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Identification of pancreatic subtypes and prognostic markers on the basis of changes in immunologic signature gene sets activity

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Abstract

The heterogeneity of pancreatic adenocarcinoma (PAAD) tumors is complex. The purpose of this work was to use immunologic signature gene sets (ISGS) activity alterations to identify subtypes of PAAD and to develop valid prognostic indicators. We discovered tumor-related differentially expressed gene sets and their associated subtypes by analyzing changes in ISGS activity via the cancer genome Atlas and the genotype-tissue expression datasets. To evaluate the relationship between these subtypes and clinical characteristics, the immunological microenvironment, and tumor immune dysfunction and exclusion, we conducted survival studies. Furthermore, we identified subtype-specific gene sets and developed a prognostic risk score (RS) model and a corresponding nomogram. The robustness and generalizability of the RS model were validated using the gene expression omnibus dataset. Additionally, differences in drug sensitivity and tumor mutation burden were compared between risk groups. On the basis of variations in ISGS activity, 2 subtypes of PAAD were distinguished. The prognosis is worse for subtype 1 patients, and there is no statistically significant difference between the clinicopathologic characteristics of the 2 subtypes. However, subtype 1 patients do not respond well to immunotherapy, and the immunological microenvironment varies significantly across subtypes. Additionally, patients were assigned to distinct risk groups by a prognostic RS model based on differentially expressed genes linked to both subtypes. Individuals belonging to distinct risk categories exhibited varying degrees of medication sensitivity; high-risk patients also had shorter survival periods and greater tumor mutation burden. For patients with pancreatic cancer, nomograms that include RS and clinicopathologic variables are may provide useful tools. Our research used the ISGS to identify subtypes of PAAD and created prognostic RS models. Through the integration of the RS and clinicopathological parameters into nomograms, prognostic prediction may be reinforced, and may provide new perspectives for future personalized treatment strategies.

Abbreviations: DEGs = differentially expressed genes, GEO = gene expression omnibus, GSVA = gene set variation analysis, GTEx = genotype-tissue expression, ISGS = immunologic signature gene sets, LASSO = least absolute shrinkage and selection operator, OS = overall survival, PAAD = pancreatic adenocarcinoma, RS = risk score, TCGA = the cancer genome Atlas, TIDE = tumor immune dysfunction and exclusion, TMB = tumor mutation burden.

Keywords: immunologic signature gene sets (ISGS), pancreatic adenocarcinoma (PAAD), personalized treatment strategies, prognostic, subtypes

1. Introduction

By 2030, pancreatic adenocarcinoma (PAAD) – a very deadly tumor – is estimated to place second in the US in terms of cancer-related fatalities.[1] Approximately 5,00,000 new instances of PAAD were identified globally in 2020, with a corresponding number of fatalities and an average 5-year survival rate of 12.8%.[2] PAAD death rates are still high despite notable advancements in treatment over the previous few decades.[3,4] Complex tumor heterogeneity, which is impacted by a confluence of environmental, immunological, personal health, and genetic variables, is the hallmark of PAAD. The molecular,

JF and SG contributed to this article equally.

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All data generated or analyzed during this study are included in this published article [and its supplementary information files].

This study used publicly available datasets (TCGA, GTEx, GEO), all of which had prior ethics committee approval. No new patient data were collected. Therefore, additional ethics approval and patient consent were not required.

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cellular, immunological, and tumor behavioral levels are where this complexity is exhibited.[5,6] Tumor response to treatment and prognosis are also significantly impacted by this heterogeneity, which also has an effect on carcinogenesis and progression.[7] Patients will thereby have better clinical outcomes with improved subtype identification and tailored treatment plans for PAAD.

Intricate regulation between cancer cells and the immune system plays a complicated role in the development of cancer.[8] Cancer's genesis and progression are significantly influenced by the immune system, and immune evasion has been identified as a characteristic of the disease.[9] The prognosis for pancreatic cancer patients has improved recently due to notable advancements in immunotherapy.[10] As of right now, TNM staging upon diagnosis is the main factor used to determine the prognosis of each patient, yet this method is inaccurate for making specific prognostic predictions. More sensitive and precise prognostic indicators are therefore desperately needed in clinical settings.[11]

Several molecular classification systems for PAAD have been proposed. Collisson et al identified 3 transcriptome-based subtypes – classical, quasimesenchymal, and exocrine-like.[12] Bailey et al later refined these into 4 molecular subtypes: squamous, pancreatic progenitor, immunogenic, and aberrantly differentiated endocrine exocrine.[13] More recently, Rashid et al developed the PurIST classifier, a clinically robust single-sample tool that stratifies tumors into basal-like and classical subtypes,[12] while Zhao et al reported transcriptomic subtypes with independent prognostic relevance.[13] Although these classifications have substantially advanced our understanding of PAAD heterogeneity, they were largely derived from global transcriptomic features. In contrast, our study focuses on immunologic signature gene sets (ISGS) activity, aiming to capture immune-related molecular alterations that may underlie prognosis and therapeutic response.

As such, the focus of research has turned to the identification of immunogenomic markers linked to prognosis. For instance, a model made up of 7 genes linked to M2 macrophages was effective in forecasting prognosis and immunotherapy response in colon cancer, which improved patient management during immunotherapy.[14] Li et al showed that immunogenomic profiling could stratify PDAC patients according to prognosis and predict immunotherapy responsiveness, correlating with immune infiltration, tumor mutation burden (TMB) and immune checkpoint expression.[15] These results imply that the immune-related genome is a highly promising biomarker for prognosticating patients. Studies on immune-related genes and important indicators linked to the prognosis of pancreatic cancer are still few, nonetheless.

The prognosis may be significantly impacted by changes in ISGS activity, which may also have an effect on the proliferation of tumor cells following therapy and the colonization of circulating tumor cells in the circulation.[16] Few research, nonetheless, have used variations in ISGS activity to determine prognosis or identify PAAD subtypes. Using the cancer genome Atlas (TCGA) and the genotype-tissue expression (GTEx) datasets, we examined changes in ISGS activity in PAAD in this work. We then determined prognostically significant differentially expressed genes (DEGs) sets and the corresponding PAAD subtypes. On these subtypes, we conducted tumor immune dysfunction and exclusion (TIDE), immune infiltration, and survival assessments. Subsequently, we created nomograms and prognostic risk score (RS) models and determined DEGs between subtypes. GSE28735 and GSE62452, 2 gene expression omnibus (GEO) datasets, were utilized to verify the RS models' prediction accuracy. We also examined medication sensitivity and TMB levels across risk groups. Figure 1 illustrates our bioinformatics analytical methodology. These results offer further information for precisely predicting prognosis and enhancing treatment approaches for pancreatic cancer.

2. Materials and methods

2.1. Data collection

The TCGA database (https://portal.gdc.cancer.gov/) provided the clinicopathologic characteristics and gene sequencing data for PAAD. The data included 178 cancer cases and 4 normal samples. 167 samples of normal pancreatic tissue with gene sequencing data were collected from the GTEx database (https://www.gtexportal.org/home/). GSE28735 and GSE62452, 2 PAAD sequencing datasets containing prognostic data, were acquired from the GEO database (https://www.ncbi.nlm.nih.gov/geo/). Batch effects were corrected using the ComBat function in the "sva" R package, with default parameters. Following batch effect correction, 114 samples in all were ready for further examination. Furthermore, the MSigDB database (https://www.gsea-msigdb.org/gsea/index.jsp) included 4872 ISGS.

2.2. Gene set variation analysis (GSVA)

The gene set variation analysis (GSVA) approach estimates the relative enrichment of genomes in a sample to examine changes in gene pathway activity under particular biological settings.[17] GSVA used sample data from the TCGA and GTEx datasets to examine the enrichment scores (ES) of 4872 ISGSs. The results showed variations in the activity of these gene sets in PAAD compared to normal.

2.3. Identification of DEGs

The "limma" tool in R4.4.0 was used to assess DEGs between cancer and noncancer samples based on the ES of the genomes in the samples, with a cutoff value of $P < .01$.[18] The list of prognostically important genes was screened by univariate Cox regression analysis, utilizing the "survival" package in R4.4.0 with a threshold value of $P < .01$. By intersecting the DEGs with the collection of prognostically relevant genes, prognostically relevant DEGs were discovered.

2.4. Subtype analysis

The "ConsensusClusterPlus" tool in R4.4.0 was used to perform unsupervised clustering of the data based on prognostically significant DEGs.[19] To investigate which k value is best for cluster stability, the samples were divided into k (2–9) groups. proportion of ambiguous clustering were used to further validate the ideal k value.

2.5. Survival analysis of subtypes

Using the "Survival" package in R4.4.0, we conducted a Kaplan–Meier survival analysis of patients with the various subtypes to see if there were any changes in prognosis across the subtypes.

2.6. Relationship between subtypes and clinicopathological characteristics

Using chi-square tests based on the clinical data of each subtype sample, correlations between subtypes and other clinicopathologic variables (TNM stage, clinical stage) were evaluated.

2.7. Immune infiltration and TIDE analysis of subtypes

Using the CIBERSORT, QUANTIseq, and TIMER algorithms, the fraction of immune cell types was calculated on the basis of the expression level of each sample in the dataset.[20–22] We studied the variations in immune cells among subtypes. To assess the differences in immunotherapy response between subtypes, the TIDE score was computed for each sample using the mRNA expression matrix.

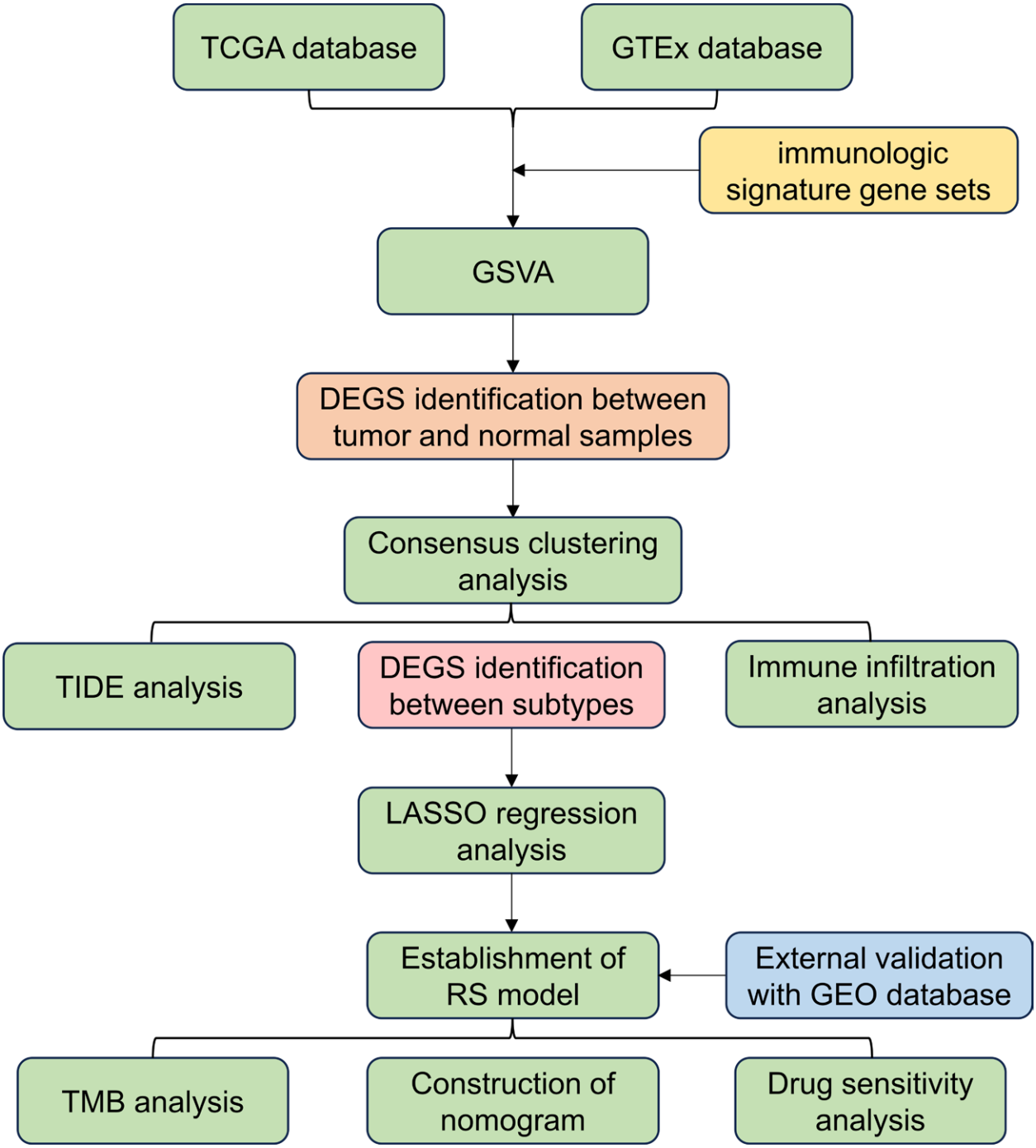


Figure 1. Flow chart of bioinformatic analysis. DEGs = differentially expressed genes, GEO = gene expression omnibus, GSVA = gene set variation analysis, GTEx = genotype-tissue expression, LASSO = least absolute shrinkage and selection operator, RS = risk score, TCGA = The cancer genome Atlas, TIDE = tumor immune dysfunction and exclusion, TMB = tumor mutation burden.

2.8. Identification of DEGs between subtypes

Using the "limma" package in R4.4.0 and a threshold value of $P < .01$ for tumor subtype information, DEGs between subtypes was evaluated.

2.9. Construction and evaluation of the RS model

The "glmnet" package in R4.4.0 was used to perform least absolute shrinkage and selection operator (LASSO) regression analysis, based on the DEGs between subtypes.[23] To build RS models, gene sets that were chosen via the LASSO regression analysis were utilized. The following formula was computed:

$$\text{Risk score} = \sum \text{n}_i = \sum (\text{coef i} * x_i)$$

where coef i is the coefficient and x_i is the ES of each selected gene set.

The samples in the GEO validation dataset and the TCGA training dataset have their scores determined using this algorithm. Based on the median score, the complete cohort was then divided into high and low risk categories. So as to evaluate

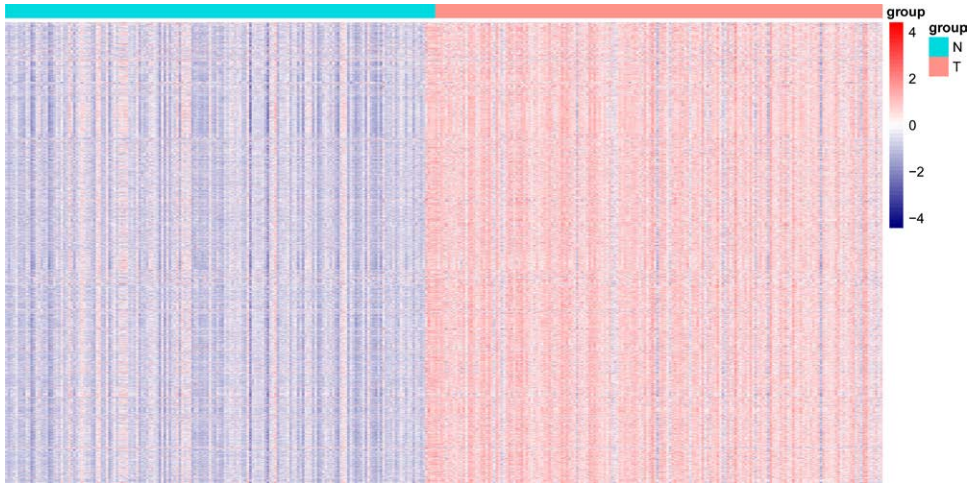


Figure 2. Enrichment score heatmap for 4872 immunogenomes in normal and pancreatic tumor samples. Based on the TCGA and GTEx databases, GSVA was carried out. GSVA = gene set variation analysis, GTEx = genotype-tissue expression, TCGA = the cancer genome atlas.

prognostic differences between risk categories, survival analyses were conducted.

2.10. TMB and drug sensitivity analysis

Every gene in the TCGA dataset samples had its mutation information gathered, and the top 20 genes with the greatest amount of mutations were chosen. Using the "maftools" package in R4.4.0, the mutation frequency of these highly modified genes was evaluated in all samples.[24] Every tumor sample had its TMB levels determined, and the results were compared across several risk categories. The "oncoPredict" program in R4.4.0 was used to evaluate each sample's sensitivity to common medications, and the variations between risk categories were compared.[25]

2.11. Construction of the nomogram

RS and clinicopathologic factors (age, sex, pathologic M, N, and T stages, and clinical stage) in patients with TCGA were the subjects of univariate and multivariate Cox regression analyses to examine the factors influencing patient prognosis. Variables with $P < .05$ were regarded as independent prognostic factors. Using the "rms" package, a nomogram was created.[26] Survival analysis, calibration curves, and receiver operating characteristic curves were used to assess the nomogram model's performance in more detail.

2.12. Statistical analysis

Based on R4.4.0, bioinformatics analysis were carried out. "limma" package was used to search for DEGs between samples with and without cancer as well as between various subtypes. "survival" package was used to carry out Cox regression and survival studies. Samples with incomplete or missing clinical information were excluded from relevant statistical analyses.

3. Results

3.1. Changes in ISGS activity in PAAD tissues

GSVA analysis was carried out on the ES of 4872 ISGS to identify the variations in ISGS activity between cancer samples and normal samples. Figure 2 displays the differential RS of ISGS in tumor and non-tumor samples.

3.2. Identification of prognosis-related differential gene sets

Four thousand three hundred thirty-three difference gene sets between tumor and non-tumor samples were found using the ES of 4872 ISGS. There were 1501 prognostically relevant differential gene sets found by taking the intersection with 1583 prognostically relevant gene sets.

3.3. PAAD subtype analysis

Using the prognostically relevant differential genes, unsupervised clustering of PAAD samples was carried out (Fig. 3A). Subtypes 1 (116 samples) and 2 (60 samples) were derived from the ideal k value of 2, according to further proportion of ambiguous clustering analysis (Fig. 3B, C).Patients in subtype 1 had poorer prognosis ($P < .001$, Fig. 3D) and higher TIDE scores ($P < .05$, Fig. 3E). No significant differences in clinicopathological characteristics were observed between the 2 subtypes ($P > .05$, Fig. 3F–I).

3.4. Differences in the immune microenvironment between subtypes

The algorithms CIBERSORT, quanTIseq, and TIMER were used to quantify the proportions of immune cells. There were 6 distinct kinds of differentiated immune cells found: M0 macrophages, M1 macrophages, neutrophils, and dendritic cells were highly raised in subtype 1, whereas CD8 T cells and monocytes were highly elevated in subtype 2 (Fig. 4).

3.5. Construction of the prognostic RS model

A total of 968 DEGs were found between the 2 subtypes in the TCGA dataset (Fig. 5A). LASSO regression analysis of subtype-associated DEGs yielded twenty-three defined gene sets (Fig. 5B, C). Using univariate Cox regression analysis, 16 of these 23 gene sets were shown to be risk factors and 7 to be protective factors (Table S1, Supplemental Digital Content, https://links.lww.com/MD/Q518).

3.6. The RS model accurately predicts overall survival (OS)

The 23 described gene sets were used to construct RS for each sample, and patients were subsequently divided into high-risk and low-risk groups (Table S2, Supplemental Digital Content, https://links.lww.com/MD/Q518). Figure 5D, E shows that patients in the high-risk group had considerably shorter OS

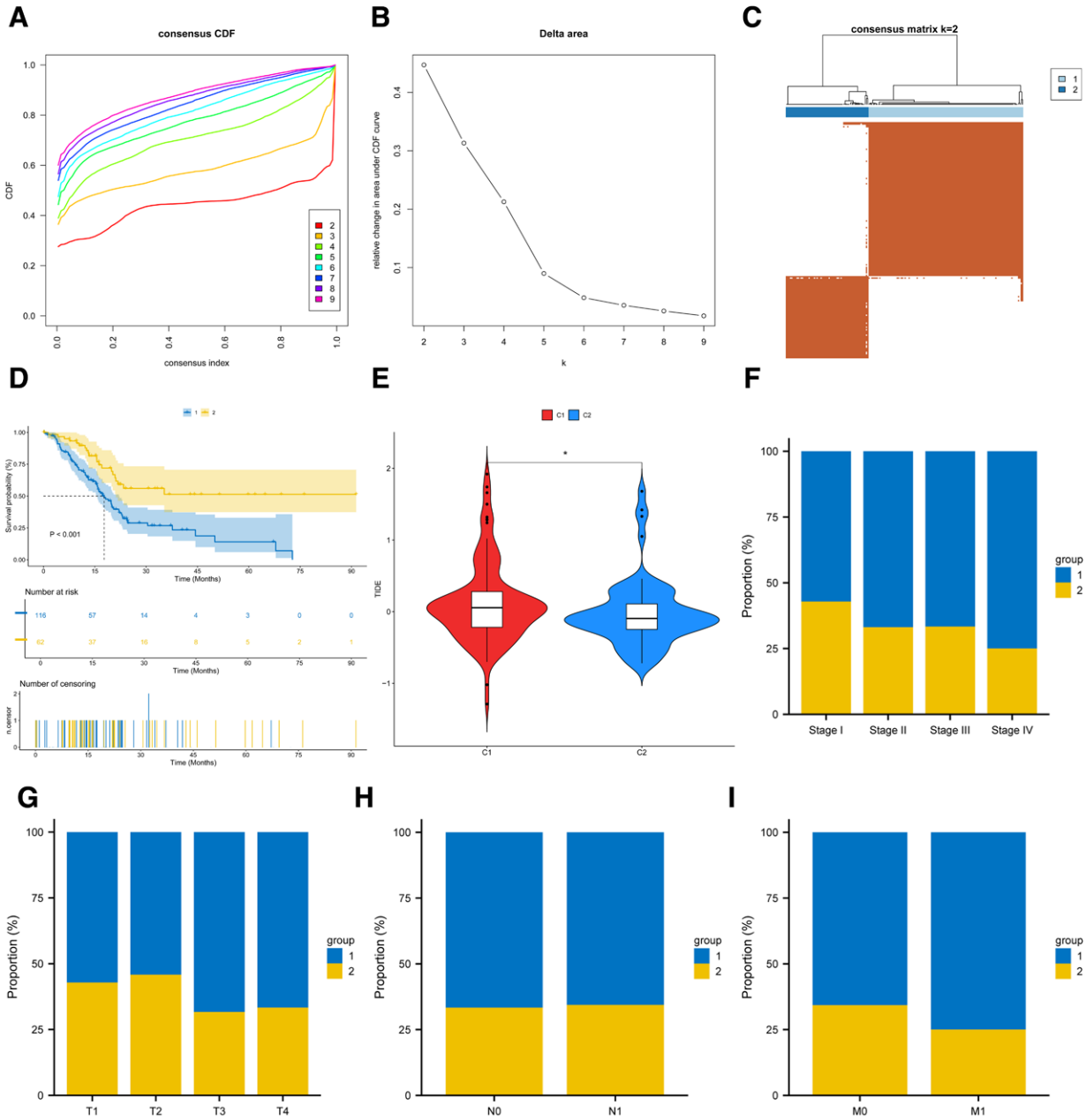


Figure 3. Identification of molecular subtypes, survival analysis, and clinical correlations. (A) Cumulative distribution function (CDF) curve for consensus clustering. (B) Proportion of ambiguous clustering (PAC) analysis. (C) Consensus matrix heatmap of clustering at $k = 2$. (D) Kaplan–Meier survival curves of the identified subtypes. (E) Comparison of TIDE scores between subtypes. (F–I) Distribution of subtypes across clinical stage (F), primary tumor stage (T) (G), lymph node stage (N) (H), and metastasis stage (M) (I). CDF = cumulative distribution function, PAC = proportion of ambiguous clustering.

than patients in the low-risk group. Figure 5F shows that the RS model demonstrated moderate predictive performance for overall survival (AUC = 0.73). Furthermore, GEO dataset was used to verify the RS model's accuracy and generalizability. High-risk patients had much shorter survival durations (Fig. 5G, H), and In the external GEO validation cohort, the RS model yielded an AUC of 0.739, consistent with the moderate predictive performance observed in the TCGA training set (Fig. 5I).

3.7. TMB and drug sensitivity analysis

All genes' mutation frequencies were evaluated using mutation data from the TCGA dataset; genes like TP53 and SMAD4 had the highest mutation frequency in the high-risk group (Fig. S1, Supplemental Digital Content, https://links.lww.com/MD/Q517). The TMB for each tumor sample was calculated, revealing that TMB was significantly higher in the high-risk group compared to the low-risk group ($P < .005$, Fig. 6A). TMB levels were found to be significantly positively correlated with RS ($P < .001$, Fig. 6B). Drug sensitivity analysis revealed notable differences in drug sensitivity between the 2 groups: the low-risk group showed higher sensitivity to gemcitabine, navitoclax-gemcitabine, and oxaliplatin, while the high-risk group was more sensitive to paclitaxel (Fig. 6C).

3.8. Construction of the nomogram

Age, stage_N, stage_T, and RS were all substantially correlated with OS ($P < .05$), according to univariate Cox regression

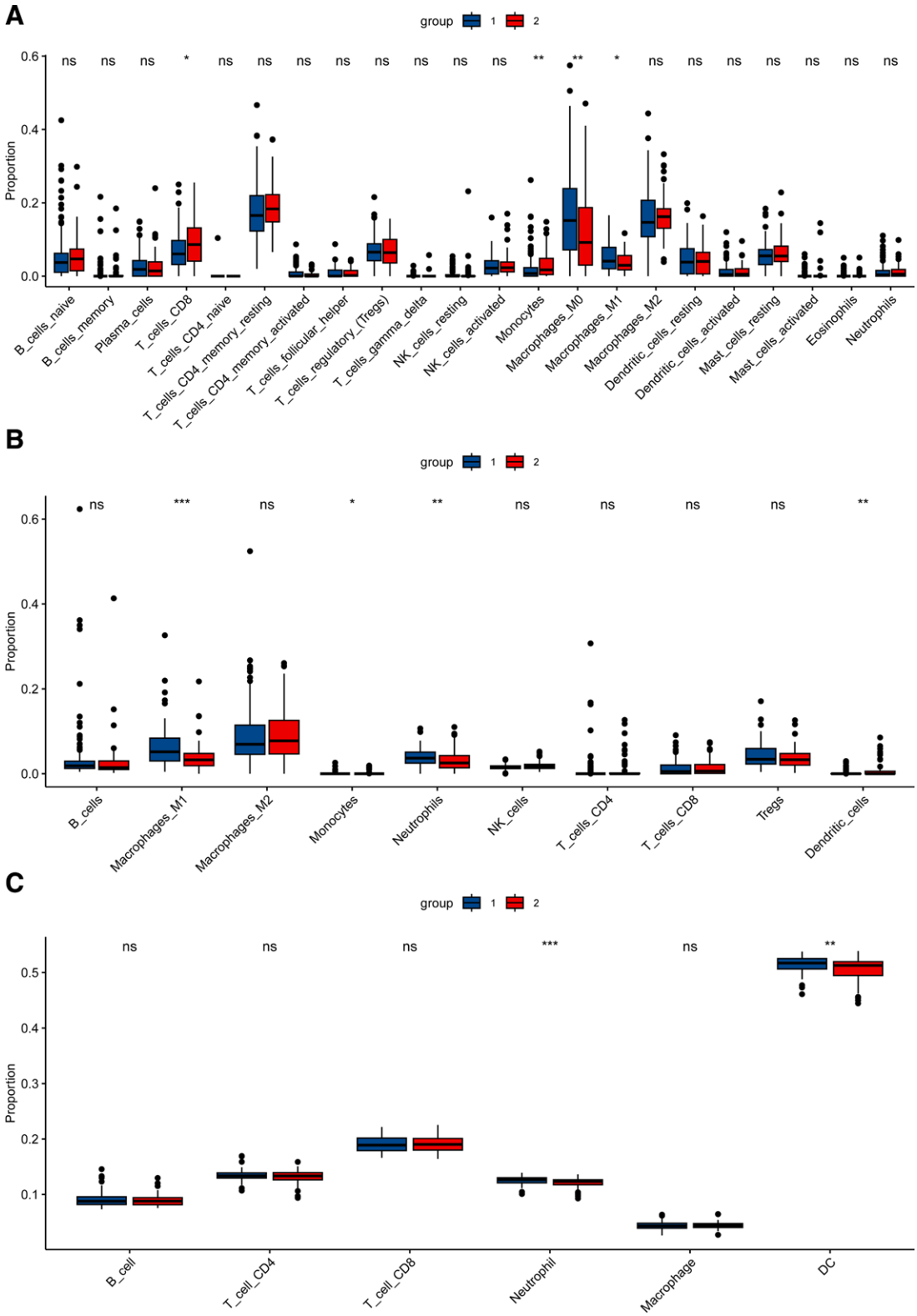


Figure 4. Immune infiltration analysis of the 2 subtypes. Relative abundance of immune cells was compared using different algorithms: (A) CIBERSORT, (B) quanTIseq, and (C) TIMER. Statistical significance: *$P < .05$, **$P < .01$, ***$P < .001$.

analysis (Fig. 7A). RS ($P < .001$) was found to be an independent predictive factor for PAAD by multivariate Cox regression analysis (Fig. 7B). By integrating RS and stage_N ($P < .005$), a nomogram was created (Fig. 7C). Patients were divided into high and low categories after scores were determined for each sample using the nomogram. The outcome was poorer for those in the high grouping (Fig. 7E). The nomogram achieved an AUC of 0.684 for OS prediction, indicating modest-to-moderate discriminative ability (Fig. 7D). The nomogram's strong prediction accuracy was further supported by calibration curve analysis (Fig. 7F).

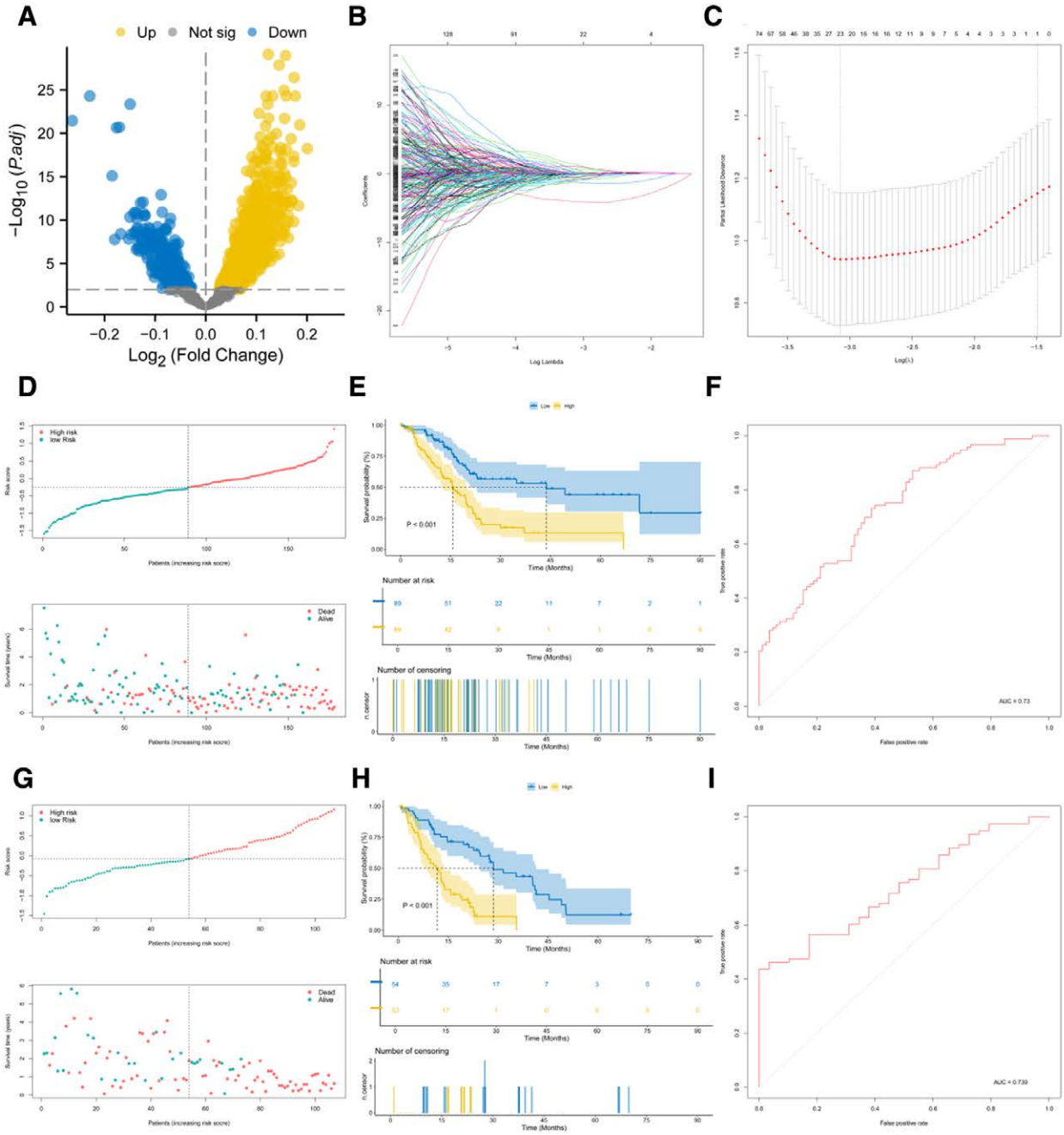


Figure 5. Identification of subtype-related DEGs and construction of a prognostic risk score (RS) model. (A) Volcano plot showing DEGs between subtypes. (B–C) Feature selection by LASSO regression. (D) Distribution of RS in TCGA cohort. (E) Kaplan–Meier survival analysis of TCGA cohort. (F) Receiver operating characteristic (ROC) curves evaluating RS performance in TCGA. (G–I) Validation of RS in GEO datasets: RS distribution (G), survival analysis (H), and ROC curves (I). DEGs = differentially expressed genes, GEO = gene expression omnibus, LASSO = least absolute shrinkage and selection operator, RS = risk score, TCGA = the cancer genome atlas, ROC = receiver operating characteristic.

4. Discussion

Patients at the same pathologic stage frequently have diverse results due to the complexity of PAAD therapy and prognosis. After therapy, some patients may experience a quick relapse, while others may survive for an extended period of time.[27,28] Thanks to developments in sequencing technology, genomes can now be sequenced and analyzed considerably more quickly to identify therapeutic targets for specific individuals. This may facilitate the identification of novel therapeutic targets and, in the long term, could contribute to improvements in prognosis pending clinical validation.[29] To improve therapeutic approaches and clinical results, researchers have been concentrating on the genetic subtypes of PAAD in recent years.[12,13]

Based on alterations in ISGS activity, we classified PAAD patients in the TCGA dataset into 2 molecular subgroups for this investigation. These subtypes' immune infiltration study revealed 6 different immune cell populations. Research has demonstrated that elevated macrophage counts are linked to worse survival and recurrence rates. This association may be explained by the stimulation of a pro-inflammatory response that results in tissue damage and changes the tumor microenvironment, which encourages tumor growth and metastasis.[5,30]

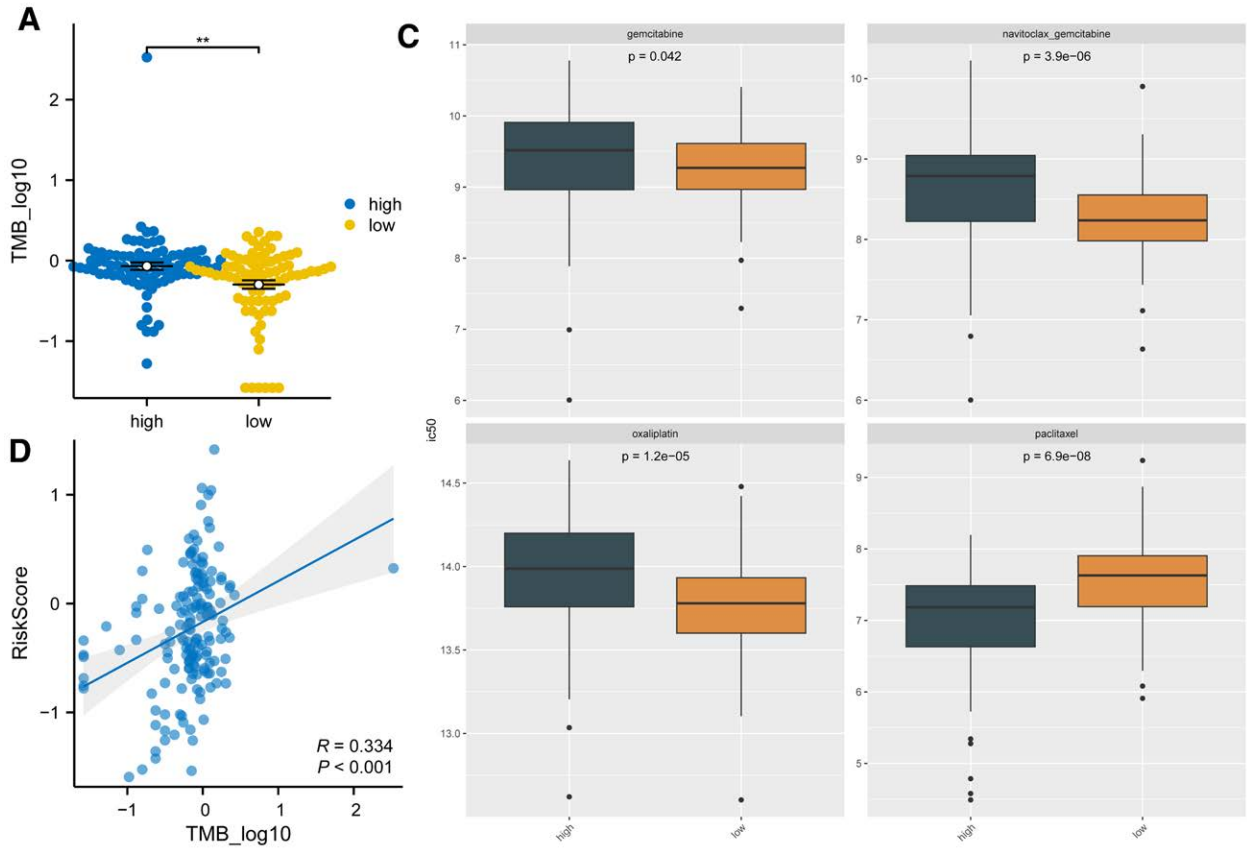


Figure 6. Tumor mutation burden (TMB) and drug sensitivity analyses. (A) Comparison of TMB levels between high- and low-risk groups. (B) Correlation between TMB levels and RS. (C) Drug sensitivity analysis between high- and low-risk groups. RS = risk score, TMB = tumor mutation burden.

Because of their pro-tumorigenic properties in the tumor microenvironment, which include promoting tumor development, metastasis, and immunosuppression, elevated neutrophil expression has been linked to poor clinical outcomes in a number of malignancies.[31] Important antigen-presenting cells, dendritic cells often activate the immune system and set off antitumor immune responses.[32] Nonetheless, immunosuppressive elements within the tumor microenvironment might compromise dendritic cell performance, impeding efficient antigen presentation and T-cell stimulation, ultimately diminishing the immune response against the tumor.[33] Because CD8 + T cells can identify and eliminate tumor cells, they are essential for antitumor immunity, and a positive expression of these cells is often linked to a favorable prognosis for cancer patients.[34,35]

Subtype 1 exhibited a lesser response to immunotherapy, according to a more thorough analysis of each subtype's reaction to the treatment. Even though there were no appreciable differences between subtypes 1 and 2, there was a worse outcome for individuals with subtype 1 according to survival analysis. This implies that immune-related variables could be responsible for the variation in prognosis.

Furthermore, we created an RS model with moderate prognostic prediction capabilities based on subtype-related DEG. Patients with lower overall survival (OS) who were classified as high risk based on the RS model may need more frequent follow-up to track the course of their disease. The GEO dataset provided additional validation of the RS model's prognostic value, demonstrating the model's resilience and generalizability.

immune checkpoint blockade (ICB) therapies, such as anti-PD-1 and anti-PD-L1 medications, have demonstrated positive therapeutic responses and long-lasting disease remission in a subset of cancer patients.[36] But ICB treatment only helps a small percentage of patients.[37] Finding novel approaches to identify patients who could be susceptible to ICB treatment is therefore critically needed. PD-1/PD-L1 inhibitor response rates were greater in patients with high tumor mutational burden (TMB) malignancies, according to a thorough review of 27 tumors.[38] According to our research, patients at higher risk typically have higher TMB levels, which may indicate that ICB treatment will work better in these situations.

Additionally, we found that SMAD4 and TP53 commonly had mutations in pancreatic cancer, with the frequency of mutations being greater in high-risk samples than in low-risk ones. Two significant tumor suppressor genes are SMAD4 and TP53.[39] By controlling the expression of several genes, TP53 suppresses tumor angiogenesis, preserves genetic stability, and encourages apoptosis.[40] SMAD4 Through the TGF-β signaling pathway, SMAD4 causes apoptosis in precancerous cells and blocks the tumor-promoting effects of pro-inflammatory cytokines.[41] The prognosis for patients with pancreatic cancer who have mutations in TP53 and SMAD4 has been shown in related research,[42,43] which helps to explain why individuals in high-risk groups have a worse prognosis.

Chemotherapy is still a cornerstone in the treatment of PAAD since it increases survival and enhances quality of life, particularly in advanced PAAD.[44] Medications such as gemcitabine, navitoclax, paclitaxel, and oxaliplatin are frequently utilized.[45,46] In drug-sensitivity predictions suggested differential sensitivity between risk groups; however, whether these predictions translate to differential clinical response requires experimental validation and prospective clinical studies before they can inform treatment selection.

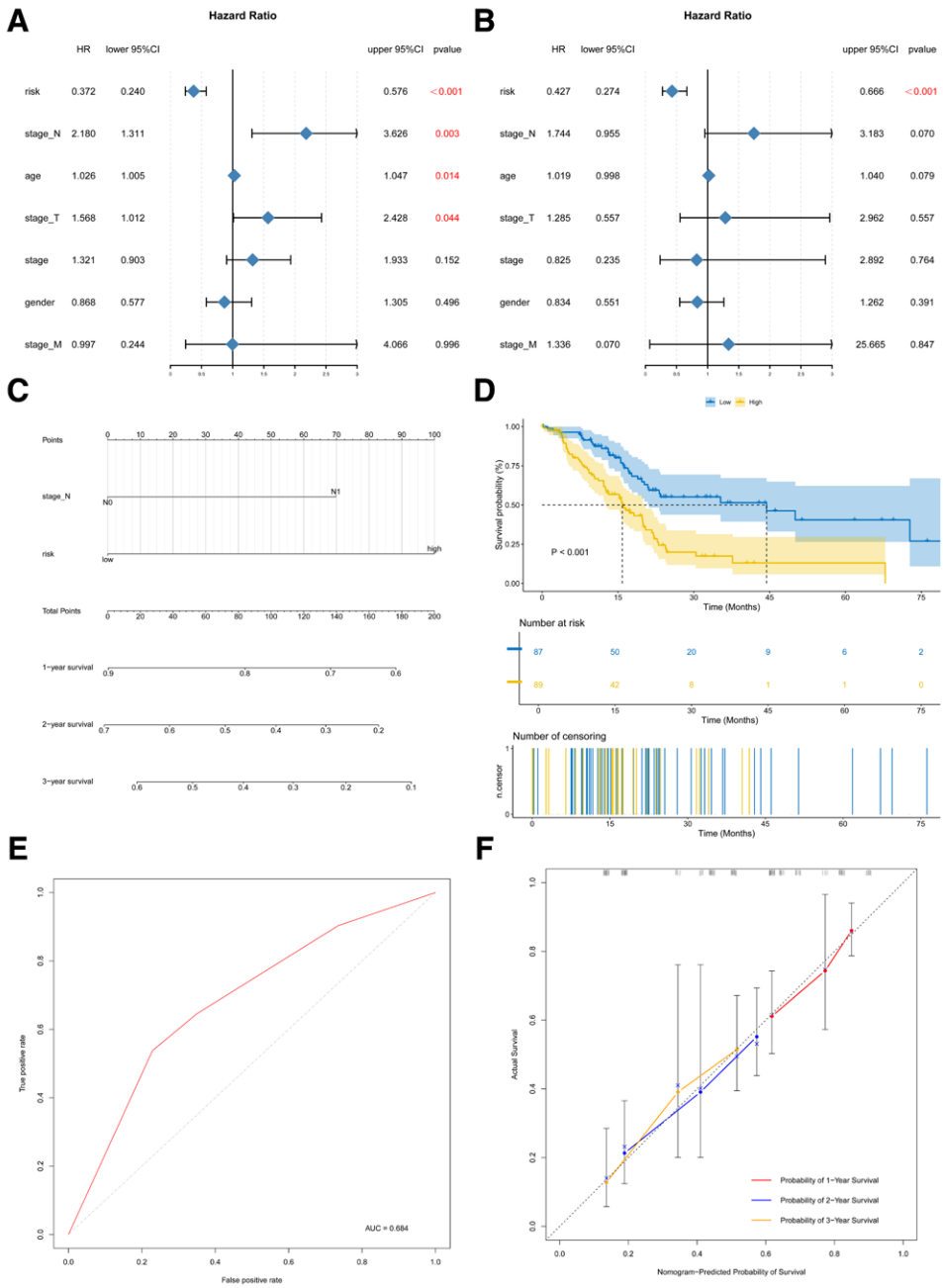


Figure 7. Construction and evaluation of a prognostic nomogram. (A) Univariate Cox regression of clinical variables and RS. (B) Multivariate Cox regression confirming RS as an independent prognostic factor. (C) Nomogram integrating RS and clinical features to predict 1-, 2-, and 3-year survival. (D) Kaplan–Meier survival analysis stratified by nomogram. (E) ROC curves evaluating nomogram performance. (F) Calibration plots assessing agreement between predicted and observed survival. ROC = receiver operating characteristic, RS = risk score.

Lastly, we found that RS was independently associated with OS in multivariate analysis. We therefore developed a nomogram integrating RS and clinicopathologic variables to facilitate risk stratification. Although the nomogram showed acceptable calibration and moderate discrimination in retrospective cohorts, its clinical utility and potential to guide individualized treatment should be evaluated in prospective studies.

In summary, we identified 2 PAAD subtypes based on ISGS activity and developed an RS model that is associated with overall survival. Integration of RS with clinicopathologic variables produced a nomogram with moderate predictive performance. These results provide a framework for immune-focused stratification in PAAD and generate hypotheses for future translational and clinical validation.

We are aware of our study's shortcomings, nevertheless. First, the robustness of our model has to be further confirmed in more independent cohorts, given our data originated from publicly available internet sources. Secondly, there is no experimental validation for the expression and function of these ISGS in patient tissues. Subsequent research endeavors ought to go further into the interplay among these gene sets and verify our conclusions using experimental means. Additionally, the predictive performance of the RS (AUC ≈ 0.73) and nomogram (AUC ≈ 0.68) was moderate; therefore, further refinement and prospective

validation in independent clinical cohorts are required before considering clinical implementation.

Author contributions

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Formal analysis: Junwei Fang, Meili Lin.
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Investigation: Junwei Fang, Shuang Gao, Weibin Lin.
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Project administration: Junwei Fang, Meiping Wang.
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Validation: Junwei Fang, Junhao Du.
Visualization: Junwei Fang, Weixiang Ni, Weibin Lin.
Writing – original draft: Junwei Fang, Weixiang Ni, Weibin Lin.
Writing – review & editing: Junwei Fang, Weixiang Ni.

References

[1] Park W, Chawla A, O'Reilly EM. Pancreatic cancer: a review. JAMA. 2021;326:851–62.
[2] Cai J, Chen H, Lu M, et al. Advances in the epidemiology of pancreatic cancer: trends, risk factors, screening, and prognosis. Cancer Lett. 2021;520:1–11.
[3] Klein AP. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. Nat Rev Gastroenterol Hepatol. 2021;18:493–502.
[4] He R, Jiang W, Wang C, Li X, Zhou W. Global burden of pancreatic cancer attributable to metabolic risks from 1990 to 2019, with projections of mortality to 2030. BMC Public Health. 2024;24:456.
[5] Yang K, Yang T, Yu J, Li F, Zhao X. Integrated transcriptional analysis reveals macrophage heterogeneity and macrophage-tumor cell interactions in the progression of pancreatic ductal adenocarcinoma. BMC Cancer. 2023;23:199.
[6] Bernard V, Semaan A, Huang J, et al. Single cell transcriptomics of pancreatic cancer precursors demonstrates epithelial and microenvironmental heterogeneity as an early event in neoplastic progression. Clin Cancer Res. 2019;25:2194–205.
[7] Juiz NA, Iovanna J, Dusetti N. Pancreatic cancer heterogeneity can be explained beyond the genome. Front Oncol. 2019;9:1–8.
[8] Thol K, Pawlik P, McGranahan N. Therapy sculpts the complex interplay between cancer and the immune system during tumour evolution. Genome Med. 2022;14:137.
[9] Hanahan D. Hallmarks of cancer: new dimensions. Cancer Discov. 2022;12:31–46.
[10] Zheng R, Liu X, Zhang Y, et al. Frontiers and future of immunotherapy for pancreatic cancer: from molecular mechanisms to clinical application. Front Immunol. 2024;15:1–20.
[11] Mizrahi JD, Surana R, Valle JW, Shroff RT. Pancreatic cancer. Lancet. 2020;395:2008–20.
[12] Rashid NU, Peng XL, Jin C, et al. Purity Independent Subtyping of Tumors (PurIST), a clinically robust, single-sample classifier for tumor subtyping in pancreatic cancer. Clin Cancer Res. 2020;26:82–92.
[13] Zhao S, Nicolle R, Augustin J, et al. Prognostic relevance of pancreatic adenocarcinoma whole-tumor transcriptomic subtypes and components. Clin Cancer Res. 2021;27:6491–9.
[14] Xu X, Zhang X, Kou R, et al. Prognosis and immunotherapy response prediction based on M2 macrophage-related genes in colon cancer. J Cancer Res Clin Oncol. 2024;150:31.
[15] Li A, Ye B, Lin F, Wang Y, Miao X, Jiang Y. A novel immunogenomic signature to predict prognosis and reveal immune infiltration characteristics in pancreatic ductal adenocarcinoma. Precis Clin Med. 2022;5:pbac010.
[16] Zhong X, Zhang H, Zhu Y, et al. Circulating tumor cells in cancer patients: developments and clinical applications for immunotherapy. Mol Cancer. 2020;19:15.
[17] Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. BMC Bioinf. 2013;14:7.
[18] Ritchie ME, Phipson B, Wu D, et al. limma powers differential expression analyses for RNA-sequencing and microarray studies. Nucleic Acids Res. 2015;43:e47.
[19] Wilkerson MD, Hayes DN. ConsensusClusterPlus: a class discovery tool with confidence assessments and item tracking. Bioinformatics. 2010;26:1572–3.
[20] Li T, Fan J, Wang B, et al. TIMER: a web server for comprehensive analysis of tumor-infiltrating immune cells. Cancer Res. 2017;77:e108–10.
[21] Chen B, Khodadoust MS, Liu CL, Newman AM, Alizadeh AA. Profiling tumor infiltrating immune cells with CIBERSORT. Methods Mol Biol. 2018;1711:243–59.
[22] Plattner C, Finotello F, Rieder D. Deconvoluting tumor-infiltrating immune cells from RNA-seq data using quanTIseq. Methods Enzymol. 2020;636:261–85.
[23] Engebretsen S, Bohlin J. Statistical predictions with glmnet. Clin Epigenetics. 2019;11:123.
[24] Mayakonda A, Lin D-C, Assenov Y, Plass C, Koeffler HP. Maftools: efficient and comprehensive analysis of somatic variants in cancer. Genome Res. 2018;28:1747–56.
[25] Maeser D, Gruener RF, Huang RS. oncoPredict: an R package for predicting in vivo or cancer patient drug response and biomarkers from cell line screening data. Brief Bioinform. 2021;22:bbab260.
[26] Wu J, Zhang H, Li L, et al. A nomogram for predicting overall survival in patients with low-grade endometrial stromal sarcoma: a population-based analysis. Cancer Commun (Lond). 2020;40:301–12.
[27] Gemenetzis G, Groot VP, Blair AB, et al. Survival in locally advanced pancreatic cancer after neoadjuvant therapy and surgical resection. Ann Surg. 2019;270:340–7.
[28] Versteijne E, Suker M, Groothuis K, et al; Dutch Pancreatic Cancer Group. Preoperative chemoradiotherapy versus immediate surgery for resectable and borderline resectable pancreatic cancer: results of the dutch randomized phase III PREOPANC trial. J Clin Oncol. 2020;38:1763–73.
[29] Dorman K, Zhang D, Heinrich K, et al. Precision oncology in pancreatic cancer: experiences and challenges of the CCCMunichLMU molecular tumor board. Target Oncol. 2023;18:257–67.
[30] Zhu S, Yi M, Wu Y, Dong B, Wu K. Roles of tumor-associated macrophages in tumor progression: implications on therapeutic strategies. Exp Hematol Oncol. 2021;10:60.
[31] Shaul ME, Fridlender ZG. Tumour-associated neutrophils in patients with cancer. Nat Rev Clin Oncol. 2019;16:601–20.
[32] Hato L, Vizcay A, Eguren I, et al. Dendritic cells in cancer immunology and immunotherapy. Cancers (Basel). 2024;16:981.
[33] Gardner A, Ruffell B. Dendritic cells and cancer immunity. Trends Immunol. 2016;37:855–65.
[34] Pagès F, Mlecnik B, Marliot F, et al. International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study. Lancet. 2018;391:2128–39.
[35] McGuigan AJ, Coleman HG, McCain RS, et al. Immune cell infiltrates as prognostic biomarkers in pancreatic ductal adenocarcinoma: a systematic review and meta-analysis. J Pathol Clin Res. 2021;7:99–112.
[36] Chan TA, Yarchoan M, Jaffee E, et al. Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic. Ann Oncol. 2019;30:44–56.
[37] Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell. 2017;168:707–23.
[38] Yarchoan M, Hopkins A, Jaffee EM. Tumor mutational burden and response rate to PD-1 inhibition. N Engl J Med. 2017;377:2500–1.
[39] Qian Y, Gong Y, Fan Z, et al. Molecular alterations and targeted therapy in pancreatic ductal adenocarcinoma. J Hematol Oncol. 2020;13:130.
[40] Blandino G, Di Agostino S. New therapeutic strategies to treat human cancers expressing mutant p53 proteins. J Exp Clin Cancer Res. 2018;37:30.
[41] Liu L, Wang Y, Yu S, et al. Transforming growth factor beta promotes inflammation and tumorigenesis in Smad4-deficient intestinal epithelium in a YAP-dependent manner. Adv Sci (Weinh). 2023;10:e2300708.
[42] Aguirre AJ, Nowak JA, Camarda ND, et al. Real-time genomic characterization of advanced pancreatic cancer to enable precision medicine. Cancer Discov. 2018;8:1096–111.
[43] Shugang X, Hongfa Y, Jianpeng L, et al. Prognostic value of SMAD4 in pancreatic cancer: a meta-analysis. Transl Oncol. 2016;9:1–7.
[44] Mahmood U, Lynch J, Sandhu SK, et al. Impact of long-term chemotherapy on outcomes in pancreatic ductal adenocarcinoma: a real-world UK multi-centre study. Cancers (Basel). 2025;17:1896.
[45] Morimoto Y, Takada K, Takeuchi O, et al. Bcl-2/Bcl-xL inhibitor navitoclax increases the antitumor effect of Chk1 inhibitor prexasertib by inducing apoptosis in pancreatic cancer cells via inhibition of Bcl-xL but not Bcl-2. Mol Cell Biochem. 2020;472:187–98.
[46] Wainberg ZA, Melisi D, Macarulla T, et al. NALIRIFOX versus nab-paclitaxel and gemcitabine in treatment-naive patients with metastatic pancreatic ductal adenocarcinoma (NAPOLI 3): a randomised, open-label, phase 3 trial. Lancet. 2023;402:1272–81.