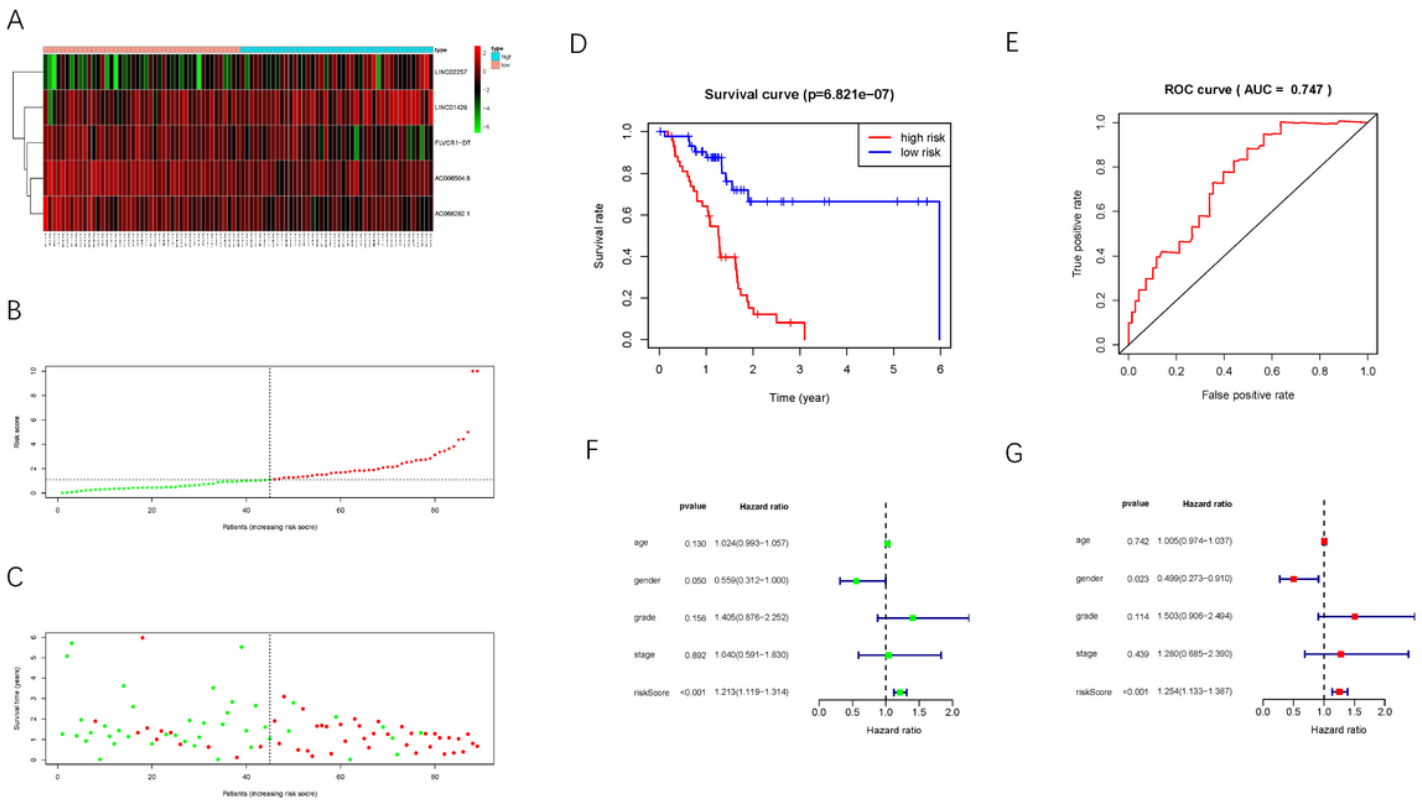


Figure 3

Confirmation of this immune-correlated lncRNA risk model for forecasting PDCA prognosis in the training set. A) Heatmap of the 5 immune-related lncRNAs expression in PDCA. The color from red to green shows a trend from high expression to low expression. B) Distribution of lncRNA model risk score. C) The survival status and duration of PDCA patients. D) Kaplan–Meier curve presenting survival in the high-risk and low-risk sets. E) ROC analysis of the risk scores for overall prognosis prediction. The AUC was calculated for ROC curves, and sensitivity and specificity were calculated to assess score performance. Proportional hazards model results. F) Univariate Cox regression results in the training set. G) Multivariate Cox regression results in the training set.



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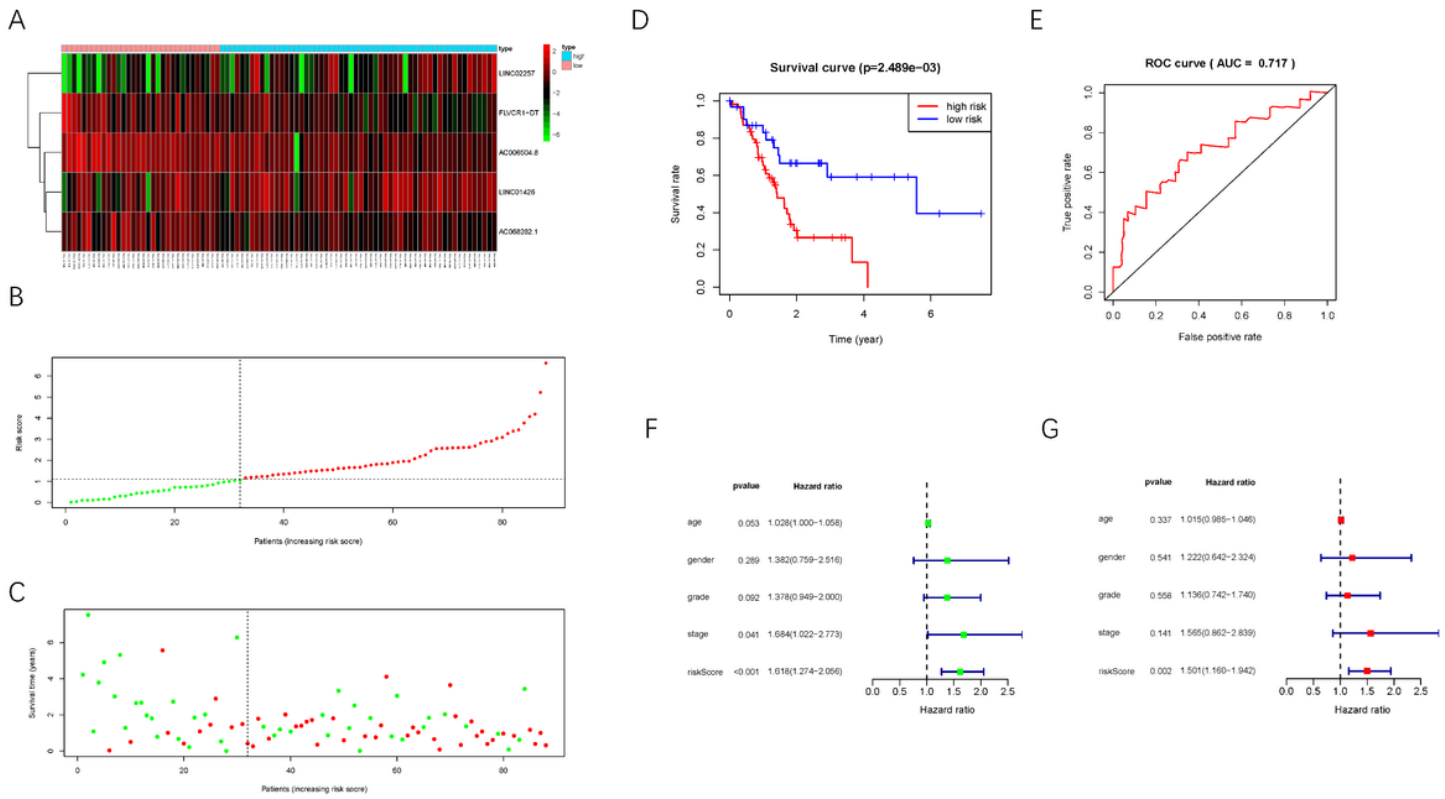

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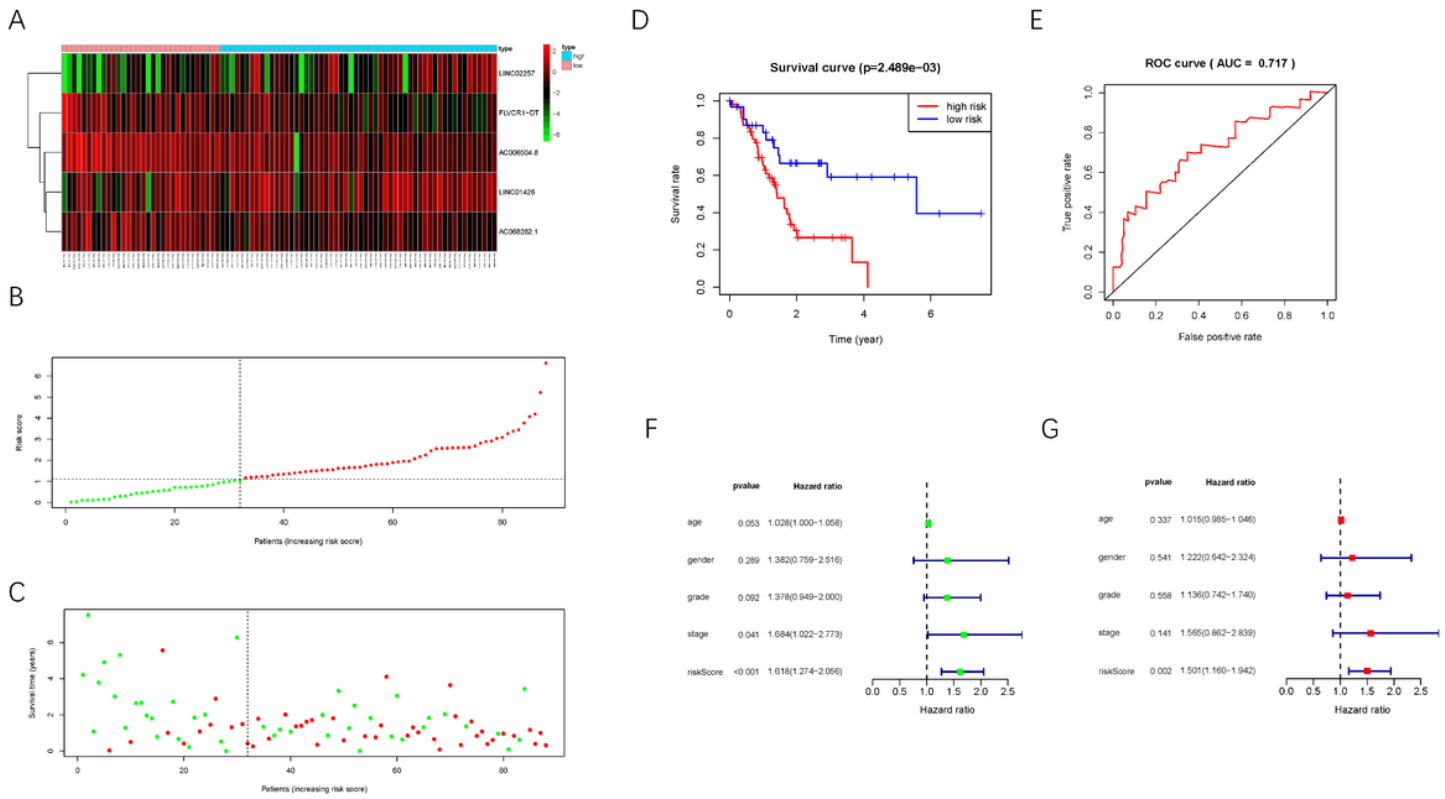

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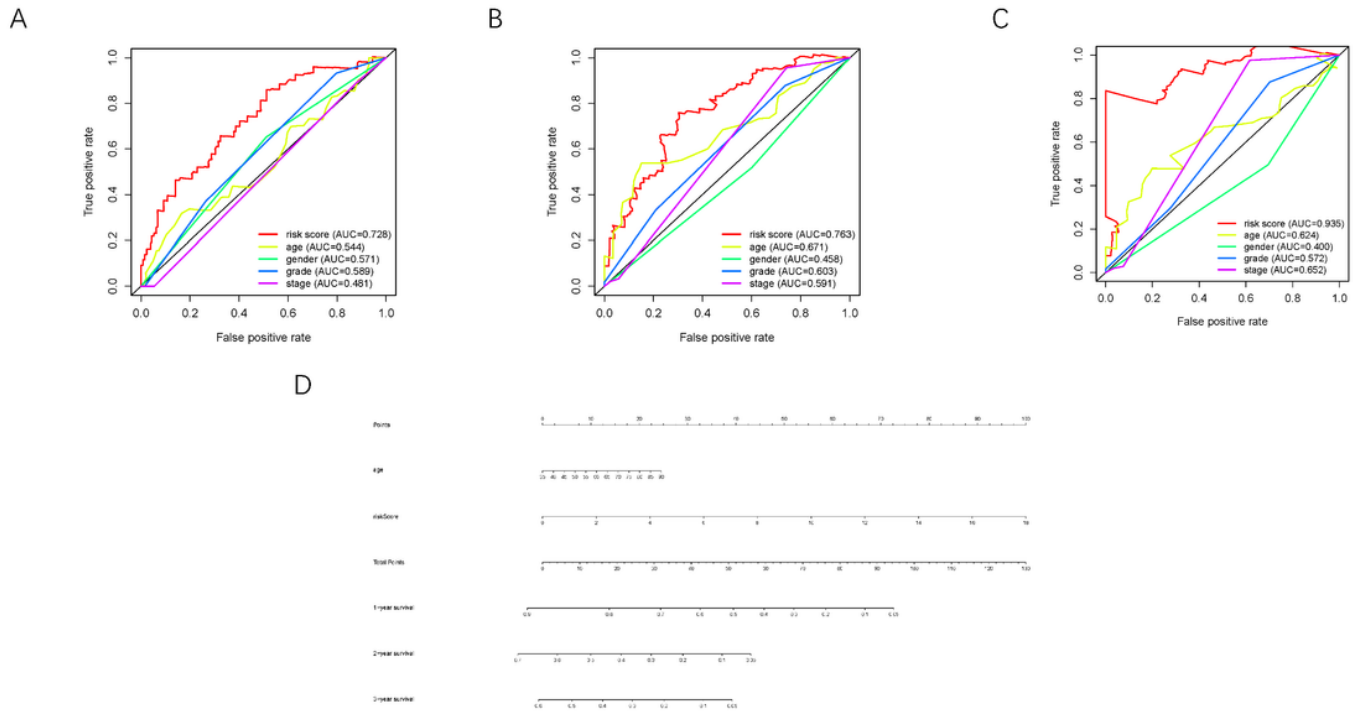

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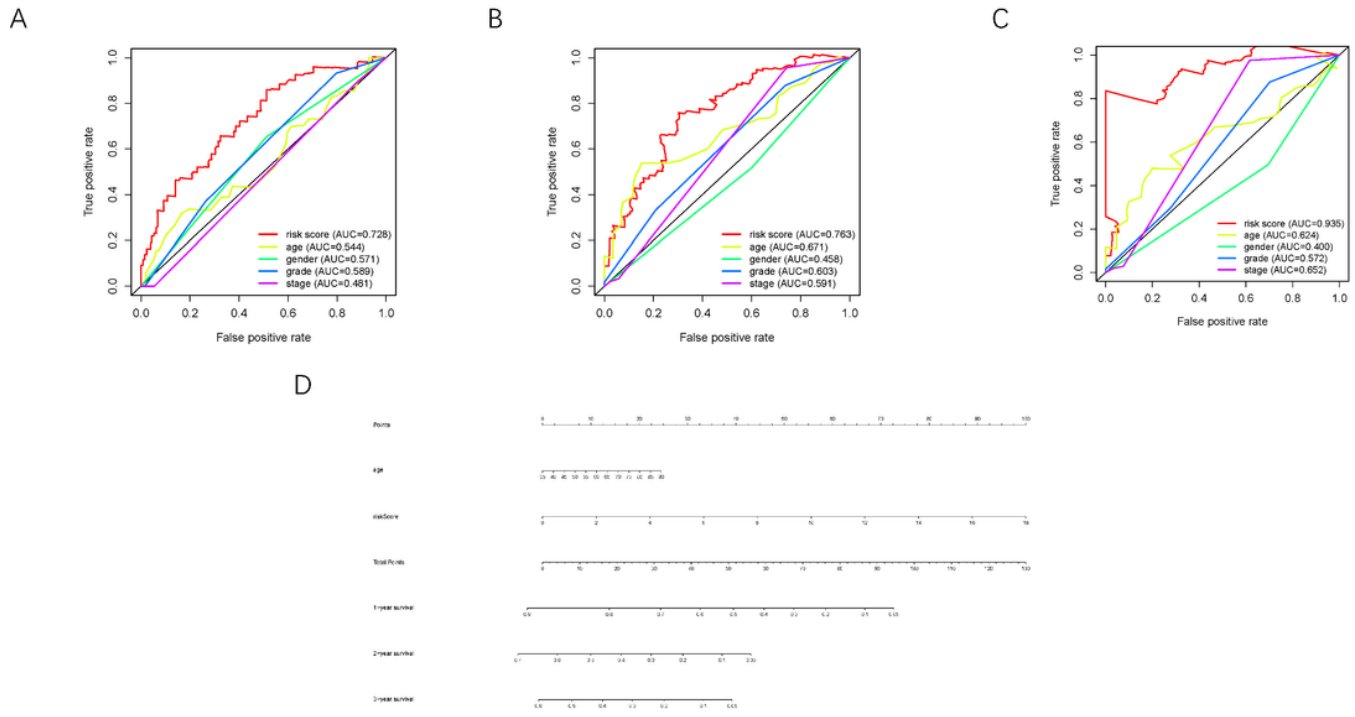

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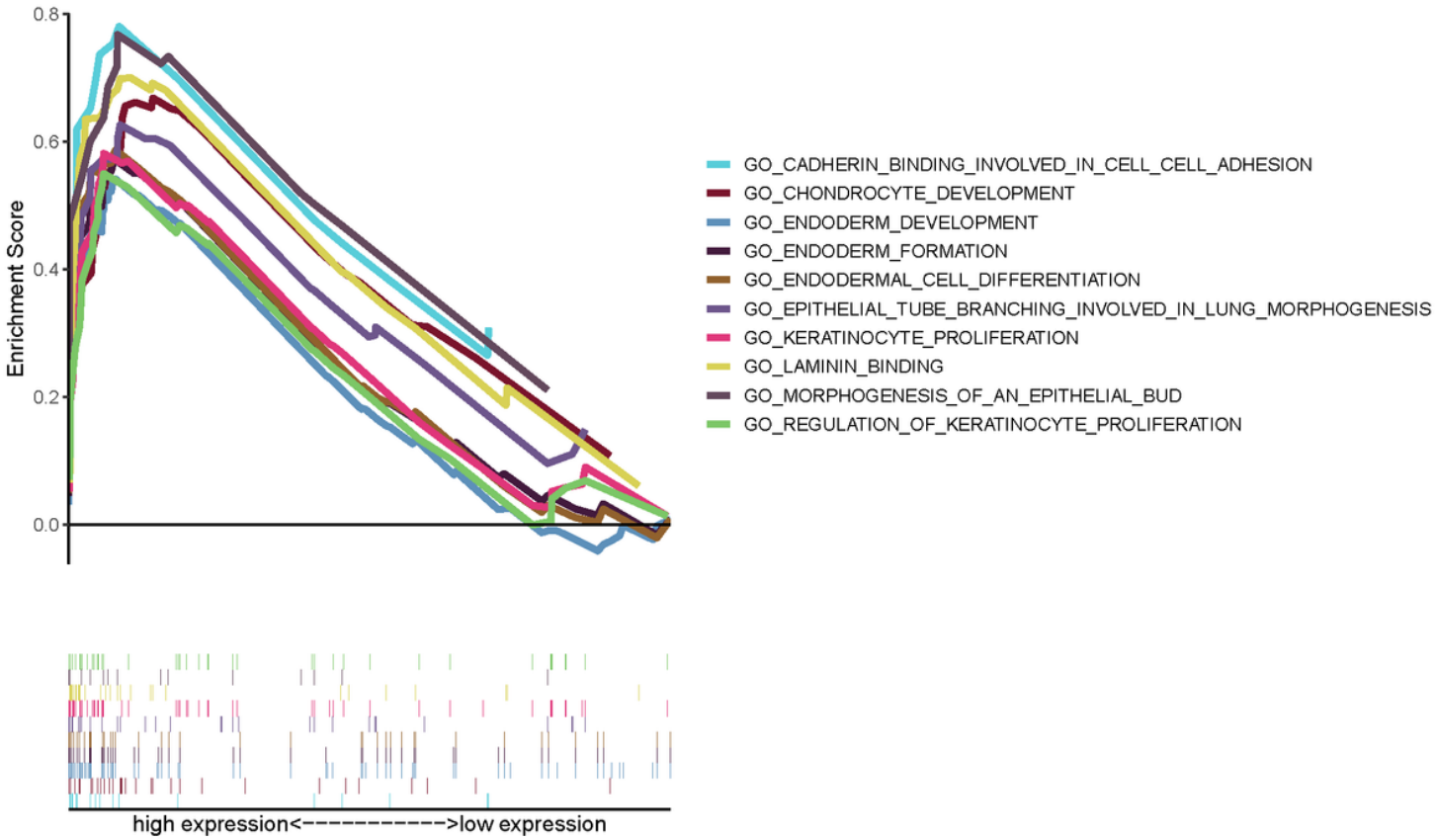
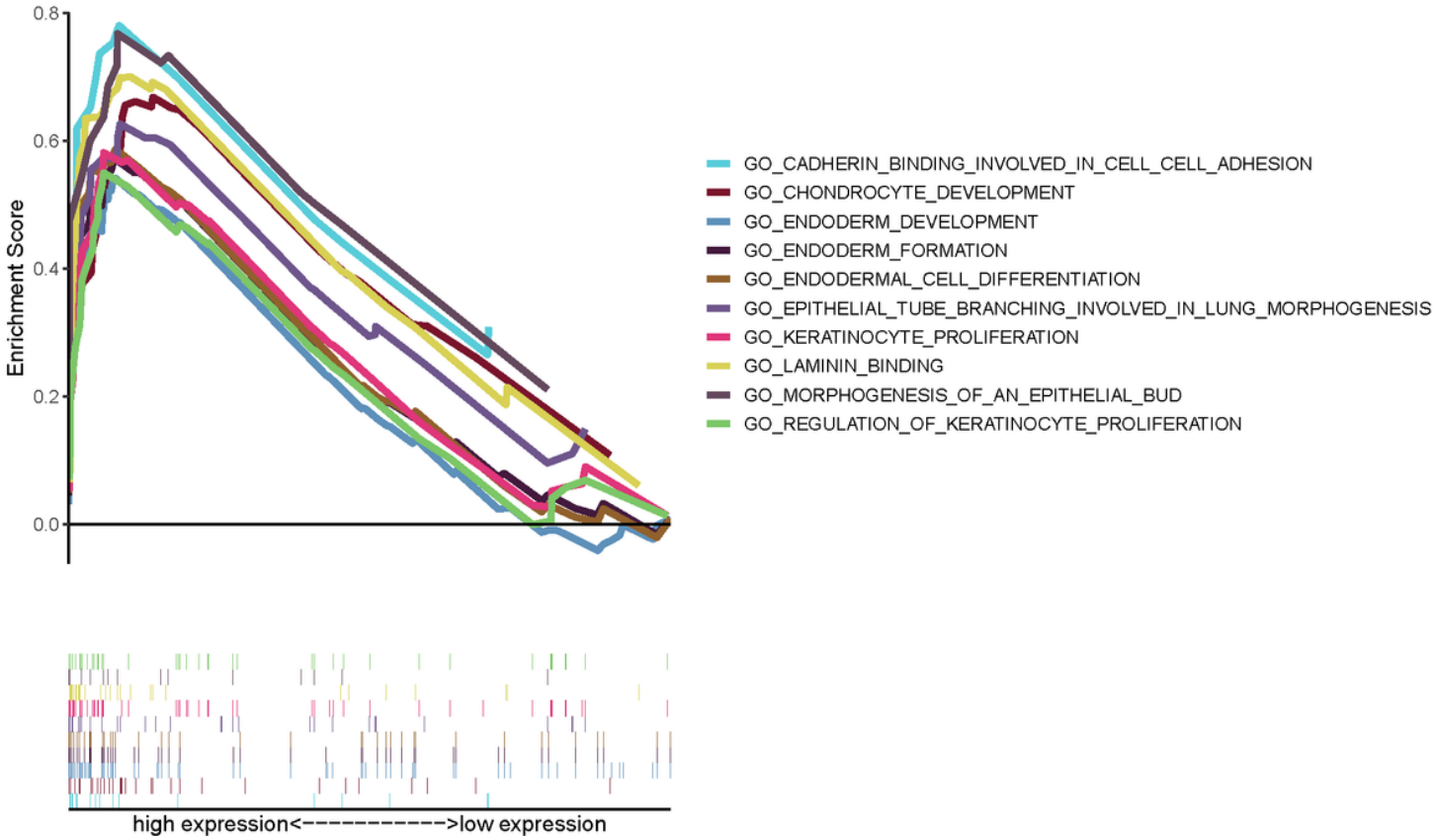


Figure 6

GSEA delineation of the biological pathways associated with the risk scores of this lncRNA signature utilizing the gene set “c5.go.v7.2.symbols.gmt”.



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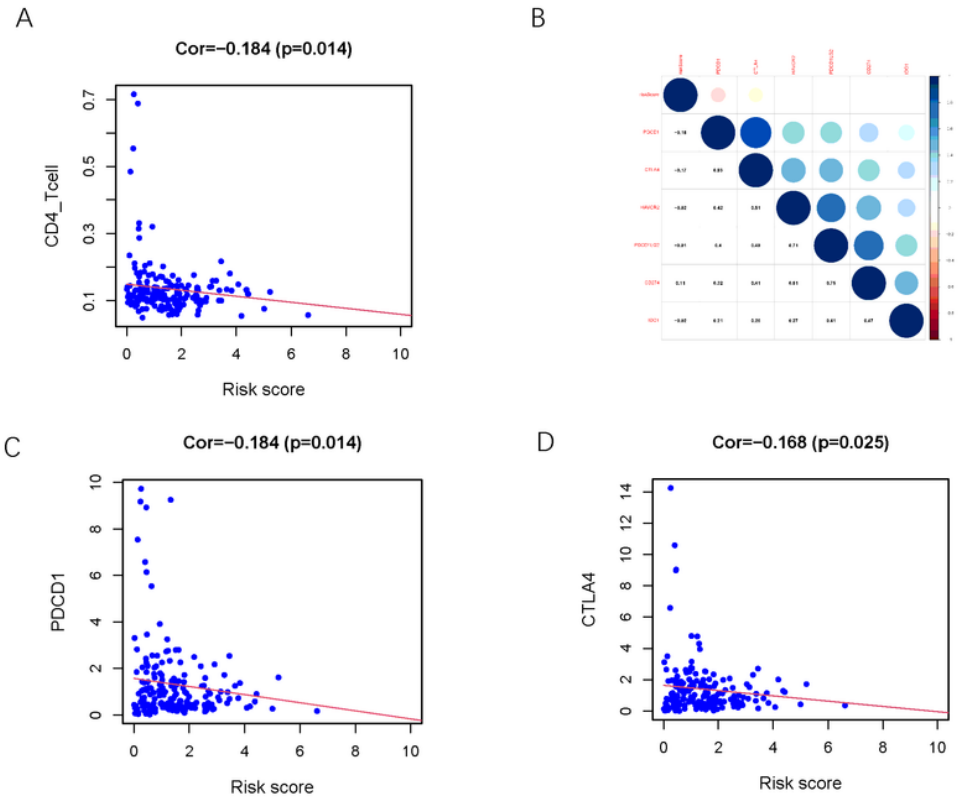
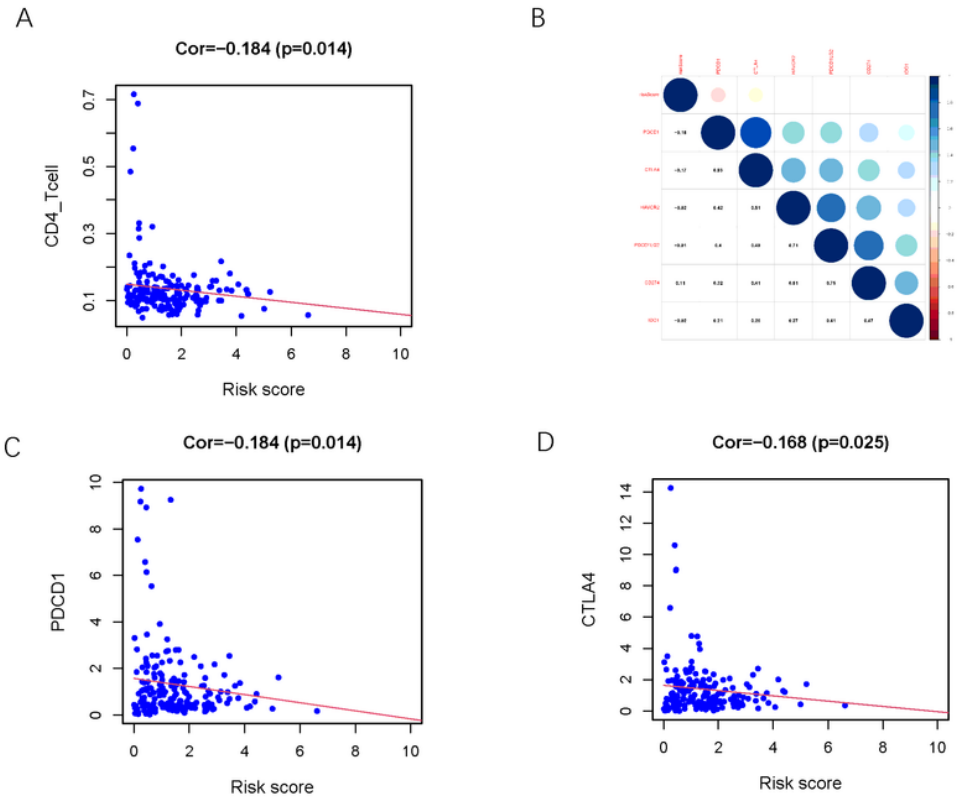


Figure 7

Correlation between tumor immune infiltration and this immune-related lncRNA signature. A) association between this signature and CD4+T cells. Association between our immune-related lncRNA signature and crucial immune checkpoint genes. A) association analyses between immune checkpoint inhibitors CD274, PDCD1, PDCD1LG2, CTLA4, HAVCR2, and IDO1 and our immune-related lncRNA signature. B) association between our risk model and PDCD1, C) association between our risk model and CTLA4.



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