



# Development and validation of a gene signature for pancreatic cancer: based on inflammatory response–related genes

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## Abstract

Pancreatic cancer (PC) is one of the most common malignant tumors in the world with a poor prognosis. There were limited studies investigating the genetic signatures associated with inflammatory responses, tumor microenvironment (TME), and tumor drug sensitivity prediction. In the Cancer Genome Atlas (TCGA) dataset, we constructed an inflammatory response–related genes prognostic signature for PC, and predictive ability of the model was assessed via the International Cancer Genome Consortium (ICGC) database. Then, we explored the differences of TME, immune checkpoint genes and drug resistance genes, and the cancer cell sensitivity to chemotherapy drugs between different risk score group. Based on the TCGA and ICGC databases, we constructed and validated a prognostic model, which consisted of 5 genes (including AHR, F3, GNA15, IL18, and INHBA). Moreover, the prognostic model was independent prognostic factors affecting overall survival (OS). The low-risk score group had better OS, and lower stromal score, compared with patients in the high-risk score group. The difference of antigen-presenting cells, T cell regulation, and drug resistance genes between different risk score groups was found. In addition, the immune checkpoint genes were positively correlation to risk score. The expression levels of AHR, GNA15, IL18, and INHBA were related to the sensitivity of anti-tumor chemotherapy drugs. Gene set enrichment analysis (GSEA) showed significant pathway such as calcium signaling pathway and p53 signaling pathway. We successfully constructed a 5-inflammatory response–related gene signature to predict survival, TME, and cancer cell sensitivity to chemotherapy drugs in PC patients. Furthermore, substantiation was warranted to verify the role of these genes in tumorigenesis.

**Keywords** Pancreatic cancer · Inflammatory response–related genes · Tumor microenvironment · Immune checkpoint inhibitors · Drug resistance genes · Prognostic model

## Introduction

PC is the 7th leading cause of cancer-related death globally, with 496,000 new cases and 466,000 deaths in the past year (Sung et al. 2021), and a dismal 5-year survival rate of roughly 5% (Li et al. 2019). Despite its low incidence, PC is projected to surpass breast cancer as the third leading cause of cancer death by 2025, according to a survey conducted in 28 European countries (Ferlay et al. 2016). Surgical resection is the mainstay

curative treatment modality (Okasha et al. 2017), significantly improving the 5-year survival rate by 20 to 30% (Okasha et al. 2017). However, because early PC symptoms are insidious, 20–80% of patients have locally advanced or distant metastases at diagnosis (Ansari et al. 2016); hence, only around 9% of the patients are eligible for resection (Kamisawa et al. 2016). Due to the high degree of malignancy, rapid progression, atypical clinical manifestations, and lack of effective screening methods, the prognosis of PC is poor (Mizrahi et al. 2020).

Treatment options for patients who cannot undergo radical surgery include radiation therapy, chemotherapy, immunotherapy (Houot et al. 2015; Lutz et al. 2014), stem cell therapy (Kure et al. 2012; Raj et al. 2015), and metabolism targeting therapy (Blum and Kloog 2014; Son et al. 2013). Although these methods have demonstrated therapeutic efficacy, it is debatable whether these treatments could ultimately benefit patients since side effects such as nausea, vomiting, fatigue, hypertension, renal impairment, and decreased immunity are quite common. Therefore, a model

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for predicting patient prognosis is essential for individualized treatment.

Public databases and next-generation gene sequencing facilitate further in-depth tumor studies, and prognostic gene signatures at the mRNA level can predict OS in PC (including ductal and lobular neoplasms, adenomas, and adenocarcinomas). Cystic, mucinous, and serous neoplasms have too few samples to participate in model construction. It should be noted that pancreatic neuroendocrine tumor, especially nonfunctional neuroendocrine tumor of the pancreas, was a rare case, and we may only learn about this disease from published case report (Mogoanta et al. 2015).

Studies have confirmed that inflammatory response (chronic inflammation, infection, autoimmunity, and tumor elicited inflammation) is related to the occurrence and proliferation of tumors (Dominguez et al. 2017; Greten and Grivennikov 2019; Mantovani et al. 2008; Panigrahy et al. 2019; Takahashi et al. 2010).

Inflammation, inflammatory secretion, and infiltration of immune cells have played a vital role in the launch of cancer and promoting and developing into malignant metastasis (Shadhu and Xi 2019).

In addition, high systemic inflammatory response indexes, such as the neutrophil-albumin ratio (Tingle et al. 2018), platelet-lymphocyte ratio (Lee et al. 2018), C-reactive protein-lymphocyte ratio (Fan et al. 2020), and monocyte-lymphocyte ratio (Sierzega et al. 2017), have been categorically demonstrated to contribute to poor prognosis in PC. A previous study constructed an inflammatory response-related gene signature which could reveal distinct survival outcome in PC (Xie et al. 2022); however, this study did not explore the relationship of the gene signature and immune checkpoint genes and drug-resistance genes.

To the best of our knowledge, genetic signatures associated with inflammatory responses, TME, and tumor drug sensitivity prediction have not been investigated to this date. Herein, an inflammatory response-related gene signature was constructed, and the efficacy, as well as the sensitivity of this signature, was evaluated.

## Methods

### Data collection

Gene expression information and corresponding clinical information of 182 and 262 PC patients were downloaded from the TCGA database (<https://portal.gdc.cancer.gov/>) and ICGC database (ICGC-CA) (<https://docs.icgc.org/>), respectively. The clinical information (including OS, survival state, age, gender, and AJCC stage) of PC patients in TCGA and ICGC was summarized in Table 1. These data are publicly available. The detailed information, such as when these patients been recruited, and how many years were they

followed, was not found. 200 inflammatory response-related genes were queried in the molecular signature database (Supplementary Table 1). Samples in datasets that meet the following inclusion criteria were included in this study: (1) the sample with both mRNA sequencing data and clinical information; (2) the sample with prognosis information. The preprocessing of TCGA RNA-seq was as follows: (1) PC samples without clinical information were removed; (2) data on normal PC tissue samples were removed; (3) the genes in which the “count” was zero in more than half of the PC samples were excluded. The ICGC data were preprocessed as follows: (1) data on normal PC tissue samples were removed, whereas data on primary tumor were retained; (2) the OS period was converted from day/month to year; (3) and only the expression profile of immune-related genes was included.

### Constructing and validating a prognostic inflammatory response-related gene signature

In the TCGA databases, differentially expressed genes (DEGs) in tumor and normal tissues were screened using the “limma” package in R, with the criteria  $\log_2$  fold change (FC) > 1 and adjusted  $P < 0.05$  (Lin et al. 2021). Univariate Cox regression analysis was used to screen prognostic-related inflammatory response genes, and the  $P$  value was adjusted by Benjamini and Hochberg (BH) correction method. To prevent overfitting, LASSO regression analysis was utilized to construct the model (Simon et al. 2011; Tibshirani 1997). The LASSO algorithm was used to select and shrink variables with the “glmnet” R package, so that some regression coefficients were strictly equal to 0, thereby obtaining an interpretable model. The normalized

**Table 1** Clinical features of PC patients in TCGA and ICGC cohort

| Clinical feature | TCGA, <i>n</i> (%) | ICGC, <i>n</i> (%) |
|------------------|--------------------|--------------------|
| Age              |                    |                    |
| > 65             | 89 (48.1%)         | 58 (48.7%)         |
| ≤ 65             | 96 (51.9%)         | 61 (51.3%)         |
| Gender           |                    |                    |
| Male             | 102 (55.1%)        | 61 (51.3%)         |
| Female           | 83 (44.9%)         | 58 (48.7%)         |
| Survival status  |                    |                    |
| Alive            | 84 (47.7%)         | 34 (18.3%)         |
| Dead             | 92 (52.3%)         | 152 (81.7%)        |
| AJCC TNM stage   |                    |                    |
| Stages I–II      | 173 (93.6%)        | 111 (93.3%)        |
| Stages III–IV    | 9 (4.8%)           | 8 (6.7%)           |
| Unknow           | 3 (1.6%)           | 0                  |
| Histologic grade |                    |                    |
| G1–2             | 129 (69.7%)        |                    |
| G3–4             | 53 (28.7%)         |                    |
| Unknown          | 3 (1.6%)           |                    |

expression matrix of candidate prognostic DEGs was the independent variable in regression, and the dependent variable was the OS and status of patients in the TCGA cohort. The optimal penalty parameter  $\lambda$  related to the smallest tenfold cross-validation in the TCGA dataset was confirmed.

Next, according to the constructed gene signature, the risk score of each patient was quantified. The calculation formula used is as follows: the expression level of each gene \* the corresponding regression coefficient (Huo et al. 2021; Mo et al. 2020). Patients were then stratified into high-risk and low-risk groups based on the median risk score of all patients. Principal component analysis (PCA) analysis and *t*-distributed stochastic neighbor embedding (*t*-SNE) analysis using the “Rtsne” and “ggplot2” packages in R were used to investigate the distribution of high and low risk patients.

To explore the predictive power of the prognostic model, survival analysis was conducted using the “survminer” package, and by drawing receiver operating characteristic (ROC) curve using “timeROC” in R. Subsequently, univariate and multivariate Cox analyses were performed using the “survival” package.

### Functional enrichment analysis

To explore potential molecular mechanisms, GSEA was conducted in the high- and low-risk groups with GSEA software 4.1.0 (Canzler and Hackermuller 2020; Croken et al. 2014). The enrichment pathway with  $P < 0.05$  represented significant pathway enrichment in the high- and low-risk groups. The infiltration scores of 16 immune cells and the activities of 13 immune-related pathways in different risk score groups were calculated using the single-sample GSEA computed by the “GSVA” R package (Hanzelmann et al. 2013).

### TME and chemotherapy sensitivity analysis

We estimated the stromal, immune, and estimate scores in the TME using the ESTIMATE algorithm (Charoentong et al. 2017). The immune and stromal scores were used to evaluate the infiltration of immune cells and stromal cells in tumor tissue (Yoshihara et al. 2013). The enrichment scores of different immune cells, related functions, and pathways were quantified using the ssGSEA.

Based on the NCI-60 database (<https://discover.nci.nih.gov/cellminer>), Pearson correlation analysis between prognostic gene expression and the sensitivity of anti-tumor drugs was conducted (including 216 drugs approved by FDA; see Supplementary Table 2). The specific method was as follows: (1) download the RNA-seq file and compound activity: DTP NCI-60 file from the NCI-60 database, (2) extract 216 drugs approved by FDA from the DTP NCI-60 file, (3) extract the information of prognostic gene expression, and (4) Pearson correlation analysis was performed to explore the relevance.

### Statistical analysis

Wilcoxon test was used to select DEGs. Survival analysis between high- and low-risk score groups was compared using the Kaplan–Meier analysis (log-rank test). The univariate Cox analysis was used to select variables related to OS. The multivariate Cox analyses were used to determine independent risk factors affecting patient prognosis. The Spearman or Pearson correlation analysis was used to compare the relevance between risk score and immune checkpoint genes (including PD-1, PD-L1, PD-L2, and CTLA-4) and drug resistance genes (including MRP1, MRP2, MRP3, MRP4, MRP5, and MRP6, MRP7, MRP8, and MRP9). The ssGSEA scores of immune cells or immune pathways in different risk groups were compared by the Wilcoxon test. The Wilcoxon test was used to compare the expression level of immune checkpoint genes and drug resistance genes. Wilcoxon test was used to compare the risk score in different clinical information (including age, gender, stage, and grade), TME scores (including immune score, stromal score, and estimate score), and immune components (including C1 (wound healing), C2 (INF-g dominant), C3 (inflammatory), and C6 (TGF-b dominant)). Correlation analysis between prognostic gene expression and the sensitivity of anti-tumor drugs was conducted by the Spearman or Pearson test. The R software (Version 3.6.3) with packages *venn*, *igraph*, *ggplot2*, *pheatmap*, *ggpubr*, *corrplot*, and *survminer* was used to create plots. In all statistical results, a two-tailed  $P$  value  $< 0.05$  indicated statistical significance.

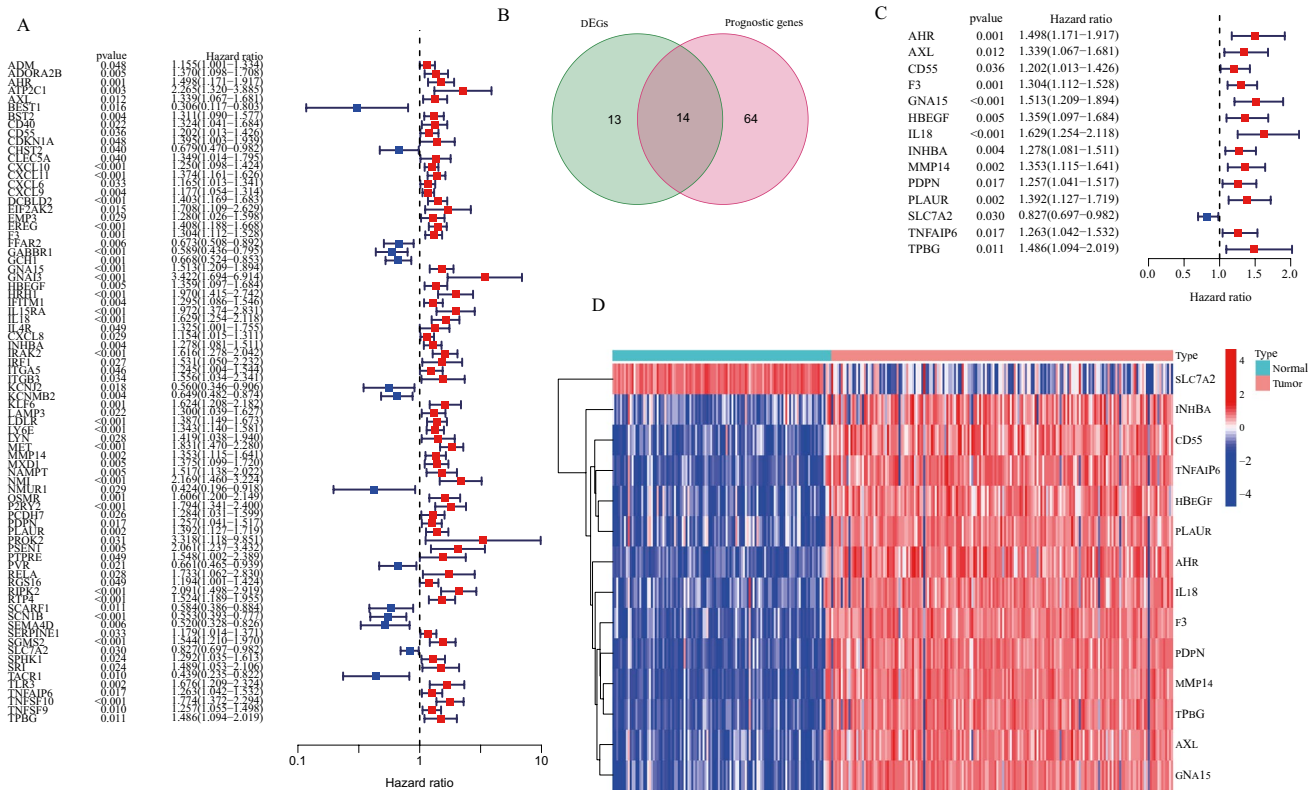
## Result

### Identification of prognostic inflammatory response-related DEGs in the TCGA cohort

A differential analysis using the “limma” package was performed with the selection criteria  $|\log_2 FC| > 1$  and  $P < 0.05$  in the TCGA database. Moreover, a total of 27 DEGs were screened (Supplementary Table 3). Next, using the univariate Cox analysis, 78 inflammatory response-related genes were analyzed based on their prognostic value (Fig. 1A). Finally, 14 overlapping genes (including AHR, AXL, CD55, F3, GNA15, HBEGF, IL18, INHBA, MMP14, PDPN, PLAUR, SLC7A2, TNFAIP6, and TPBG) between DEGs and inflammatory response-related genes were selected (Fig. 1B, C, D).

### Construction of a prognostic model in the TCGA cohort

A LASSO analysis was further conducted to construct a prognostic model, and a 5-inflammatory response-related gene signature was established (Fig. 2A–B). The risk score was calculated as follows: Risk score =  $AHR \times 0.02623 + F3 \times 0.01848 + GNA15$



**Fig. 1** Identification of the candidate inflammatory response-related genes based on TCGA cohort. **A** Forest map of 78 genes with prognostic value. **B** Venn diagram to identify 14 overlapping genes between DEGs and inflammatory response-related genes with prognostic value. **C** Forest map of the 14 overlapping genes. **D** The heatmap of 14 overlapping genes expression in PC tissues and adjacent normal tissue samples. PC, pancreatic cancer

×0.12838 + IL18 × 0.19575 + INHBA × 0.01221. According to the median value of risk scores, samples were stratified into the high- and low-risk score groups. Survival analysis (log-rank test) revealed that patients in the low-risk score group had a better prognosis than those in the high-risk group (Fig. 2C). In addition, a relatively robust area under the curve (AUC) value that was obtained for 1-, 2-, and 3-year OS was 0.679, 0.656, and 0.702, respectively (Fig. 2D). The survival status map showed that the number of mortalities increased as the risk score increased (Fig. 2E–F). Furthermore, the PCA analysis and T-SNE analysis maps could clearly distinguish patients in the high- or low-risk score groups (Fig. 2G–H).

**External validation of the 5-inflammatory response-related gene signature in the ICGC cohort**

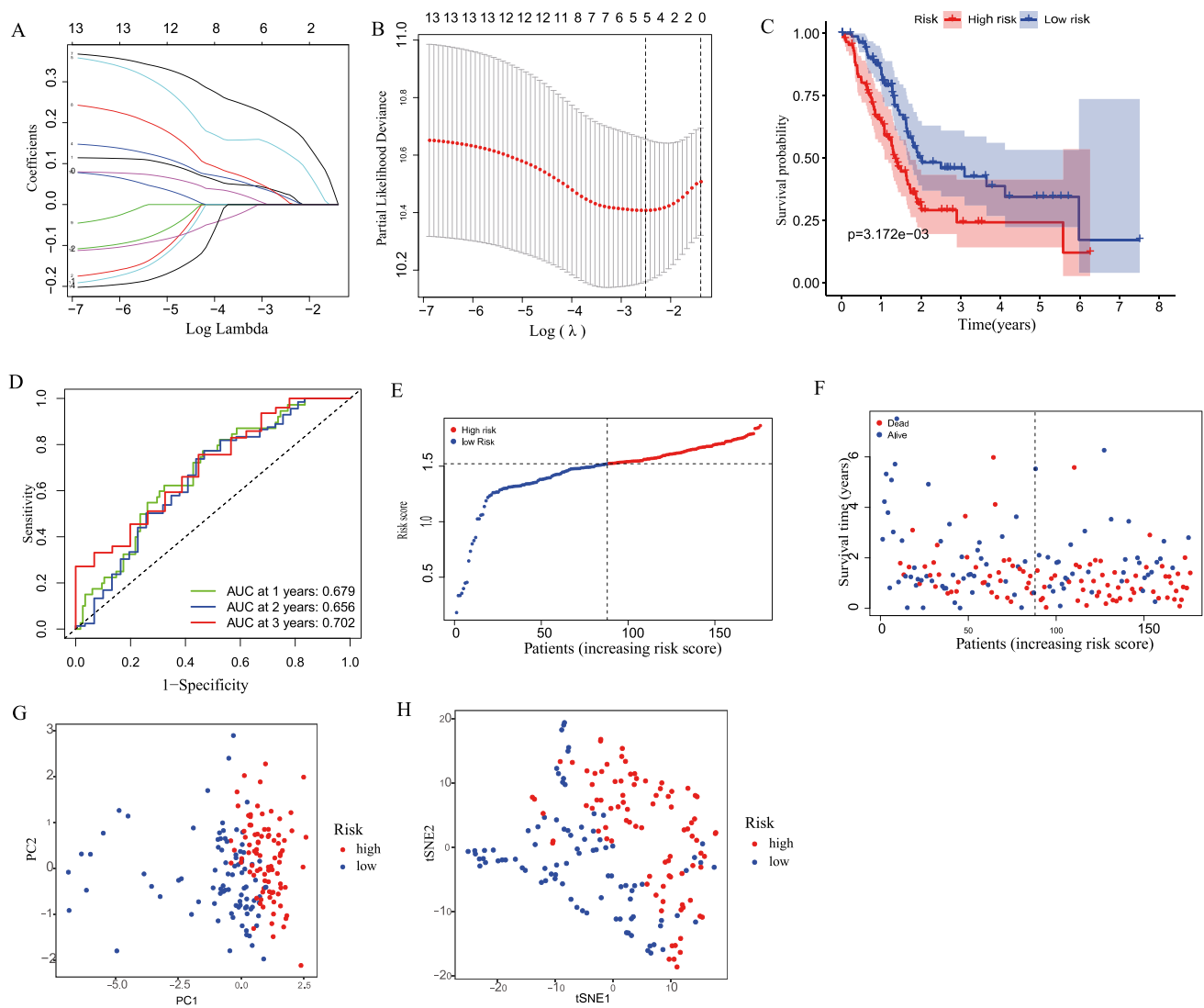
External cohort verification (186 HCC patients from the ICGC cohort) was used to confirm the stability of the above model. The formula to calculate the risk score of the ICGC cohort was the same as the TCGA cohort. Based on the median value mentioned previously, we stratified the risk score of the ICGC cohort into a high- or low-risk group.

nostic value. **C** Forest map of the 14 overlapping genes. **D** The heatmap of 14 overlapping genes expression in PC tissues and adjacent normal tissue samples. PC, pancreatic cancer

The survival curve of the low-risk score group was significantly better than the high-risk score group (Fig. 3A). The AUCs for 1, 2, and 3-year OS were 0.753, 0.722, and 0.684, respectively (Fig. 3B). The survival status map showed that the number of mortalities increased as the risk score increased (Fig. 3C–D). The PCA analysis and T-SNE analysis maps could clearly distinguish patients with different risk score groups (Fig. 3E–F), consistent with the results of the TCGA cohort.

**Analysis of independent prognostic factors affecting OS based on the TCGA cohort and the ICGC cohort**

We included the risk score and other clinical factors (age, gender, grade, and AJCC stage) in univariate and multivariate Cox regression analyses based on the TCGA cohort (clinical factors include age, gender, grade, and AJCC TNM stage) and the ICGC cohort (clinical factors include age, gender, and AJCC TNM stage.) to explore independent prognostic factors affecting OS. Interestingly, we obtained a similar result, with the risk score being an independent factor affecting patient prognosis (Figure 4A, B, C, D).



**Fig. 2** Construction of a 5-inflammatory response-related gene signature using the TCGA cohort. **A** Cross-validation for tuning parameter selection in the LASSO model. **B** LASSO coefficient profiles of 6 prognostic inflammatory response-related genes. **C** Kaplan-Meier curves of OS of PC patients in the training cohort using the risk score. **D** ROC plot. **E–F** The distribution of the risk score, and the survival status of patients. **G** The PCA plot. **H** The t-SNE plot. OS, overall survival; PC, pancreatic cancer; ROC, receiver operating characteristic; PCA, principal component analysis; t-SNE, t-distributed stochastic neighbor embedding

score. **D** ROC plot. **E–F** The distribution of the risk score, and the survival status of patients. **G** The PCA plot. **H** The t-SNE plot. OS, overall survival; PC, pancreatic cancer; ROC, receiver operating characteristic; PCA, principal component analysis; t-SNE, t-distributed stochastic neighbor embedding

### Prognostic model risk score and clinical features

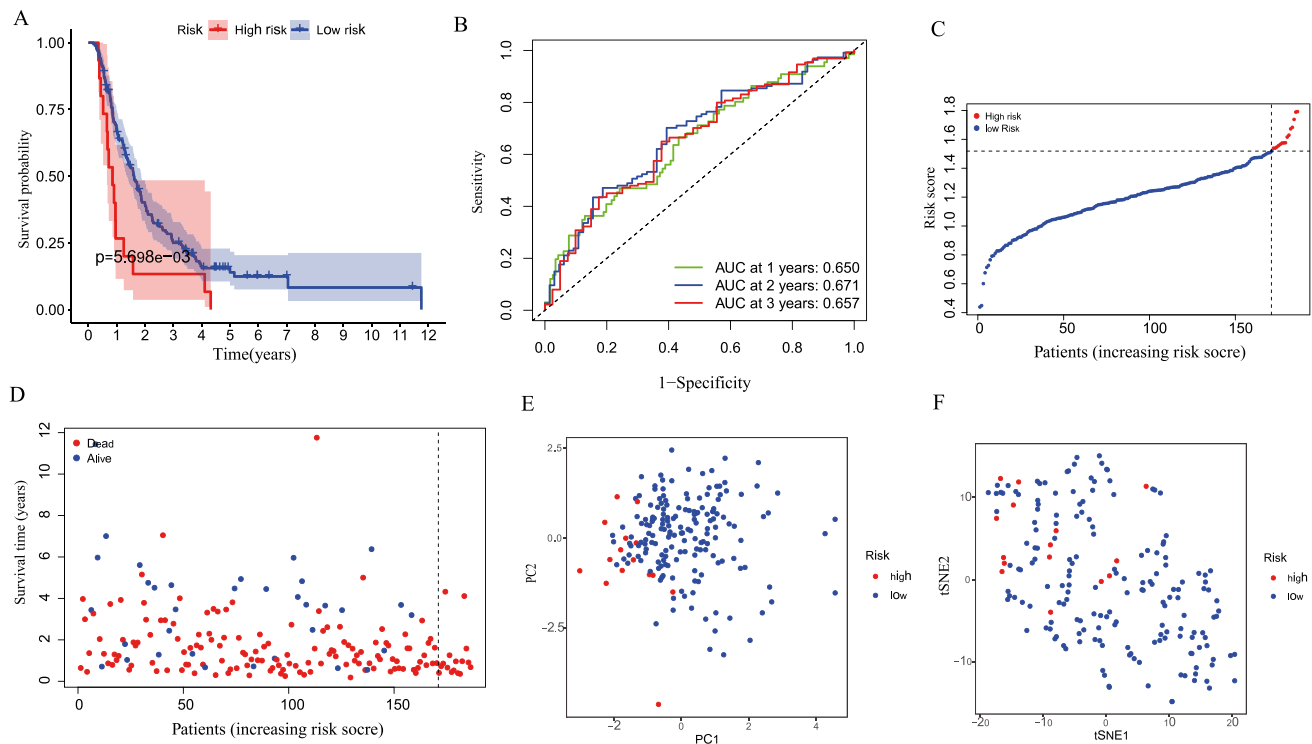
In addition, a Wilcoxon test between risk scores and clinical features was conducted. The results revealed that a higher risk score was associated with poorer tumor differentiation ( $P < 0.05$ ) (Fig. 5A, B, C, D).

### Immune status and TME analysis

ssGSEA was utilized to explore the relationship between the risk score and immune status, related functions and pathways. The results showed that the fractions of CD8+ T cells, mast cells, and NK cells in the high-risk score group are

significantly lower than those in the low-risk score group in the TCGA cohort ( $P < 0.05$ ) (Fig. 6A). In the ICGC cohort, the fractions of B cells and TIL in the high-risk score group are significantly lower compared to the low-risk score group ( $P < 0.05$ ) (Fig. 6C). Furthermore, the cytolytic activity and type-II IFN response scores in the TCGA cohort were lower in the high-risk score group than in the low-risk score group. In contrast, the type-I IFN response score was higher in the high-risk score group compared to the low-risk score group (Fig. 6B). In the ICGC cohort, the HLA, MHC class I, T cell co-stimulation, and type-II IFN response scores were lower in the high-risk score group than in the low-risk score group (Fig. 6D). This showed the difference in antigen-presenting





**Fig. 3** Validation of the 5-inflammatory response-related gene signature in the ICGC cohort. **A** Kaplan–Meier curves of OS of PC patients in the test cohort using the risk score. **B** ROC curves. **C–D** The distribution of the risk score, and the survival status of patients.

**E** The PCA plot. **F** The t-SNE plot. OS, overall survival; PC, pancreatic cancer; ROC, receiver operating characteristic; PCA, principal component analysis; t-SNE, t-distributed stochastic neighbor embedding

cells and T cell regulation between different risk score groups. Next, we explored the potential contribution of the risk score to the TME. The result showed that stromal score experienced a significantly higher trend in the risk-low group (Fig. 6E).

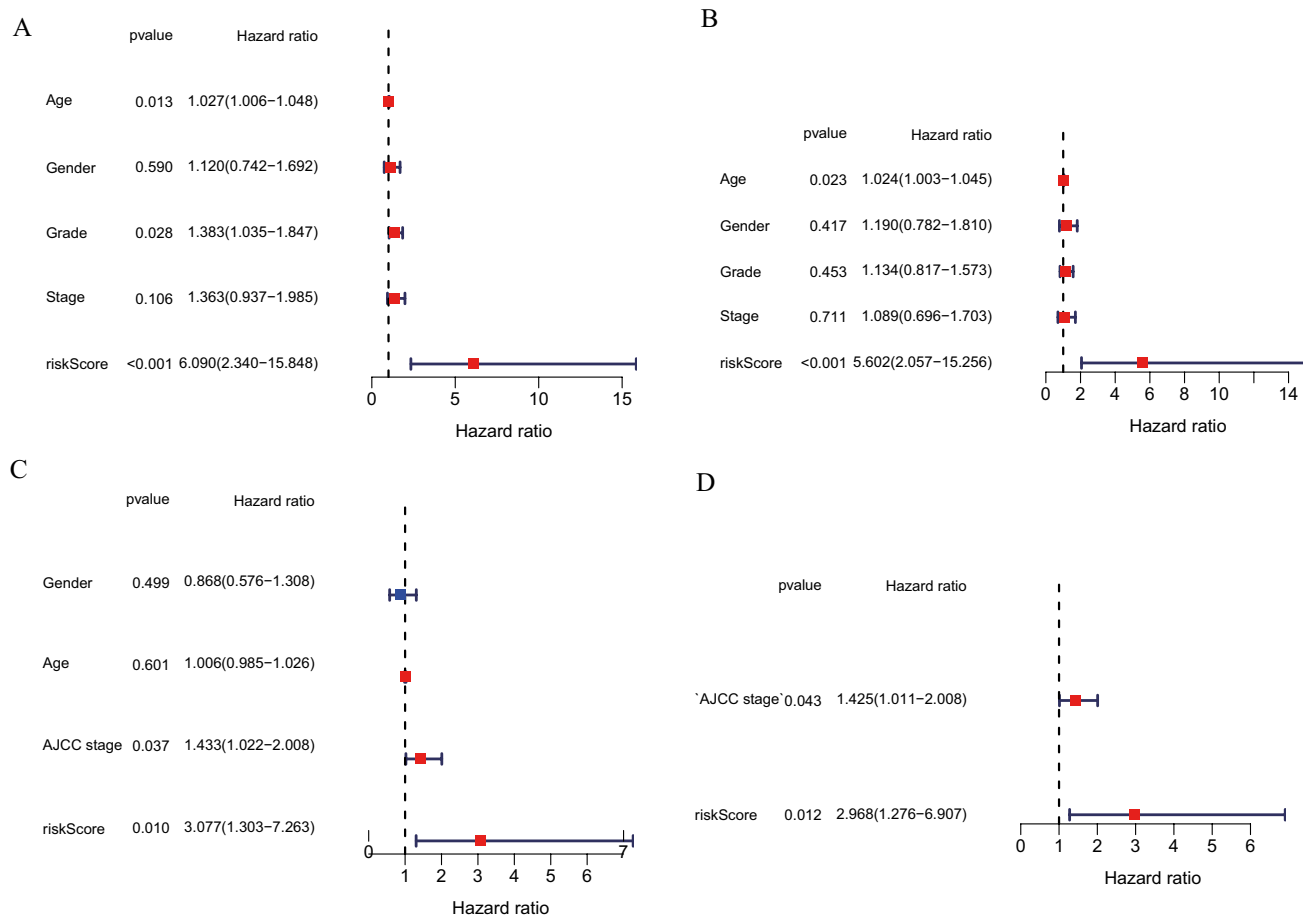
In addition, the correlation between risk scores and immune components was further studied. There are six types of immunogenic permeates in human tumors, corresponding to tumor-promoting and tumor-suppressing functions (Tamborero et al. 2018), namely, C1 (wound healing), C2 (INF-g dominant), C3 (inflammatory), C4 (lymphocyte depleted), C5 (immunologically quiet), and C6 (TGF- $\beta$  dominant). The results showed that a high-risk score was significantly related to C1, C2, and C6, while a low-risk score was significantly related to C3 (Fig. 6F).

### Relationship between risk score and immune checkpoint genes and drug resistance genes

To explore the relationship between risk scores, immune checkpoint genes, and drug resistance genes, the Spearman or Pearson correlation analysis test was performed. Although the results showed that the expression levels of cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed cell death 1 (PD-1), programmed cell death-ligand 1 (PD-L1),

programmed cell death-ligand 2 (PD-L2) had no significant difference in high and low-risk score groups ( $P > 0.05$ ), we found that the expression levels of these genes differed in different risk score groups (Supplementary Fig. 1A–1H). In addition, the expression level of PD-L1 was positively correlated with risk scores. This showed that the prognostic score could provide a theoretical basis for immunotherapy.

The expression levels of MRP1, MRP3, and MRP8 in the high-risk score group were significantly higher than that in the low-risk score group (Supplementary Fig. 2A, 2E, and 2I). The expression levels of MRP1, MRP3, and MRP8 were positively correlated with risk scores (Supplementary Fig. 2B, 2F, and 2J). In contrast, the expression levels of MRP2 and MRP4 in the low-risk score group were significantly higher compared to in the high-risk score group (Supplementary Fig. 2C and 2G). The expression levels of MRP2 and MRP4 were negatively correlated with the risk score (Supplementary Fig. 2D and 2H). However, there was no significant difference in the expression levels of MRP5, MRP6, MRP7, and MRP9 in the high- and low-risk score groups (Supplementary Fig. 3A, 3C, 3E and 3G). The expression levels of MRP6 and MRP9 were negatively correlated with the risk score (Supplementary Fig. 3D, 3H). In comparison, the expression levels of MRP5 and MRP7 were not significantly correlated with the risk score (Supplementary Fig. 3B, 3F).



**Fig. 4** Screening for independent prognostic factors affecting OS based on TCGA cohort (**A, B**), and ICGC cohort (**C, D**). **A, C** OS-related factors were screened by univariate cox regression analyses.

**B, D** OS-related factors were screened by multivariate Cox regression analysis. OS, overall survival

### Prognostic gene expression and cancer cell sensitivity to chemotherapy

Figure 7 shows the relationship between prognostic gene expression and drug sensitivity. The higher the expression level of GNA15, the more drug-sensitive cancer cells were to Nelarabine, Fludarabine, Asparaginase, Cladribine, and Hydroxyurea. The higher the expression level of AHR, the less drug-resistant cancer cells were to Oxaliplatin, Arsenic trioxide, and Lomustine ifosfamide. The higher the expression level of INHBA, the more drug-sensitive cancer cells were to Zoledronate and Dasatinib. The higher the expression, the more drug-sensitive cancer cells were to Tyrothricin. The higher the expression level of IL18, the less drug-resistant cancer cells were to Pipamperone, Bortezomib, Actinomycin D, and Estramustine.

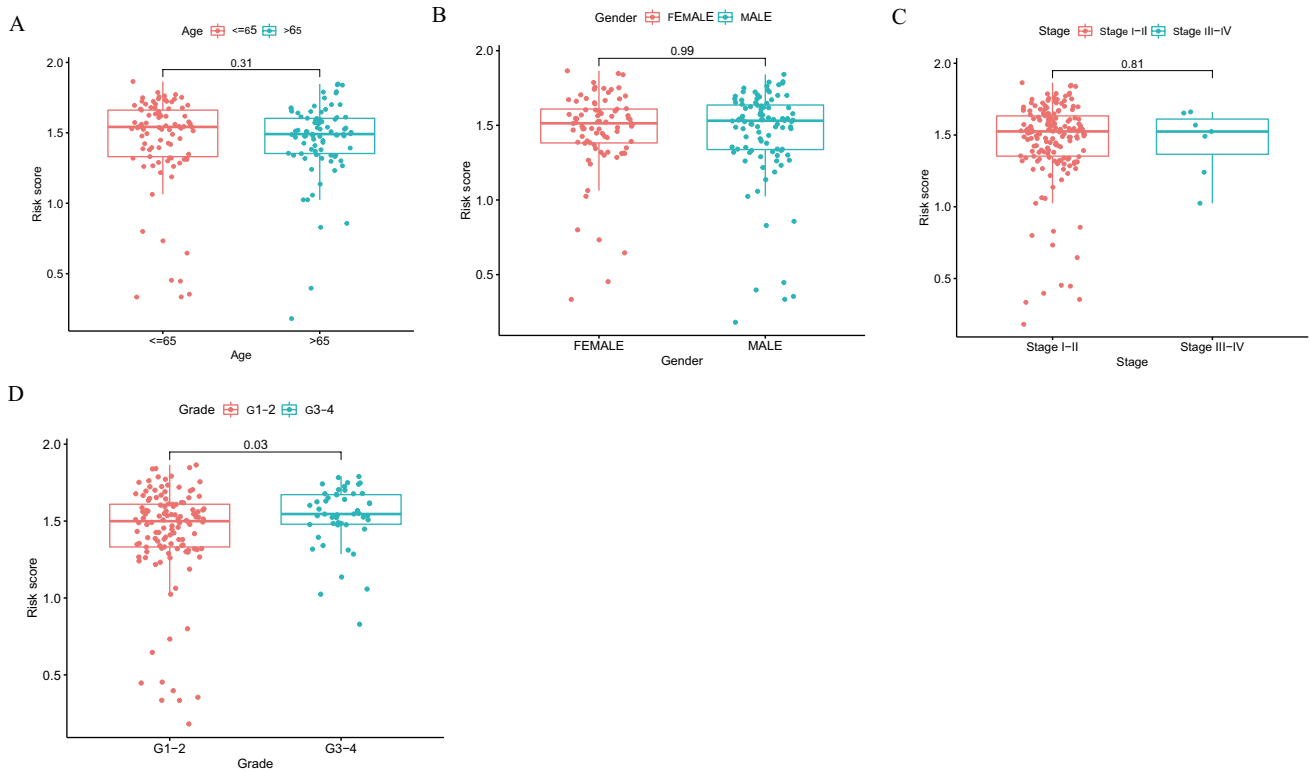
### Biological function and pathway analyses

To explore potential mechanisms, GSEA enrichment analysis was conducted, and the results showed that calcium signaling

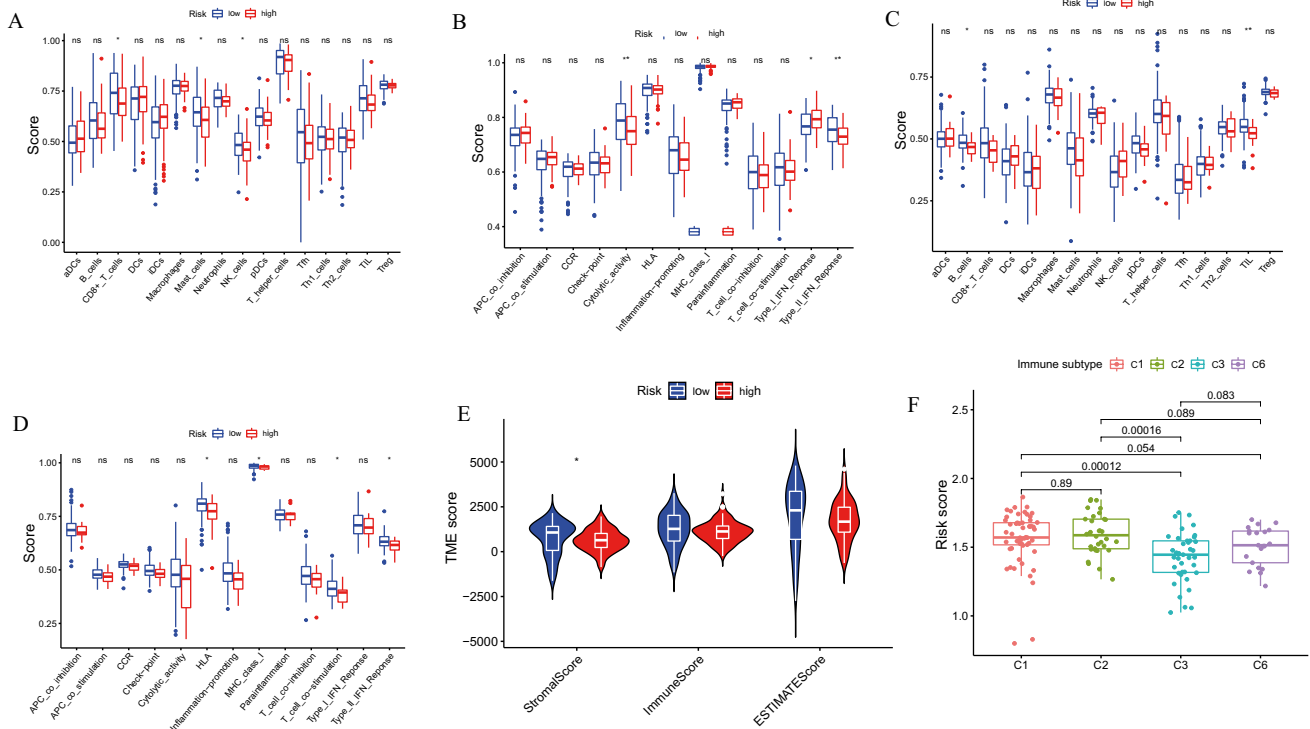
pathway, serine, and threonine metabolism, maturity-onset diabetes of the young, neuroactive ligand-receptor interaction, p53 signaling pathway, pathogenic Escherichia coli infection, pentose phosphate pathway, proteasome, thyroid cancer, and tryptophan metabolism were significantly enriched. Collectively, our results provide new ideas for further targeted gene therapy (Fig. 8).

### Validation of the six mRNA expressions

In the TCGA database, all five genes were highly expressed compared to adjacent non-tumor liver tissue. Next, as showed in Fig. 9, we further performed expression verification of the 5 genes from HPA database (The Human Protein Atlas) and GEPIA database (<http://gepia.cancer-pku.cn/>). Compared to normal pancreatic tissues, AHR, F3, GNA15, IL18, and INHBA were significantly overexpressed in PC tissues. Using clinical samples from the Human Protein Atlas, we analyzed the expression of the proteins encoded by the 5 genes to determine their clinical relevance. Compared to normal pancreatic



**Fig. 5** The relationship between the inflammatory response-related gene signature and the clinical features of the patients in TCGA cohort. **A** Age. **B** Gender. **C** TNM stage. **D** Grade



**Fig. 6** Immune status and TME analysis in different risk-score groups based on TCGA cohort (**A**, **B**) and ICGC cohort (**C**, **D**). **A**, **C** The boxplots of the scores of 16 immune cells. **B**, **D** The boxplots of the 13 immune-related functions. **E** Comparison between the risk score

and stromal score, immune score, and estimate score. **F** Comparison of the risk score in different immune infiltration subtypes. TME, tumor microenvironment



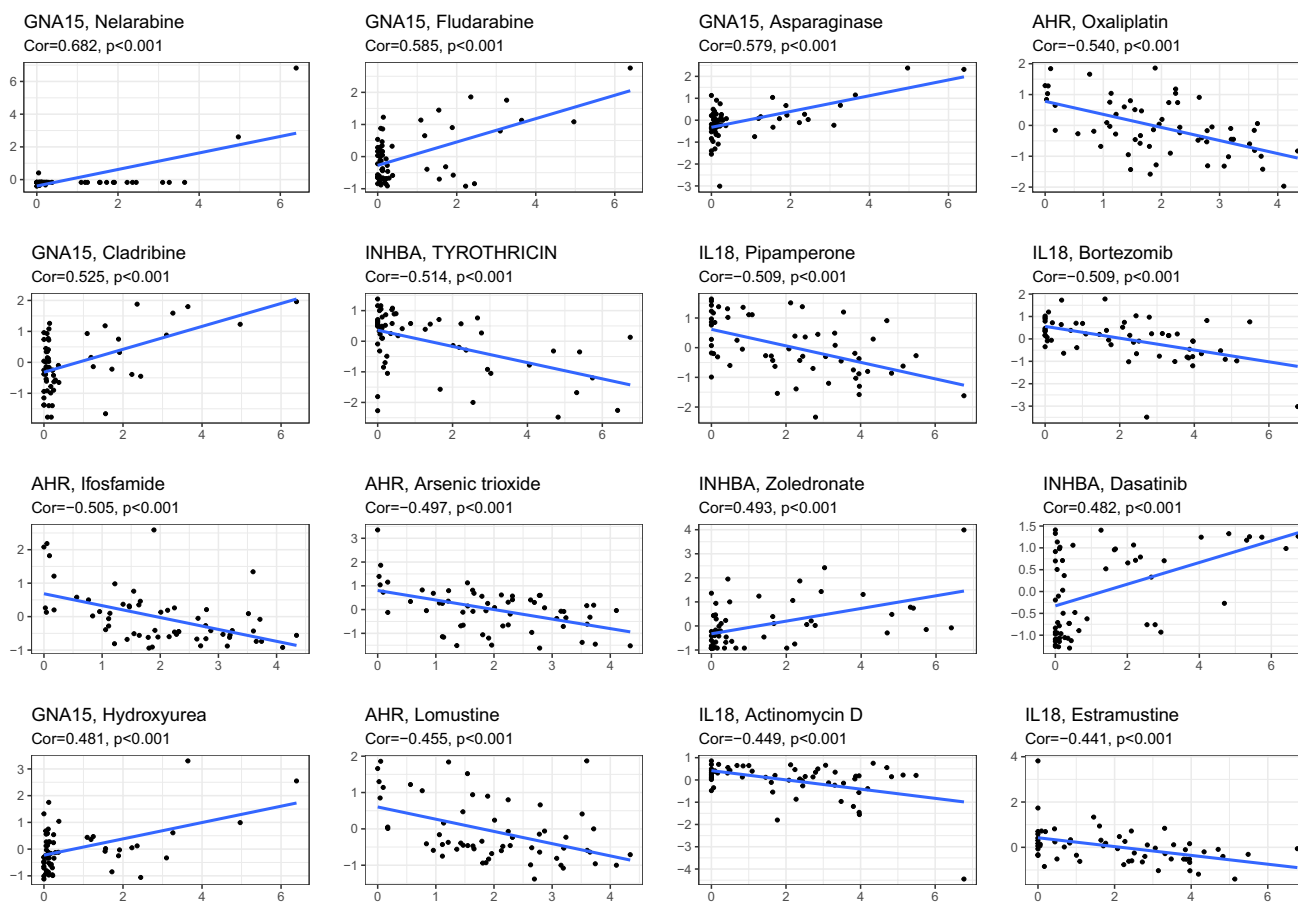
tissues, IL18 was strongly expressed, while AHR, F3, and GNA15 were moderately expressed. However, the website did not list INHBA.

## Discussion

Public databases greatly facilitate cancer research as well as treatment development and are extensively used nowadays. Inflammation-related genes are receiving more and more attention. To our knowledge, we are the first to investigate the role of inflammation-related genes in PC. Herein, we constructed a prognostic model related to inflammatory response genes using the TCGA database. Notably, we achieved a good AUC for our prognostic model. Additionally, the ICGC database was utilized to validate the prognostic model's prediction capabilities. We achieved good AUCs for the TCGA and ICGC datasets, and patients with a high-risk score had a worse OS, with the risk score being an independent predictive factor for patients. Therefore, further investigation of genes

associated with the 5-inflammatory response can shed light on the mechanism underlying our prognostic model.

AHR is a ligand-activated transcription factor that promotes cancer cell growth and malignancy (Puga et al. 2009; Safe et al. 2013). AHR activation results in immune-tolerant cells such as a dendritic cell (DCS) and Tregs, which co-cultivates the TME, which is defective in identifying and eliminating cancer cells (Cheong and Sun 2018). AHR can activate KYN to further increase the growth and invasiveness of PC cells (Wang et al. 2020). The reallocation of methylated cysteines in the GNA15 gene is associated with the presence of Gα15 in pancreatic ductal adenocarcinoma (PDAC), starting with precursor lesions and continuing through all stages of PDAC (Innamorati et al. 2021). Studies have confirmed that IL-18 plays an essential role in the pathogenesis of chronic and eosinophilic pancreatitis (Manohar et al. 2018, 2017). In addition, macrophage-induced IL-18-mediated inflammatory responses play a crucial function in malignant pancreatic tumors initiation and progression (Kandikattu et al. 2021). Studies have confirmed that INHBA plays a vital role in different tumors



**Fig. 7** Relationship between prognostic gene expression and drug sensitivity. UCK2. PFKFB4. PDE6A. ME1. LCAT. PDE6C. G6PD. LPCAT1. DTYMK. POLR3G

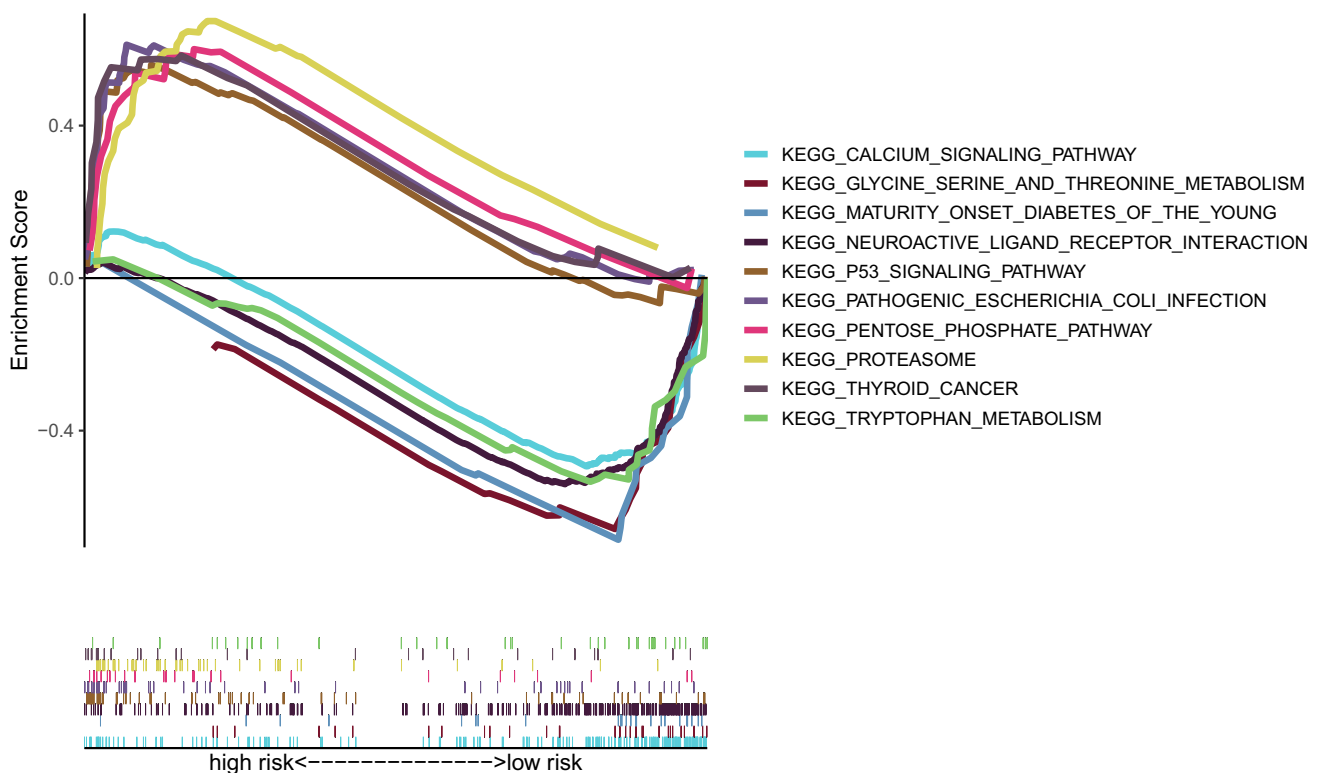
(Chen et al. 2019; Lyu et al. 2018). High levels of INHBA expression were positively associated with lower 5-year survival rates in colorectal and gastric cancer patients (Okano et al. 2013; Zhao et al. 2021). The role of F3 in the occurrence and development of PC was still unclear, and further research was needed.

This study conducted a correlation analysis between the immune status and the TME to show the differences in antigen-presenting cells and T cell regulation in different risk groups. We found that type-II IFN response scores in the high-risk group were significantly lower than the low-risk score group. Notably, Type II interferon plays a key role in the killing of tumor cells (Mitra-Kaushik et al. 2004; Street et al. 2002; Wang et al. 2015). We also found that the stromal score of patients in the low-risk group were higher than in the high-risk group. Furthermore, the relationship between the risk score and immune components was investigated. The results revealed that the high-risk scores were associated with C1, C2, and C6, while low-risk scores were associated with C3. These results suggest that C1 could promote tumor initiation and progression, while C3 could have a protective factor, consistent with the findings of a previous study (Tamborero et al. 2018). GSEA analysis revealed an enrichment of oncogenic pathways in the high-risk group. We hypothesized that changes in inflammatory response-related

gene expression levels could enhance tumor incidence and growth by altering the TME. The Wilcoxon test of risk score and clinical characteristics revealed that the greater the risk score, the less differentiated the tumor. Together, our findings provided more contexts for the poor outcome of patients in the high-risk group.

To explore the clinical significance of the model, a correlation analysis between prognostic models and the expression levels of immune checkpoint genes and drug resistance genes was carried out. Although the expression levels of CTLA-4, PD-1, PD-L1, and PD-L2 had no significant difference in the high- and low-risk score groups ( $P > 0.05$ ), we found that the expression levels of immune checkpoint genes were different in different risk-score groups. In addition, the results showed that PD-L1 expression levels were positively correlated with risk scores. This showed that the prognostic score could provide a theoretical basis for immunotherapy.

MRP1 to MRP9 was the primary drug resistance gene against the anti-tumor drug, and the major mechanism is to discharge the anti-tumor chemotherapy drug outside the cells, resulting in tumor drug resistance (Sodani et al. 2012). So, the relationship between risk score and drug resistance genes, including MRP1, MRP2, MRP3, MRP4, and MRP8, indicated that targeting tumor resistance genes appears to have a therapeutic potential for high-risk patients.



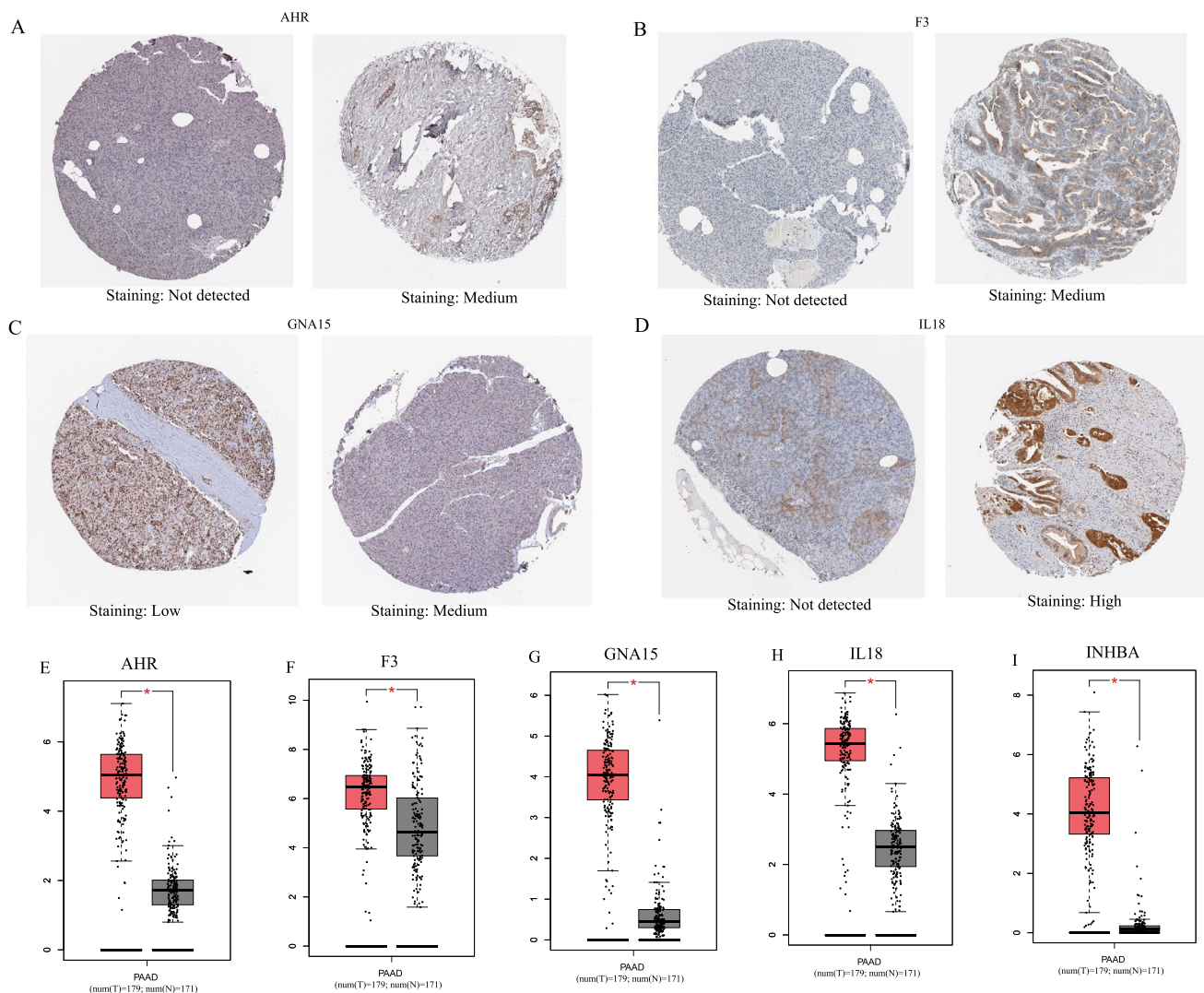
**Fig. 8** Gene set enrichment analysis between different risk groups in the TCGA cohort

Using the NCI-60 database, we uncovered that some genes, such as GNA15 and INHBA, were upregulated. Importantly, these genes were negatively associated with increased drug resistance to Nelarabine, Fludarabine, Asparaginase, Cladribine, Hydroxyurea, Zoledronate, and Dasatinib in cancer cells. However, increased expression of AHR, INHBA, and IL18 was associated with increased drug resistance to Oxaliplatin, Tyrothricin, Pipamperone, Bortezomib, Arsenic trioxide, Lomustine, Actinomycin D, and Estramustine *in vivo*. Collectively, the above findings might be an important foundation for tumor treatment.

The risk factors for pancreatic cancer include alcohol consumption, smoking, a diet rich in saturated fats, and viral infections such as chronic infection with hepatitis B and C viruses (Gheorghe et al. 2022). The symptomatology remains

nonspecific until its advanced stages, and the retroperitoneal location of the pancreas makes imaging diagnosis more difficult. With the establishment of next-generation sequencing technology and the beginning of the era of precision medicine, various treatments for PC have been developed. Large-scale gene sequencing is another heavy economic burden for PC patients. We successfully constructed and validated a 5-inflammatory response-related prognostic model. According to the relationship between prognostic model and immune checkpoint genes or drug resistance genes, the corresponding immunotherapy or chemotherapy may be applied to patients in different risk groups.

Also, the 5-inflammatory response-related gene signature (including AHR, F3, GNA15, IL18, and INHBA) could not be used for the diagnosis of pancreatic cancer in early stages.



**Fig. 9** Differences in protein expression induced by five genes were verified in human tissue samples. **A–D** The mRNA expression levels in TCGA pancreatic cancer tumor tissue and matching normal tissue from data of TCGA and the genotype-tissue expression (GTEx) data-

base. **E–I** The representative protein expression of the five genes in pancreatic cancer tumor tissue and normal tissue. Data was obtained from the human protein atlas (<https://www.proteinatlas.org/>)

Clinical manifestations, endoscopic ultrasound, endoscopic retrograde cholangiopancreatography (ERCP), CT, MRI, and PET, and methylation of ADAMTS1 and BNC1 showed a certain sensitivity and specificity for the early diagnosis of PC (Gheorghe et al. 2020).

We have not performed further experiments to explore the mechanisms of these inflammatory response-related genes, but we will address this in the future work. Moreover, as for the PC patients who were mostly diagnosed by imaging modalities and treated with nonsurgical methods, the value of this model may be limited, because our model need to quantify the expression levels of five specific genes in tumor tissues. Therefore, it is necessary to continue performing multicenter prospective research on this subject.

## Conclusion

In conclusion, we successfully constructed a 5-inflammatory response-related gene signature based on the TCGA database and verified the stability of the model using the ICGC database. Nevertheless, further substantiation is warranted to verify the role of these genes in tumorigenesis. Taken together, our findings might shed new light on immune responses, the TME, and drug resistance to promote the development of personalized cancer therapies.

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**Author contribution** All authors contributed to the study conception and design. Manjiang Li wrote the article. Yuxu Wang and Yongbiao Ma processed the data analysis. Wei Ding and Futian Du designed the study and reviewed the article.

**Data availability** The datasets analyzed for this study were obtained from The Cancer Genome Atlas (TCGA) (<https://portal.gdc.cancer.gov/>) and International Cancer Genome Consortium (ICGC) (<https://icgc.org/>).

## Declarations

**Ethics approval and consent to participate** Not applicable

**Consent for publication** Not applicable

**Conflict of interests** The authors declare no competing interests.

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