



Comprehensive analysis of disulfidptosis-related lncRNAs for predicting prognosis and response of immunotherapy in pancreatic adenocarcinoma

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Background: Pancreatic adenocarcinoma (PAAD) is a common and deadly tumor. Currently, there is a severe lack of therapeutic options. As a novel mode of cell death, increasing evidence reveals the important role of the disulfidptosis in cancer. However, few studies have utilized disulfidptosis-related long-stranded non-coding RNAs (DRlncRNAs) to investigate the prognosis of PAAD.

Methods: We comprehensively analyzed the expression and prognostic value of 958 DRlncRNAs in PAAD using data from The Cancer Genome Atlas (TCGA). We established and validated a new DRlncRNAs-related prognostic index by least absolute shrinkage and selection operator (LASSO) and COX regression analysis. In addition, we built a nomogram consisting of risk score, age, gender, tumor grade and stage to validate the clinical feasibility of the index. We further evaluated the value of the index in terms of PAAD functional pathways, tumor microenvironment (TME) and tumor mutations.

Results: We designed a risk model for five DRlncRNAs and demonstrated its accuracy using receiver operating characteristic (ROC) curves. COX regression suggested that the model may be an independent predictor of cancer prognosis. Tumor immune infiltration analysis revealed that low-risk subgroups had higher extent of immune infiltration, higher sensitivity to immunotherapy and a higher TME score. This is helpful for us to discover more precise immunotherapy for PAAD patients.

Conclusions: In conclusion, we established a DRlncRNA index comprising of five DRlncRNAs, which may provide new insights for clinical diagnosis and precision therapy.

Keywords: Pancreatic adenocarcinoma (PAAD); disulfidptosis; long-stranded non-coding RNA (lncRNA); prognosis; immunotherapy response

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Introduction

Pancreatic cancer is a malignant tumor that is difficult to diagnose and treat, and ranks fourth in cancer-related deaths worldwide, with a 5-year survival rate of less than 9% (1). In recent years, the incidence and mortality rates of

pancreatic cancer have been on the rise, and it is expected that it will overtake colorectal cancer to become the second most common cause of cancer deaths by 2040 (2). For borderline resectable pancreatic cancer (RPC), surgical treatment is undoubtedly the most effective. However,

only 20% of patients have the opportunity to undergo surgery at the time of diagnosis due to the lack of early symptoms of pancreatic cancer and the presence of lymph node invasion or distant metastasis at the early stage of the disease (2). For junction RPC, neoadjuvant therapy based on FOLFIRINOX chemotherapy regimen can reduce the tumor volume or downstage, and improve the surgical resection rate of pancreatic cancer patients, but it does not significantly improve the prognosis of patients (3). Due to the “cold tumor” characteristics of pancreatic cancer tumor cells, conventional chemotherapy and radiotherapy are not effective in the treatment of locally advanced pancreatic cancer (LAPC). Immunotherapy, as a novel therapeutic strategy, has achieved encouraging results in melanoma, lung cancer, renal cancer, uroepithelial cancer and other malignant tumors. Immunotherapy for pancreatic cancer, including monoclonal antibody therapy, tumor vaccines, and chimeric antigen receptor T cell (CAR-T cell) therapy, has made some progress and demonstrated potential therapeutic effects in clinical trials. However, there are no clear biological markers to predict which patients are more likely to benefit from immunotherapy (4). Therefore, the search for a biomarker that more accurately predicts the prognosis of pancreatic cancer patients and the efficacy of immunotherapy is imminent.

Apoptosis is involved in several physiological and pathological processes in tumors. Resistance to normal death is considered to be the basis of cancer cell genesis

and progression (5). In recent years, many newly discovered apoptotic cell deaths such as ferroptosis, cuproptosis, and immunogenic cell death have attracted attention (6,7). Disulfidptosis is a special mode of cell death that is different from the programmed forms of cell death that have been discovered so far, and its mechanism is that the abnormal accumulation of intracellular disulfide will cause disulfide stress, which ultimately leads to rapid cell death. A recent study has found that cells with high expression of *SLC7A11*, under glucose starvation, abnormally accumulate intracellular disulfide bonds such as cystine, which affects the binding between cytoskeletal proteins, leading to the collapse of the cytoskeletal protein network and rapid cell death (8). In the era of precision oncology, targeting the weakness of tumor metabolism by inducing Disulfidptosis provides new ideas in the field of cancer metabolic therapy (9). The study of Li *et al.* found that the disulfidptosis-related genes (DRGs), *SLC7A11* and *LRPPRC*, play an important role in predicting the prognosis of hepatocellular carcinoma patients (10). DRGs can affect the prognosis and therapeutic effects of hepatocellular carcinoma patients by regulating the tumor microenvironment (TME), drug sensitivity and immune infiltration (11).

Long-stranded non-coding RNA (lncRNA) is a type of non-coding RNAs (ncRNAs) with a length of more than 200 nucleotides that participates in the regulation of a range of physiological and pathological processes but cannot encode proteins. It has been demonstrated that lncRNA is implicated in the genesis and development of several different tumors by way of regulating the biological behavior of tumor cells. For example, *MALAT1*, one of the most studied lncRNAs, is up-regulated in pancreatic cancer cells and plays a significant part in epithelial-to-mesenchymal transition (EMT)-mediated programs to promote pancreatic tumor invasion and metastasis, and its expression has been linked to patients' chances of survival from pancreatic cancer (12). *LUCAT1* is associated with non-small cell lung cancer prognosis and is involved in the proliferation, invasion and metastasis of a variety of cancer cells (13). The regulation of prostate cancer cell growth is carried out by *PCGEM1*. Overexpression of *PCGEM1* inhibits DOX-induced apoptosis in cancer cells (14). However, the relationship between disulfidptosis-related lncRNAs (DRlncRNAs) and the diagnosis, prognosis, and therapeutic efficacy of immunotherapy in pancreatic adenocarcinoma (PAAD) still needs to be further explored.

In this research, we combined the transcription and clinical data of 178 patients with PAAD to develop a novel DRlncRNAs index (DRLI). We further elucidated the

Highlight box

Key findings

- A predictive prognosis model based on disulfidptosis-related lncRNAs was established to predict overall survival in pancreatic adenocarcinoma (PAAD).

What is known and what is new?

- It has been demonstrated that lncRNA is implicated in the genesis and development of several different tumors by way of regulating the biological behavior of tumor cells. However, the relationship between disulfidptosis-related lncRNAs and the diagnosis, prognosis, and therapeutic efficacy of immunotherapy in PAAD still needs to be further explored.
- A novel prognosis model based on disulfidptosis-related lncRNAs of PAAD has been successfully developed and verified.

What is the implication, and what should change now?

- This model could be used as a tool for predicting overall survival in PAAD, provide a new view for the clinical treatment of pancreatic cancer.

potential of the associated lncRNAs as PAAD biomarkers to predict patient prognosis, guide clinical decision-making, and specify personalized therapy. We present this article in accordance with the TRIPOD reporting checklist (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-23-1706/rc>).

Methods

Dataset

One hundred and seventy eight PAAD patients' RNA sequencing and related clinical details were gathered from The Cancer Genome Atlas (TCGA) (<https://portal.gdc.cancer.gov/>). Relevant clinical characteristics included information on age, sex, clinical grade, clinical stage and overall survival (OS). Patients lacking survival information were excluded. Subsequently, we obtained 31 DRGs from the previously published literature (10,11,15). The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Identification of DRlncRNAs

Pearson correlation analysis was utilized to identify DRlncRNAs. LncRNAs with P value <0.01 and an absolute Pearson correlation coefficient ≥ 0.4 were selected as DRlncRNAs.

Construction and validation of prognostic DRLI

We randomized PAAD patients from the TCGA database into training and validation sets. From the training sets, we screened lncRNAs having prognostic significance using univariate Cox regression analysis ($P < 0.05$). Least absolute shrinkage and selection operator (LASSO) regression is an approach for selecting and shrinking variables in Cox proportional risk models, which improves stability and usability while reducing model complexity (16). The LASSO regression was performed in the training set by using the R package "glmnet" to identify the DRlncRNAs with the most prognostic value. Subsequently, multifactorial COX regression analysis was further used to find DRlncRNAs independently affecting prognosis to establish a DRLI.

The following is the DRLI equation:

$$\text{DRLI score} = \sum [\text{Exp}(\text{LncRNA}) \times \text{Coef}(\text{LncRNA})] \quad [1]$$

In the equation, Coef (LncRNA) denotes the coefficient

of survival-related lncRNAs, exp (LncRNA) denotes the expression of lncRNA. Depending on the median DRLI score, PAAD patients were categorized into low- and high-DRLI subgroups. Survival analysis was conducted using the R package "Survival" to verify the availability of prognostic signatures. By the R package "survivalroc", the receiver operating characteristic (ROC) curves was plotted to verify the diagnostic value of the DRLI for 1-, 2- and 3-year survival. Subsequently, we performed analyses in the validation sets and in the whole sets to validate these results.

Independence evaluation of DRLI

The independent predictive power of the DRLI was verified by univariate and multivariate Cox regression analyses. The criterion of independence for both analyses was $P < 0.05$. In oncology treatment, nomograms are widely used in the assessment of patient prognosis, and they facilitate clinical decision-making by condensing prediction models to quantified probabilities of an event occurring in a given circumstance for a specific patient (17). We used the R package "RMS" to establish a nomogram combining DRLI scores and clinical factors to verify the clinical viability of the DRLI. Correction curves at the 1-, 2-, and 3-year intervals were employed to validate how well their estimated and actual survival probability agreed with one another. Then, using data from the TCGA cohort, we additionally examined at the association between DRLI scores and clinical features. OS determined by DRLI score was compared in each clinical subgroup (age, sex, grade, stage) to verify the predictive value of our DRLI.

Functional enrichment analysis

To further investigate the possible mechanisms leading to the prognostic differences between the high- and low-DRLI groups, we analyzed the differentially expressed genes (DEGs, $|\log_2\text{FC}| > 1$ and $P < 0.05$) between the two subgroups. Functional enrichment analysis of DEGs was performed to explore the differences in biological processes (BPs), cellular components (CCs), molecular functions (MFs) and signaling pathways between the high- and low-DRLI groups.

Immune checkpoint analysis and immune cell infiltration analysis

By using single-sample gene set enrichment analysis (ssGSEA) based on the "gsva" R package, we evaluated distinction in

immune-related functional pathways and tumor-infiltrating immune cell (TIIC) subsets between the high- and low-DRLI subgroups (18). With the help of the ESTIMATE algorithm, we examined the abundance of immune and mesenchymal stromal cells in PAAD samples to assess TME differences between the high- and low-DRLI subgroups (19). Also, immune checkpoint inhibitors' (ICIs') therapeutic effects might be influenced by the expression of genes associated with immunological checkpoints. By using the "ggpubr" R package, we compared the activation of immune checkpoint genes between the high- and low-DRLI subgroups.

Association between DRLI scores and tumor mutations

Patients with poorer prognosis tend to be associated with more genetic mutations. Therefore, tumor mutation burden (TMB) analysis was performed on PAAD tumor mutation data from the TCGA database in order to compare TMB values between low- and high-DRLI groups throughout the whole cohort. The R package "maftools" was used to assess mutation data. The relationship between survival and TMB status was also explored, and subgroup survival analysis was performed by combining TMB status and DRLI score.

Statistical analysis

All statistical analyses were performed with R software (4.2.2) and Perl. $P < 0.05$ differences were statistically significant. The accuracy of the DRLI in assessing prognosis was confirmed using ROC curves and Kaplan-Meier survival analysis. Subgroups with different clinical characteristics were analyzed to fully assess the stability of the DRLI. Differences between subgroups were explored using *t*-tests and Wilcoxon signed rank tests.

Results

Determination of prognosis-related DRlncRNAs

We obtained the expression profiles of 31 DRGs and 16,901 lncRNAs from 178 PAAD patients from the TCGA database. Further 958 DRlncRNAs were obtained by Pearson correlation analysis (Figure 1A).

Construction of the DRLI

We randomized 178 patients with PAAD containing clinical information in the TCGA database into a training group

($n=89$) and a validation group ($n=89$). Cross-validation showed no considerable variation in clinical characteristics between the groups, demonstrating that the groups were independent of each other ($P > 0.05$) (Table 1). In combination with clinical data, we determined the prognostic value of these DRlncRNAs by univariate Cox regression in the training subgroup. The results showed that among the 958 DRlncRNAs, 84 lncRNAs significantly linked to patient survival in PAAD ($P < 0.05$), and a forest plot (Figure 1B) was produced to show their expression. Seven prognostic DRlncRNAs associated with OS in PAAD were identified by LASSO regression analysis in the training subgroup (Figure 1C, 1D). Multifactorial COX regression analysis showed that five DRlncRNAs could independently assess the prognosis of PAAD, and the DRLI was established using these five DRlncRNAs. The DRLI was then used to assess the distribution of DRLI scores, survival status and survival time of the training set, validation set and the overall patients (Figure 2A-2C). The accuracy of the DRLI for prediction at 1, 2 and 3 years was validated by ROC curves (Figure 2D).

To verify the excellent predictive value of the prognostic signature associated with disulfidptosis, we compared the DRLI score with other clinical elements. The C-index of all factors of interest was calculated, and the results showed that the C-index of the DRLI score was superior to most other clinical elements (Figure 2E). These results imply that the DRlncRNAs-based index might provide a more accurate prognosis assessment for PAAD patients.

Relationship between DRLI scores and clinicopathologic features

Subgroup survival analysis validated the prognostic value of DRLI scores in patients with different clinical characteristics. According to the calculated DRLI scores, PAAD patients with different clinicopathological characteristics were categorized into low-DRLI and high-DRLI groups, respectively. Kaplan-Meier survival analyses showed that patients in the low-DRLI subgroups were significantly relevant to poorer survival outcomes except those with stages III-IV (Figure 3A-3H). The Figures 3I further shows the distribution of clinicopathologic features and DRLI scores.

Independent prognostic analysis and the establishment of nomogram

DRLI scores were analyzed as a factor influencing the

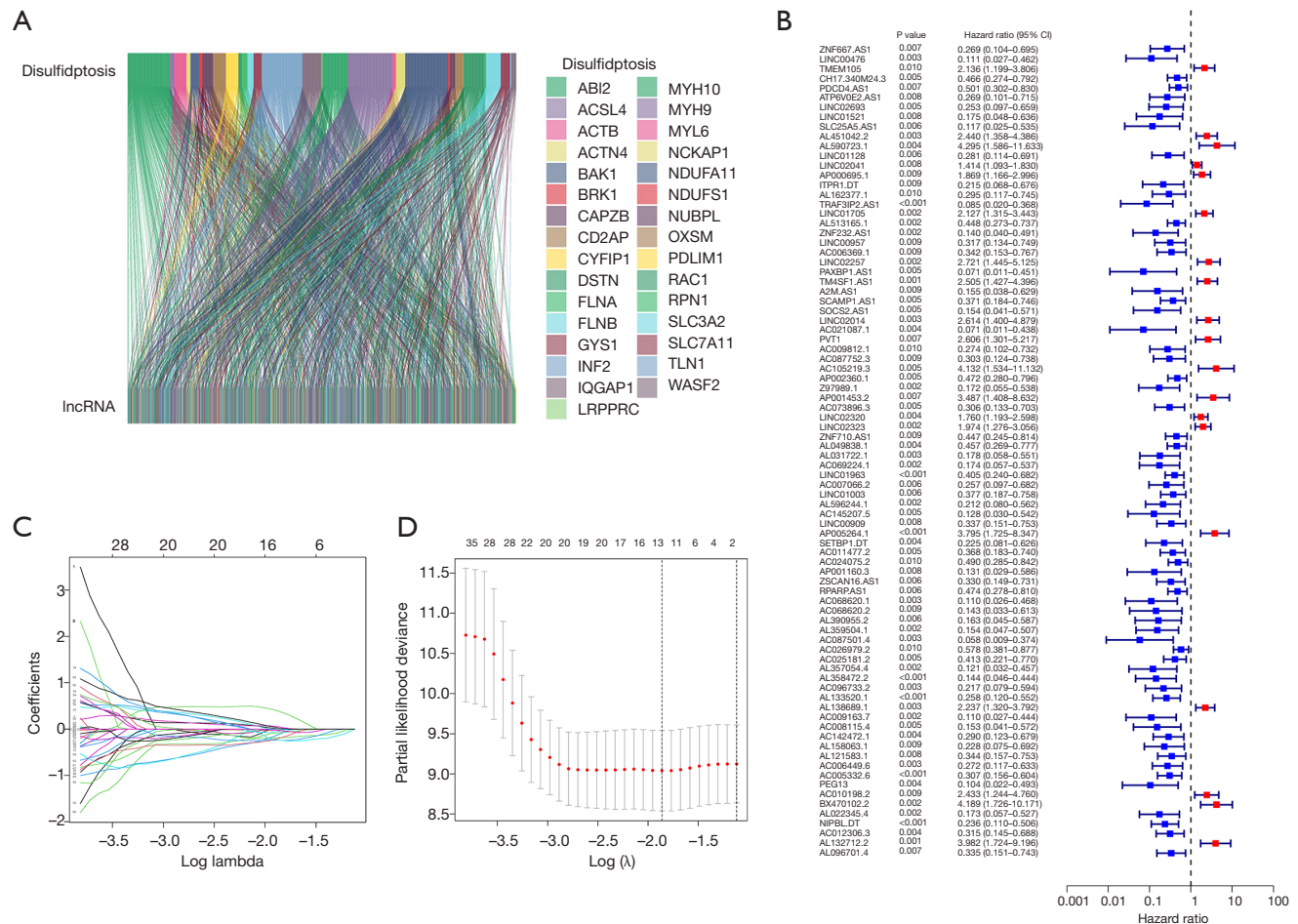


Figure 1 Determination of prognosis-related DRlncRNAs. (A) Sankey diagram displayed the relationship between DRGs and DRlncRNAs. (B) DRlncRNAs with different hazard ratios. (C,D) Selection of seven most prognostic DRlncRNAs. The two dashed lines in the figure D indicate two particular values of λ , lambda.min on the left and lambda.1se on the right. lambda between these two values of λ are both considered appropriate. lambda.1se constructs the simplest model, using a small number of genes. The model constructed by lambda.min, on the other hand, is more accurate and uses a larger number of genes. DRlncRNAs, disulfidptosis-related long-stranded non-coding RNAs; DRGs, disulfidptosis-related genes.

prognosis of PAAD patients in univariate and multivariate COX regression analyses along with traditional clinical parameters. The results showed that DRLI score could be used as an independent prognostic predictor [univariate hazard ratio (HR) =1.167, 95% confidence interval (CI): 1.102–1.236, P<0.001; multivariate HR =1.168, 95% CI: 1.099–1.241, P<0.001] (Figure 4A,4B). Compared to the other clinicopathologic parameters, the DRLI score's area under the curve (AUC) value was greater (Figure 4C). Several clinical factors and DRLI scores were included in the nomogram to better predict survival outcomes in PAAD patients (Figure 4D). Subsequently, a calibration curve was

plotted to investigate the accuracy of this nomogram, and the results that followed indicated that the actual values were generally consistent with the predicted values (Figure 4E). Overall, our nomogram is a useful tool to estimate a patient's prognosis for PAAD.

Exploration of cellular processes and signaling pathways affected by DRlncRNAs

By analyzing the functional enrichment of 897 DEGS between the high- and low-DRLI subgroups, we investigated the potential biological pathways and functions

Table 1 The clinical information of the training group and the validation group

| Clinical feature | Type | Total | Validation | Train |
|------------------|---------|-------------|------------|------------|
| Age, years | ≤65 | 94 (52.81) | 47 (52.81) | 47 (52.81) |
| | >65 | 84 (47.19) | 42 (47.19) | 42 (47.19) |
| Gender | Female | 80 (44.94) | 42 (47.19) | 38 (42.7) |
| | Male | 98 (55.06) | 47 (52.81) | 51 (57.3) |
| Grade | G1 | 31 (17.42) | 18 (20.22) | 13 (14.61) |
| | G2 | 95 (53.37) | 41 (46.07) | 54 (60.67) |
| | G3 | 48 (26.97) | 29 (32.58) | 19 (21.35) |
| | G4 | 2 (1.12) | 0 (0) | 2 (2.25) |
| | GX | 2 (1.12) | 1 (1.12) | 1 (1.12) |
| Stage | I | 21 (11.8) | 9 (10.11) | 12 (13.48) |
| | II | 147 (82.58) | 73 (82.02) | 74 (83.15) |
| | III | 3 (1.69) | 2 (2.25) | 1 (1.12) |
| | IV | 4 (2.25) | 3 (3.37) | 1 (1.12) |
| | Unknown | 3 (1.69) | 2 (2.25) | 1 (1.12) |
| T | T1 | 7 (3.93) | 4 (4.49) | 3 (3.37) |
| | T2 | 24 (13.48) | 10 (11.24) | 14 (15.73) |
| | T3 | 142 (79.78) | 72 (80.9) | 70 (78.65) |
| | T4 | 3 (1.69) | 2 (2.25) | 1 (1.12) |
| | TX | 1 (0.56) | 0 (0) | 1 (1.12) |
| | Unknown | 1 (0.56) | 1 (1.12) | 0 (0) |
| M | M0 | 80 (44.94) | 38 (42.7) | 42 (47.19) |
| | M1 | 4 (2.25) | 3 (3.37) | 1 (1.12) |
| | MX | 94 (52.81) | 48 (53.93) | 46 (51.69) |
| N | N0 | 49 (27.53) | 21 (23.6) | 28 (31.46) |
| | N1 | 120 (67.42) | 63 (70.79) | 57 (64.04) |
| | N1b | 4 (2.25) | 2 (2.25) | 2 (2.25) |
| | NX | 4 (2.25) | 2 (2.25) | 2 (2.25) |

Data are presented as n (%).

between the two subgroups. Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis point to a strong relationship between DEGs and a variety of activities and processes, including those involved in the control of chemical synaptic transmission, the cyclic adenosine monophosphate (cAMP) signaling pathway, and the mitogen-activated protein kinase (MAPK) signaling network (*Figure 5A,5B*).

Analysis of tumor immune infiltration

We further investigated the tumor immune landscape of PAAD in the TCGA database. We explored the infiltration profiles of 16 immune cells by ssGSEA algorithm. The results are shown in *Figure 6A*: between the high- and low-DRLI subgroups, there were appreciable differences in the infiltration profiles of five TIICs. It is interesting to note that none of these cells were as high as those in the

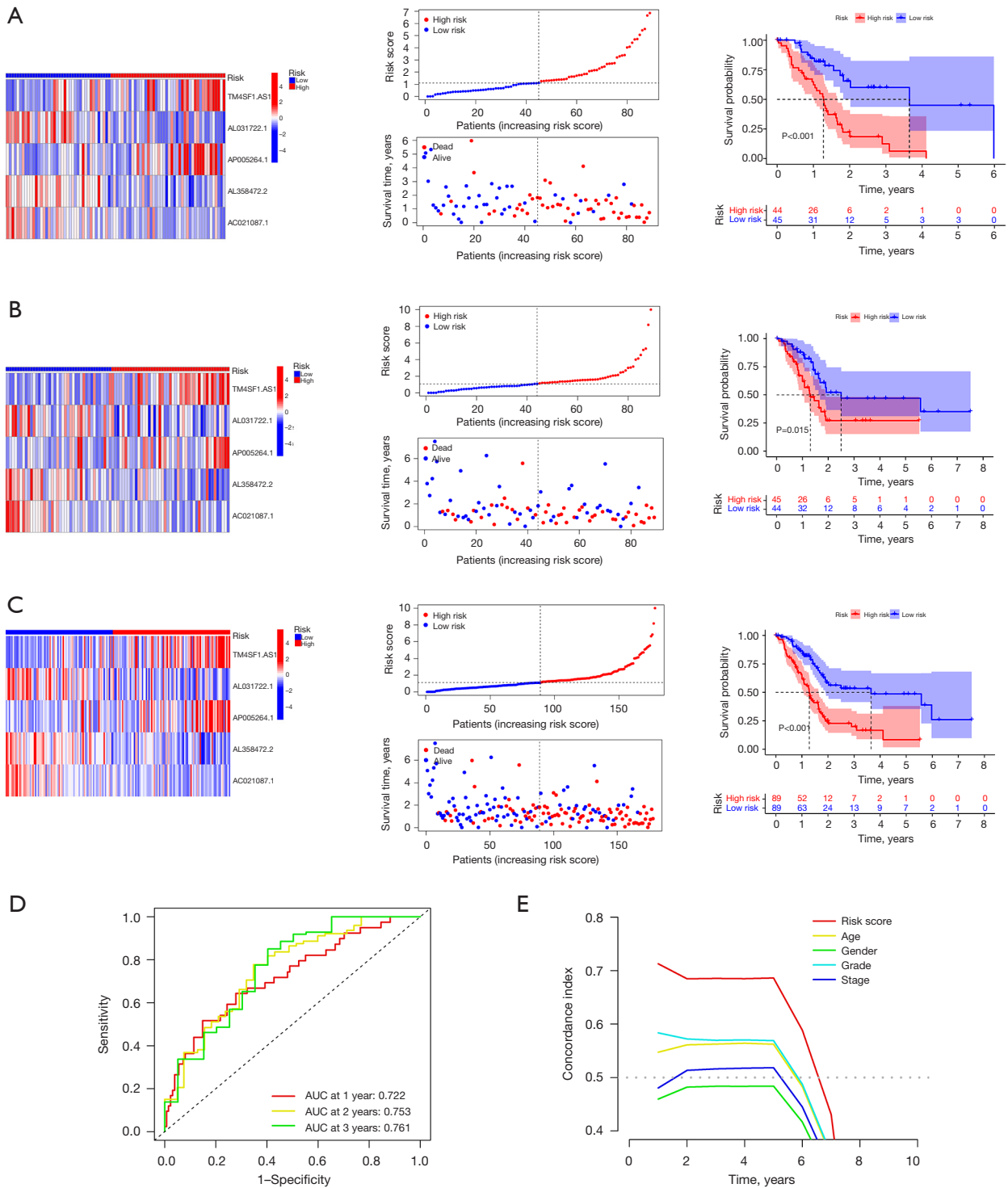


Figure 2 Predictive value of the DRLI. (A) Predictive value of the DRLI in the training cohort. (B) Predictive value of the DRLI in the validation cohort. (C) Predictive value of the DRLI in the entire cohort. (D) Time-dependent receiver operating characteristic curves in 1-, 2- and 3-year. (E) The C-index of the risk score and other clinical parameters. A C-index of 0.5 represents complete inconsistency, indicating that the index has no predictive role (dashed lines). DRLI, disulfidptosis-related long-stranded non-coding RNA index; AUC, area under the curve.

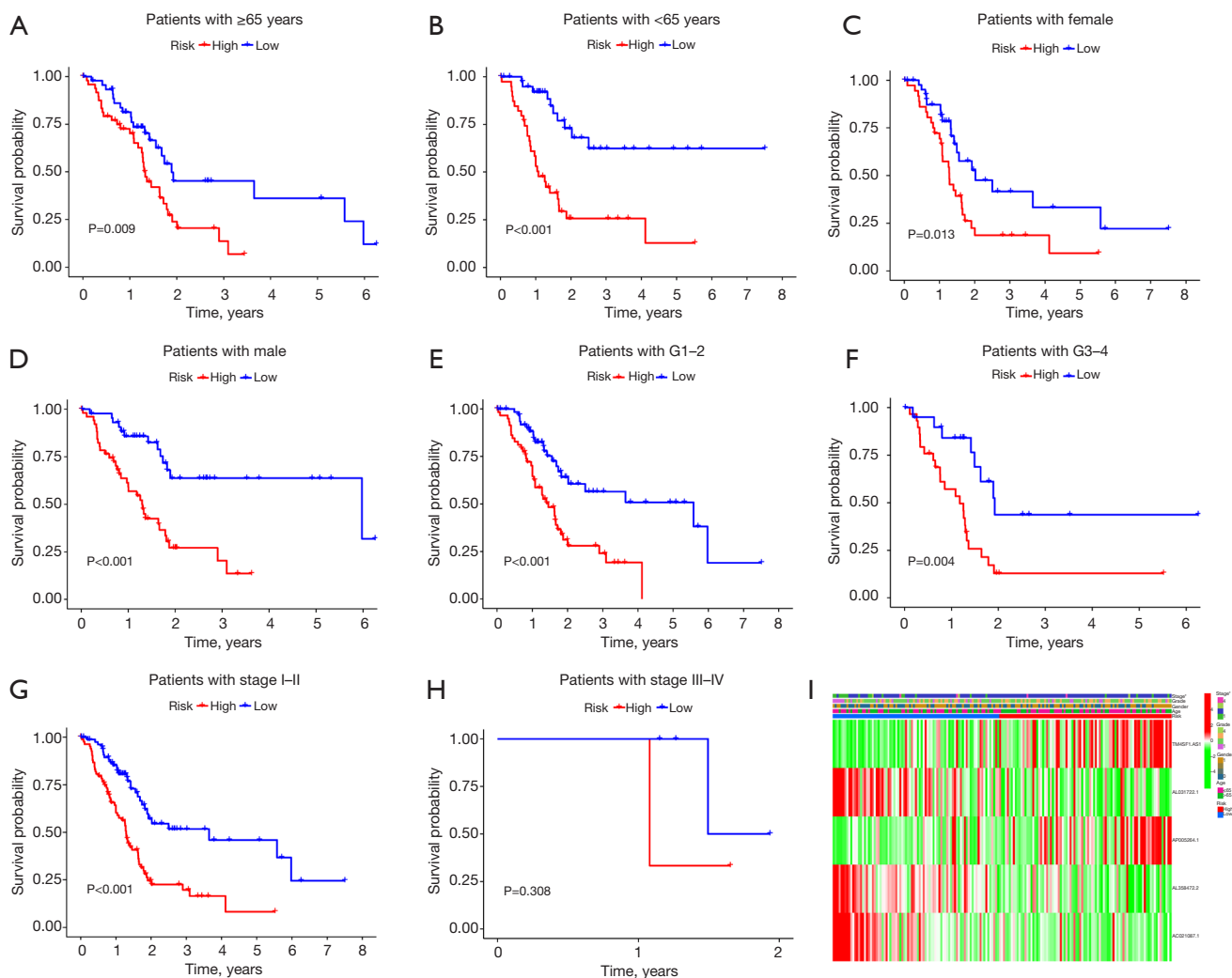


Figure 3 Kaplan-Meier survival curves layered by age (A,B), gender (C,D), tumor grade (E,F), and stage (G,H) between two risk groups. (I) The heatmap of clinicopathological features and five DRlncRNAs. *, P<0.05. DRlncRNAs, disulfidptosis-related long-stranded non-coding RNAs.

PAAD high-risk subgroup. The ssGSEA algorithm was subsequently applied to assess the immune-related functions between the two subgroups. The high-DRLI subgroups showed less levels of immune function (Figure 6B). The ESTIMATE algorithm revealed that the low-DRLI subgroups had higher immunity scores, stroma scores, and estimated scores (Figure 6C-6E). This suggests that the purity of tumors in the high-DRLI subgroup was higher. The above results suggest that an immunosuppressive tumor immune microenvironment exists in the high-DRLI subgroup, which may be a contributing factor to the poorer prognosis of this patient subgroup. In addition, the expression analysis of immune checkpoint genes was also negatively correlated with DRLI score (Figure 6F). In

conclusion, these results provide new insights for future individualized and precise treatment of patients in different risk subgroups.

Tumor mutation analysis

We examined the TMB profiles of two subgroups and identified the top 20 mutated genes (Figure 7A). The low TMB group had better survival outcomes (Figure 7B). The combined analysis of TMB and DRLI score showed that the low DRLI + low TMB group had better survival than the other three groups (Figure 7C). These results suggest that DRLI score has better prognostic predictive ability compared to TMB.

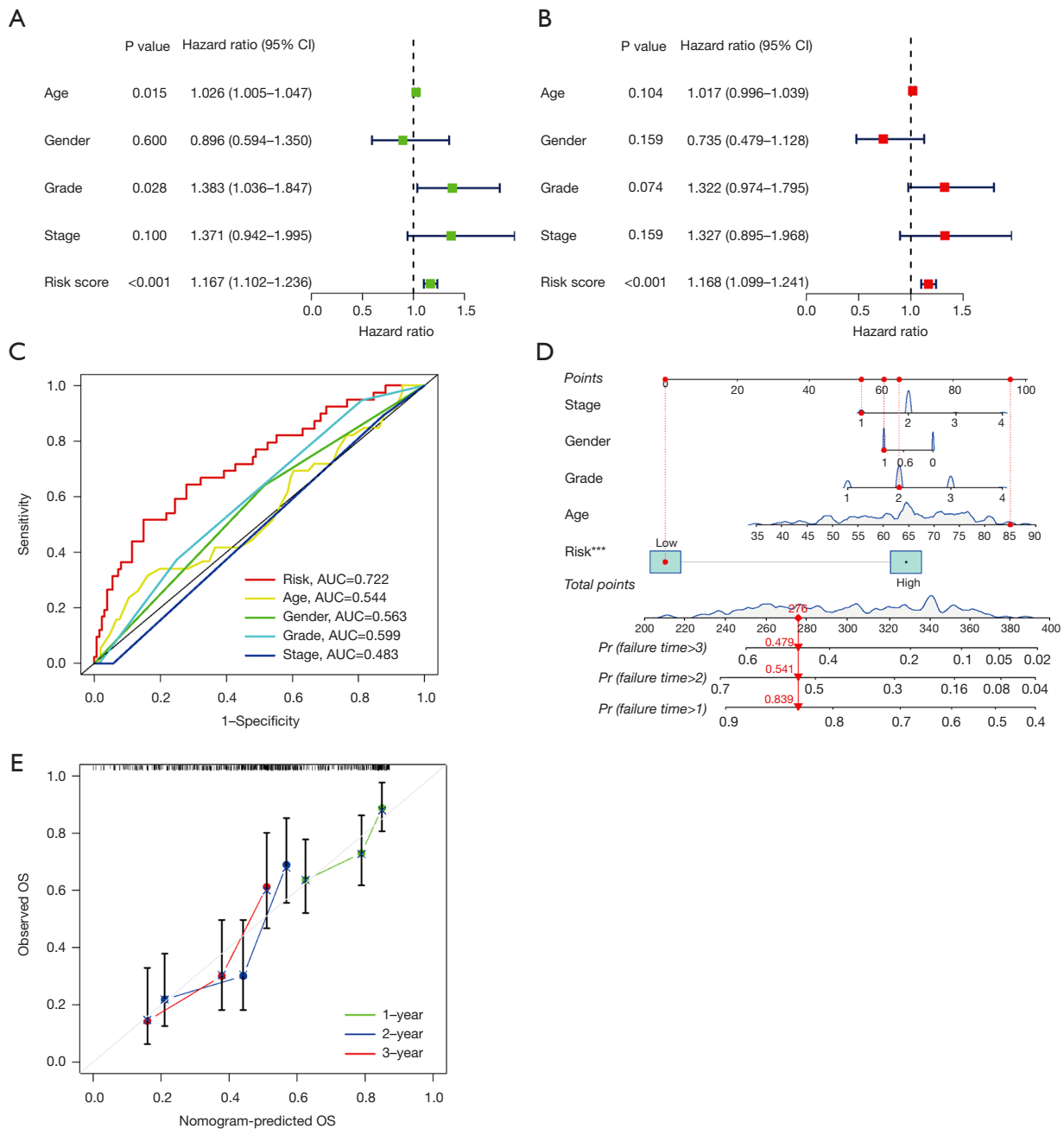


Figure 4 Independent analyses and establishment of nomogram. (A,B) Univariate and multivariate Cox regression analyses. (C) The receiver operating characteristic curves. (D) Nomogram. (E) Calibration curves for the nomogram. ***, $P < 0.001$. AUC, area under the curve; Pr, probability; OS, overall survival.

Discussion

To date, the molecular mechanisms underlying the pathogenesis of PAAD are not fully understood. LncRNAs are crucial in the incidence and progression of PAAD

according to several studies (20). PAAD-associated lncRNAs can act as oncogenic or tumor suppressor lncRNAs and serve as potential markers for assessing the survival and diagnosis of patients with PAAD (21). For example, *LNC00976*, a tumor suppressor lncRNA, is a good

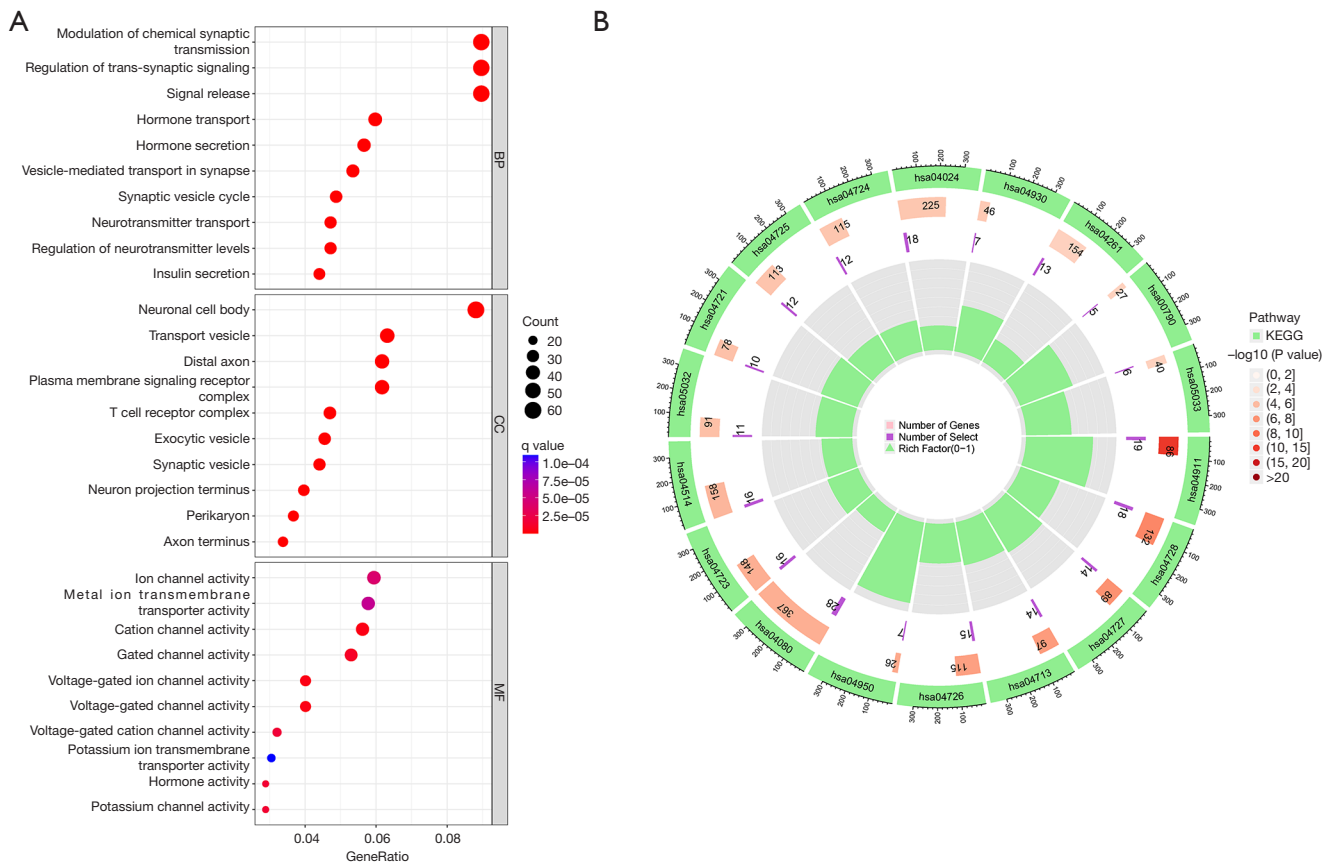


Figure 5 Functional enrichment. (A) GO function enrichment. (B) KEGG pathway enrichment. GO, Gene Ontology; BP, biological process; CC, cellular compartment; MF, molecular function; KEGG, Kyoto Encyclopedia of Genes and Genomes.

predictor of PAAD prognosis. Further studies have shown down-regulation of *LINC00976* expression would result in reduced proliferation, migration potential and invasiveness of pancreatic cancer cells *in vivo* and *in vitro* (22). Upregulation of *LINC00462* resulted in EMT of PAAD cells and could interact with miR-665 to increase the metastatic and invasive power of PAAD cells (23). On the contrary, *LINC01111*, a newly discovered lncRNA, was shown to inhibit various biological functions of PAAD cells, such as proliferation, metastasis, cell survival, and cell cycle progression *in vitro* experiments (24). However, studies on disulfidptosis-related lncRNAs are few, particularly in PAAD.

In this study, we included 178 patients with PAAD from the TCGA dataset. First, we identified 84 lncRNAs with prognostic significance associated with disulfidptosis by univariate Cox regression analysis. Five lncRNAs (including *TM4SF1.AS1*, *AL031722.1*, *AP005264.1*, *AL358472.2*, *AC021087.1*) were selected by LASSO regression and

multifactorial Cox regression analysis to develop a predictive signature for estimating OS in PAAD patients. On the basis of their median DRLI scores, patients were classified into low- and high-DRLI groupings. The results of survival analysis revealed a substantial difference between the high-DRLI and low-DRLI groups in survival. ROC curves were applied to confirm the validity and dependability of the DRLI's prediction capability. To test its validation furtherly, we repeated all analyses in validation set and entire set. We also used DRLI scores together with other clinical factors to construct nomogram plots to quantitatively predict 1-, 2-, and 3-year survival rate in individual PAAD patients, thereby providing recommendations for clinical diagnosis and treatment.

Among the five lncRNAs used to construct the model, *TM4SF1.AS1* and *AP005264.1* were potential risk factors, while *AL031722.1*, *AL358472.2* and *AC021087.1* were protective factors. A previous study has found that *TM4SF1-AS1* contributes to tumorigenesis by inducing

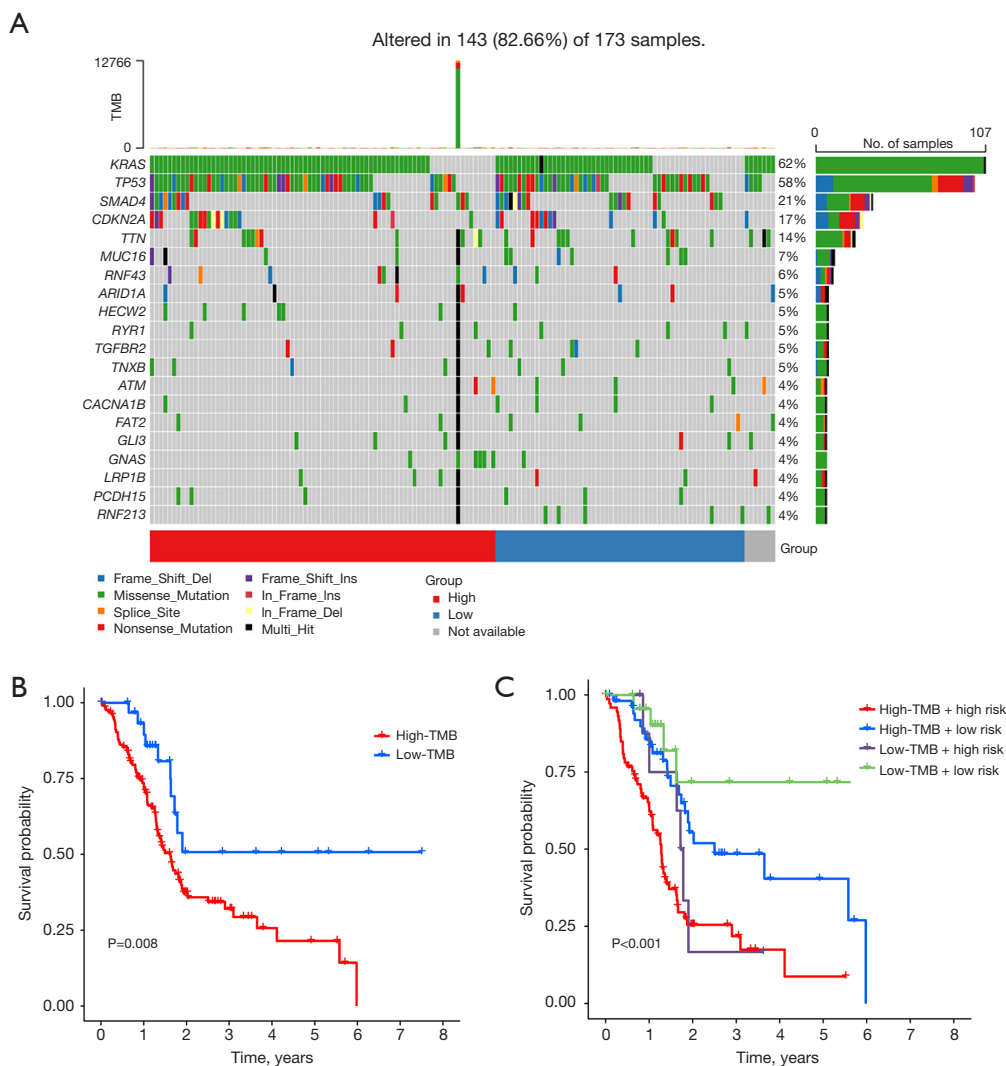


Figure 7 Tumor mutation analysis. (A) Top 20 mutation genes' information in two risk groups. (B) Kaplan-Meier curves of OS stratified by TMB. (C) Kaplan-Meier curves of OS stratified by both TMB and risk score. TMB, tumor mutation burden; OS, overall survival.

stress granule formation and inhibiting apoptosis (25). In hepatocellular carcinoma, *TM4SF1-AS1* promotes tumor cell proliferation, migration, and invasion by enhancing *TM4SF1* expression (26). *M4SF1-AS1* is upregulated in gastric cancer tissues, and affects the ability of cells to proliferate, invade and transition from epithelium to mesenchyme through the *PI3K-AKT* signaling pathway (27). Lin *et al.* found that *AL031722.1* may be associated with the prognosis of lower-grade gliomas (28). Alterations of metabolism-related lncRNA *AP005264.1* expression were strongly correlated with poor prognosis in osteosarcoma (29). In addition, other lncRNAs in this study have not been reported in the relevant literature.

The TME is a complex and evolving system composed mainly of non-tumor cells, extracellular matrix (ECM), vascular system, and non-cellular components (30). The immune microenvironment, composed of multiple immune cells, is an important component of the TME and can serve as a predictor of tumor immunotherapy efficacy (31). The high- and low-DRLI subgroups exhibited significantly different immune cell infiltration and immune function scores. Interestingly, the scores of CD8+ T cells, Th cells, B cells, Tfh cells, and TIL cells were significantly higher in the low-DRLI group than in the high-DRLI group. CD8+ T lymphocytes can differentiate into effector CTLs upon stimulation with tumor-associated antigens.

CTLs can participate in the tumor immune response by transferring from the peripheral immune organs to the tumor site through chemokines. Tfh cells enhance CD8+ T lymphocytes functions by secreting IL-21. Th cells can participate in both CTL cell activation and secretion of cytokines with antitumor immune effects. B cells can differentiate into plasma cells with the ability to secrete antitumor antibodies upon stimulation with tumor-associated antigens. TIL cells are infiltrating lymphocytes isolated from tumor tissues. Some of these cells are T cells targeting tumor-specific mutant antigens, which are immune cells capable of penetrating deep into the interior of tumor tissues to kill tumors. As innate immune cells, NK cells, macrophages and NKT cells did not observe significant differences between the two subgroups. In summary, the anti-tumor ability of the low-DRLI group was stronger than that of the high-DRLI group. The TME results showed that the stromal score and the immune score of the low-DRLI group were higher than those of the high-DRLI group, which indicated that the high-DRLI group samples had less infiltration of immune cells, a low content of stromal cells, and a high purity of tumors, which was in line with the results assessed by the ssGSEA algorithm. These findings suggest that the tumor immune microenvironment in patients with PAAD in the high-DRLI group is immunosuppressive, and the low-DRLI group may have a stronger anti-tumor immune response, which explains the difference in prognosis between the two groups.

Today, ICIs have emerged as a novel focus for cancer treatment (32). Compared with conventional therapies, immune checkpoint inhibition therapy has shown rapid and consistent efficacy in certain cancer patients, especially in patients with advanced metastatic cancer (33,34). Our study found differences in the distribution of immune checkpoint genes in the two subgroups, with most of the genes being more highly expressed in the low-DRLI subgroups. Patients in the low-DRLI group may have a better therapeutic response to immune checkpoint inhibition therapy.

The higher the tumor mutational load, the greater the production of neoantigens that can be recognized by immune cells, and the better the efficacy of receiving immune checkpoint inhibitor therapy is likely to be (35,36). This study found higher TMB values in the high-DRLI group, suggesting that immunotherapy may be more beneficial in this subgroup. In a subsequent subgroup survival analysis, it was shown that the DRLI score was a more accurate predictor of prognosis in patients with PAAD compared to TMB.

There are some limitations in this study. First, there was no external validation of our results with other datasets due to the lack of lncRNA expression profiles. Second, we explored the potential mechanisms of different prognoses between high- and low-risk subgroups by pure bioinformatics analysis. However, the results need to be verified by more fundamental experiments.

Conclusions

The present study implicates that several DRlncRNAs (*TM4SF1.AS1*, *AL031722.1*, *AP005264.1*, *AL358472.2*, *AC021087.1*) may serve as independent prognostic biomarkers for pancreatic cancer. Our study also demonstrated that these lncRNAs are strongly correlated with the immune microenvironment, mutational burden, and response to immunotherapy in pancreatic cancer. This research may provide a new view for the clinical treatment of pancreatic cancer.

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Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-23-1706/rc>

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

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