Int J Clin Exp Pathol 2024;17(11):396-410
www.ijcep.com /ISSN:1936-2625/IJCEP0155093

Original Article

Establishment and analysis of a prognostic model of pancreatic ductal adenocarcinomas based on nerve-cancer crosstalk-related genes

Lei Jiang[1,2], Xiaozhi Lu[1,2], Yuran Dai[1,2], Kuirong Jiang[1,2], Yi Miao[1,2], Jun Yu[3,4,5], Lingdi Yin[1,2*], Jishu Wei[1,2*]

[1]Pancreas Center, The First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu, China; [2]Pancreas Institute of Nanjing Medical University, Nanjing, Jiangsu, China; [3]Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [4]The Pancreatic Cancer Precision Medicine Center of Excellence, Johns Hopkins University School of Medicine, Baltimore, MD, USA; [5]The Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins University School of Medicine, Baltimore, MD, USA. [*]Equal contributors and co-senior authors.

Received December 24, 2023; Accepted October 26, 2024; Epub November 15, 2024; Published November 30, 2024

Abstract: Pancreatic ductal adenocarcinoma (PDAC) is a highly malignant tumor with a five-year survival rate of 13%, the lowest among all malignant tumors. The work aims to use bioinformatics methods to mine Nerve-cancer crosstalk-related genes (NCCGs) in pancreatic cancer and evaluate their correlation with tumor stage and prognosis, thereby providing a new direction of development and experimental basis for pancreatic cancer treatment. This study included 185 individuals with PDAC from the TCGA database, together with clinical and RNA sequencing data. A review of prior studies revealed the mechanism of neural-cancer crosstalk and identified 42 neural-cancer crosstalk-related genes (NCCGs). Multivariate logistic regression analysis showed that NGFR (OR=39.076, 95% CI; P<0.05), CHRNB2 (OR=41.076, 95% CI; P<0.05), and CHRNA10 (OR=39.038, 95% CI; P<0.05) were identified as independent risk factors for PNI development. Pearson correlation analysis revealed that CHRNA10 was negatively connected with PDAC microsatellite instability, whereas CHRNA10, CHRNB2, and NGFR were negatively correlated with PDAC tumor mutation burden. The GEPIA database revealed that CHRNB2 expression was higher in stage I PDAC. The pancreatic cancer single-cell dataset PAAD_CRA001160 revealed that malignant tumor cells, ductal cells, endothelial cells and fibroblasts accounted for a large proportion in the tumor microenvironment of pancreatic cancer. Furthermore, the NGFR gene was shown to be more significantly expressed in various pancreatic cancer cells. Bioinformatics analysis was used to create a validated prognostic model of pancreatic cancer, which explored the critical mechanisms of neural-tumor interactions and revealed the potential of cancer-neural crosstalk-related genes as prognostic biomarkers and anti-tumor therapy targets.

Keywords: Pancreatic ductal adenocarcinoma, cancer-neural crosstalk-related genes, prognostic model, bioinformatics analysis

Introduction

The incidence of Pancreatic ductal adenocarcinoma continues to increase by 2021 [1]. Pancreatic ductal adenocarcinoma (PDAC) is the seventh leading cause of cancer-related deaths worldwide with 432,242 related deaths in 2018 [2]. Unlike other cancers, the incidence of PDAC is increasing by 0.5% to 1.0% per year, and it will be the second-leading cause of cancer-related mortality by 2030 [3]. Surgery, radiotherapy, chemotherapy and immunotherapy are the main methods for the treatment of PDAC, yet PDAC still needs an effective curative treatment [4]. Surgical resection is currently the only means to achieve long-term survival in patients with PDAC. Although only 15% to 20% of patients present with resectable disease, the increasing use of neoadjuvant therapies have broadened the pool of patients who are eligible for surgical resection. Unfortunately, the 5-year survival rate of PDAC is less than 10% [5].

The diagnosis of PDAC cannot be made based on symptoms and signs alone. The tumor mark-

https://doi.org/10.62347/GHUM8504

er sialylated Lewisa blood group antigen CA19-9 is frequently used in the workup for PDAC. In symptomatic patients, the sensitivity and specificity of CA19-9 range from 70% to 90%, making it inadequate as a diagnostic in this population. Because these markers are elevated in only a subset of patients with PDAC, their utility in diagnosis is limited. CT is the first-line imaging modality for the initial evaluation of suspected PDAC and is preferred over MRI. The primary role of EUS is to guide needle biopsies to confirm the diagnosis of PDAC. In select cases, EUS may be helpful in detecting a small pancreatic mass that may be difficult to observe on CT or MRI and thus is the preferred imaging modality in some early detection surveillance programs.

Previous studies have shown that KRAS, NRG1, NTRK and other related genes play pivotal roles in the development of PDAC [6]. Therefore, it is important for us to focus on advances in related genes and explore the molecular mechanism of PDAC.

Nerves are an important part of the tumor microenvironment. Recent studies have shown the cancer-associated neurogenesis and nerve-cancer cross-talk. This highlights the importance of nerve-cancer cross-talk in tumor progression [7]. Nerves have been shown to infiltrate the tumor microenvironment and actively stimulate cancer cell growth and dissemination [8]. Perineural invasion has an extremely poor prognosis for malignant tumors [9]. PNI is an important prognostic factor for PDAC [10].

Furthermore, new evidence suggests that cancers may reactivate nerve-dependent developmental and regenerative processes to promote their growth and survival [11]. The prognostic value of cancer-nerve crosstalk-related genes (NCCGs) in PDAC has not been studied.

In this study, we used bioinformatics analysis to study the expression and prognostic value of NCCGs. The following data may provide evidence for new biomarkers and therapeutic targets to guide clinical treatment and prolong survival time.

Materials and methods

Data and processing

We derived the RNA sequencing data and clinical information of 185 PDAC patients from The Cancer Genome Atlas (TCGA) database and then downloaded them. RNA sequencing data and survival information of PDAC patients were derived from the Gene Expression Omnibus (GEO) database. Item selection: the data are classified as clinical and the data format is bcr, xml. Rstdio (4.2.0) was used for data processing and analysis.

Identification of nerve-cancer cross-talk-related genes

In a previous study, forty-two nerve-cancer crosstalk genes were identified, and these genes are displayed in **Table 1** [12].

Research flow chart

Technical route: First, we need to download the original data of pancreatic cancer patients from TCGA. We found 42 tumor nerve-related genes. Three characteristic genes were found by lasso regression analysis. After grouping in turn, the KM survival curve was drawn to compare the differences in prognosis and survival. A formula and risk score can be obtained. Finally, the ROC analysis was verified by the GEO database.

Mutation analysis and protein-protein interaction

The cBioPortal for Cancer Genomics (http://cbioportal.org) provides a Web resource for exploring, visualizing, and analyzing multidimensional cancer genomics data [11]. We implemented the mutation analysis by this. Protein-protein interactions (PPI) are particularly important due to their versatility, specificity and adaptability. The STRING database aims to integrate all known and predicted associations between proteins, including both physical interactions and functional associations [13]. Our PPI analysis of 42 NCCGs was performed by STRING (https://string-db.org/).

Functional enrichment analysis

Previous studies investigated oncogenes based on GO terms and KEGG pathways. Some important GO terms and KEGG pathways were confirmed to be highly related to oncogenes [14]. Pathview can be easily combined with other tools for automated and efficient pathway analysis pipelines, such as Gene Ontology (GO) analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis, which maps and

Table 1. Identification of NCCGs

Gene	English full name
SEMA4F	Semaphorin 4F
ADRB2	Adrenoceptor beta 2
ADRB3	Adrenoceptor beta 3
MAP2	Microtubule associated protein 2
NGFR	Nerve growth factor receptor
NTRK1	Neurotrophic receptor tyrosine kinase 1
NTRK2	Neurotrophic receptor tyrosine kinase 2
NTRK3	Neurotrophic receptor tyrosine kinase 3
L1CAM	L1 cell adhesion molecule
GDNF	Glial cell derived neurotrophic factor
GFRA1	GDNF family receptor alpha 1
GFRA2	GDNF family receptor alpha 2
GFRA3	GDNF family receptor alpha 3
NTN1	Netrin 1
SLIT2	Slit guidance ligand 2
GRIN1	Glutamate ionotropic receptor NMDA type subunit 1
GRIN2A	Glutamate ionotropic receptor NMDA type subunit 2A
GRIN2B	Glutamate ionotropic receptor NMDA type subunit 2B
GRIN2C	Glutamate ionotropic receptor NMDA type subunit 2C
GRIN2D	Glutamate ionotropic receptor NMDA type subunit 2D
GRIN3A	Glutamate ionotropic receptor NMDA type subunit 3A
GRIN3B	Glutamate ionotropic receptor NMDA type subunit 3B
CHRM1	Cholinergic receptor muscarinic 1
CHRM2	Cholinergic receptor muscarinic 2
CHRM3	Cholinergic receptor muscarinic 3
CHRM4	Cholinergic receptor muscarinic 4
CHRNA1	Cholinergic receptor nicotinic alpha 1 subunit
CHRNA2	Cholinergic receptor nicotinic alpha 2 subunit
CHRNA3	Cholinergic receptor nicotinic alpha 3 subunit
CHRNA4	Cholinergic receptor nicotinic alpha 4 subunit
CHRNA5	Cholinergic receptor nicotinic alpha 5 subunit
CHRNA6	Cholinergic receptor nicotinic alpha 6 subunit
CHRNA7	Cholinergic receptor nicotinic alpha 7 subunit
CHRNA9	Cholinergic receptor nicotinic alpha 9 subunit
CHRNB2	Cholinergic receptor nicotinic beta 2 subunit
CHRNB4	Cholinergic receptor nicotinic beta 4 subunit
CHRNG	Cholinergic receptor nicotinic gamma subunit
CHRNB1	Cholinergic receptor nicotinic beta 1 subunit
CHRND	Cholinergic receptor nicotinic delta subunit
CHRNE	Cholinergic receptor nicotinic epsilon subunit
CHRNA10	Cholinergic receptor nicotinic alpha 10 subunit
TACR1	Tachykinin receptor 1

renders user data onto pathway graphs intuitively and informatively [15]. In this way, we succeeded in exploring the potential functions and pathways of genes.

Construction of prognostic gene model

Single-factor Cox regression analysis was carried out by using the R package survival to filter genes. The candidate genes were analyzed by lasso regression using the R package survival and R package glmnet, and the selected genes were analyzed by bidirectional stepwise multifactor Cox regression analysis.

Gene set enrichment analysis (GSEA)

GSEA enrichment using plyr, ggplot2, grid, gridExtra software package was used to explore the biological function and pathway of target genes in pancreatic cancer.

Correlation between target genes and microsatellite instability and tumor mutation load in pancreatic cancer correlation exploration

We used the plyr, ggplot2, grid, gridExtra software packages to research the correlation between target genes and microsatellite instability and tumor mutation load in pancreatic cancer.

Relationship between target genes and immune cells in pancreatic cancer Spearman correlation exploration

Software packages plyr, ggplot2, grid, gridExtra were used to research the correlation between target genes and immune cells in pancreatic cancer includes B cells, CD4+ T cells, CD8+ T cells, neutrophils, macrophages and dendritic cells.

Statistical analysis

Rstdio (4.2.0) was used to analyze the data. The association between the expression of genes and immune cell was analyzed using Spearman analysis. Survival analysis was con-

ducted using the Kaplan Meier method, and risk factor analysis was conducted using Cox proportional risk regression. P<0.05 was considered a significant difference.

Results

Expression and mutation of NCCGs in PDAC

First, we explored the expression of 42 NCCGs in PDAC and normal tissue in the TCGA database. A total of 9 genes were up- or downregulated in PDAC (**Figure 1A**). GDNF, GFRA3, NTRK1, CHRM2, CNRM3, GRIN1, CHRNA5, and CHRNB2 were upregulated, while SLIT2 was downregulated compared with normal tissue (*P<0.05, **P<0.01).

Then, we summarized the incidence of copy body mutations and somatic NCCGs in PDAC (**Figure 1B**). In the 178 samples, the most common type of mutation was a missense mutation (green band). In the figure, we only showed the top 10 genes with high mutation rates; GRIN2A and GRIN2B (25.8%) had the highest rates. Other genes were SLIT2, CHRNA10, NTRK1, MAP2, CHRM2, CHRM3, L1CAM, and GRIN3A. Single-sample gene set enrichment analysis (ssGSEA) was used to explore the content of different cells in the sample (**Figure 1C**). Then, we made high and low groups by the content of neurons. We used K-M plotter to verify that there was a significant correlation between the content of neurons and the prognosis of the patients (**Figure 1D**).

Functional enrichment of NCCGs

We used GO analysis and KEGG pathway analysis to further explore the function of NCCGs. We found that the 42 NCCGs were mainly enriched in chemical synapses and transmembrane movement through GO analysis (**Figure 2A**). In addition, KEGG pathway analysis showed that 42 NCCGs were mainly involved (**Figure 2B**). In the end, we used String to perform PPI analysis of these forty-two NCCGs, and the correlation showed a complex interaction between these genes (**Figure 2C**).

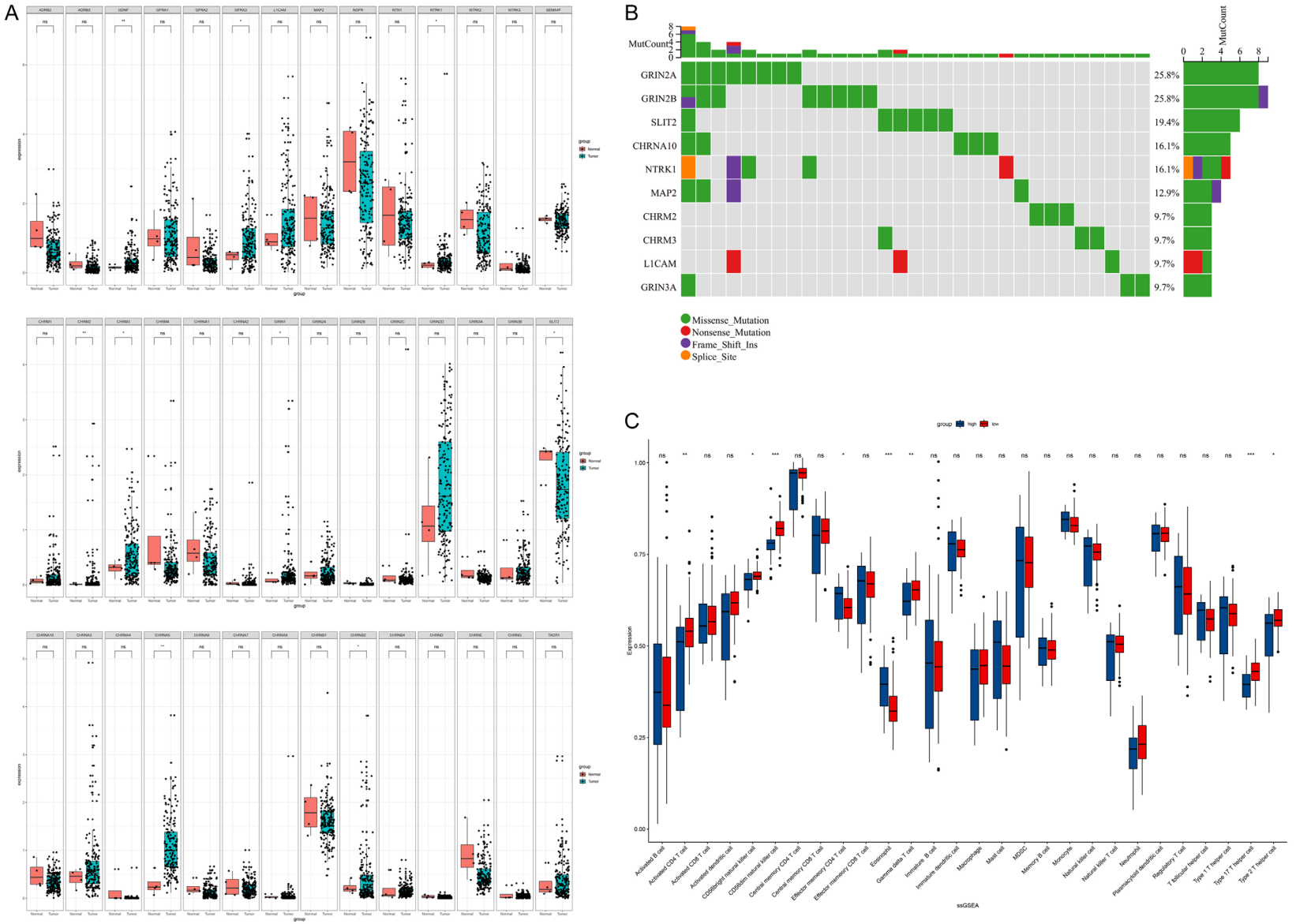
Construction and verification of the prognostic gene model

Among the 42 NCCGs, K-M plotter was used to look for prognostic genes. Three genes (CHRNA10, CHRNB2, and NGFR) are shown in **Figure 3**. Then, we used LASSO regression analysis to construct a prognostic gene model by these 3 prognostic genes (**Figure 4A**). According to the risk score, we classified each PDAC patient into a high-score group and a low-score group. The distributions of the risk score, survival time, and expression of the three genes are shown in the icon (**Figure 4B**). As shown in the third figure, the low-score group had better survival rates (**Figure 4C**). Finally, the 12-, 36-, and 60-month ROC curves were drawn to calculate the AUCs: 0.65, 0.74, and 0.88, respectively (**Figure 4D**). We used the data downloaded from GEO to verify the accuracy of the prognosis model.

Correlation was used to explore the potential relationship between CHRNA10, CHRNB2, NGFR gene expression and B cell, CD4+ T cell, CD8+ T cell neutrophil macrophage dendritic cells. The results suggested that the CHRNA10 gene was positively correlated with CD4+ T cells in pancreatic cancer, while the CHRNA10 gene was negatively correlated with CD8+ T cells in pancreatic cancer (**Figure 5A**). The CHRNB2 gene is positively correlated with CD4+ T cell macrophages in pancreatic cancer (**Figure 5B**), the NGFR gene is positively correlated with B cells, CD4+ T cell, CD8+ T cells, neutrophils, macrophages and dendritic cell in pancreatic carcinoma (**Figure 5C**).

In order to explore the association between CHRNA10, CHRNB2, the NGFR gene and pancreatic cancer genomic alterations, we investigated the association between CHRNA10, CHRNB2, the NGFR gene and microsatellite instability tumor mutation load in pancreatic cancer. The results showed that the CHRNA10 gene was negatively correlated with microsatellite instability of pancreatic cancer and the CHRNA10 gene was negatively correlated with tumor mutation load of pancreatic cancer (**Figure 6A**). The CHRNB2 gene has no correlation with microsatellite instability in pancreatic cancer, but the CHRNB2 gene has negative correlation with tumor mutation load in pancreatic cancer (**Figure 6B**). The NGFR gene has no correlation with microsatellite instability in pancreatic cancer, but the NGFR gene has negative correlation with tumor mutation load in pancreatic cancer (**Figure 6C**).

According to the GEPIA database, we explored expression of CHRNA10, CHRNB2, and the


A
B
C
MutCount
GRIN2A 25.8%
GRIN2B 25.8%
SLIT2 19.4%
CHRNA10 16.1%
NTRK1 16.1%
MAP2 12.9%
CHRM2 9.7%
CHRM3 9.7%
L1CAM 9.7%
GRIN3A 9.7%
Missense_Mutation
Nonsense_Mutation
Frame_Shift_Ins
Splice_Site
group
high
low
Expression
ssGSEA
Activated B cell
Activated CD4 T cell
Activated CD8 T cell
Activated dendritic cell
CD56bright natural killer cell
CD56dim natural killer cell
Central memory CD4 T cell
Central memory CD8 T cell
Effector memory CD4 T cell
Effector memory CD8 T cell
Eosinophil
Gamma delta T cell
Immature B cell
Immature dendritic cell
Macrophage
Mast cell
MDSC
Memory B cell
Monocyte
Natural killer cell
Natural killer T cell
Neutrophil
Plasmacytoid dendritic cell
Regulatory T cell
T follicular helper cell
Type 1 T helper cell
Type 17 T helper cell
Type 2 T helper cell

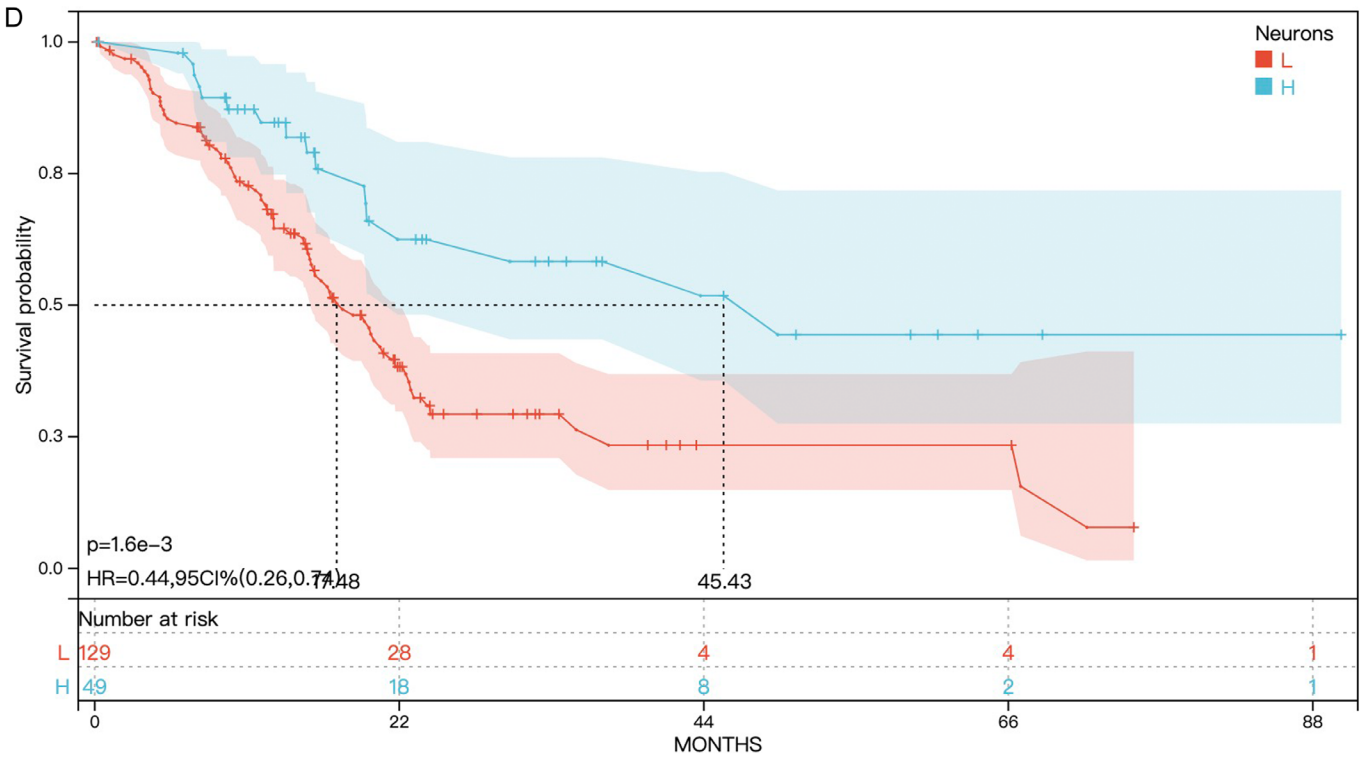


Figure 1. Expression and mutation of NCCGs. A. The expression of 42 NCCGs in PDAC and normal tissue; B. The mutation landscape of the top 10 mutation rate of NCCGs. *P<0.05, **P<0.01. NCCGs, nerve-cancer cross-talk genes; PDAC, pancreatic ductal adenocarcinomas; C. The content of different cells in the sample; D. The overall survival of PDAC patients in the high/low-neurons groups.

Figure 2. The functional enrichment analysis of NCCGs. A. The Gene Ontology (GO) analysis; B. Gene Set Enrichment Analysis (GSEA); C. The protein-protein interaction of NCCGs.

NGFR gene in stage I, stage II, stage III and stage IV of pancreatic cancer. The results showed that CHRNB2 gene expression was significantly correlated with the clinical stage of pancreatic cancer, while CHRNA10, and NGFR gene expression was not correlated with the

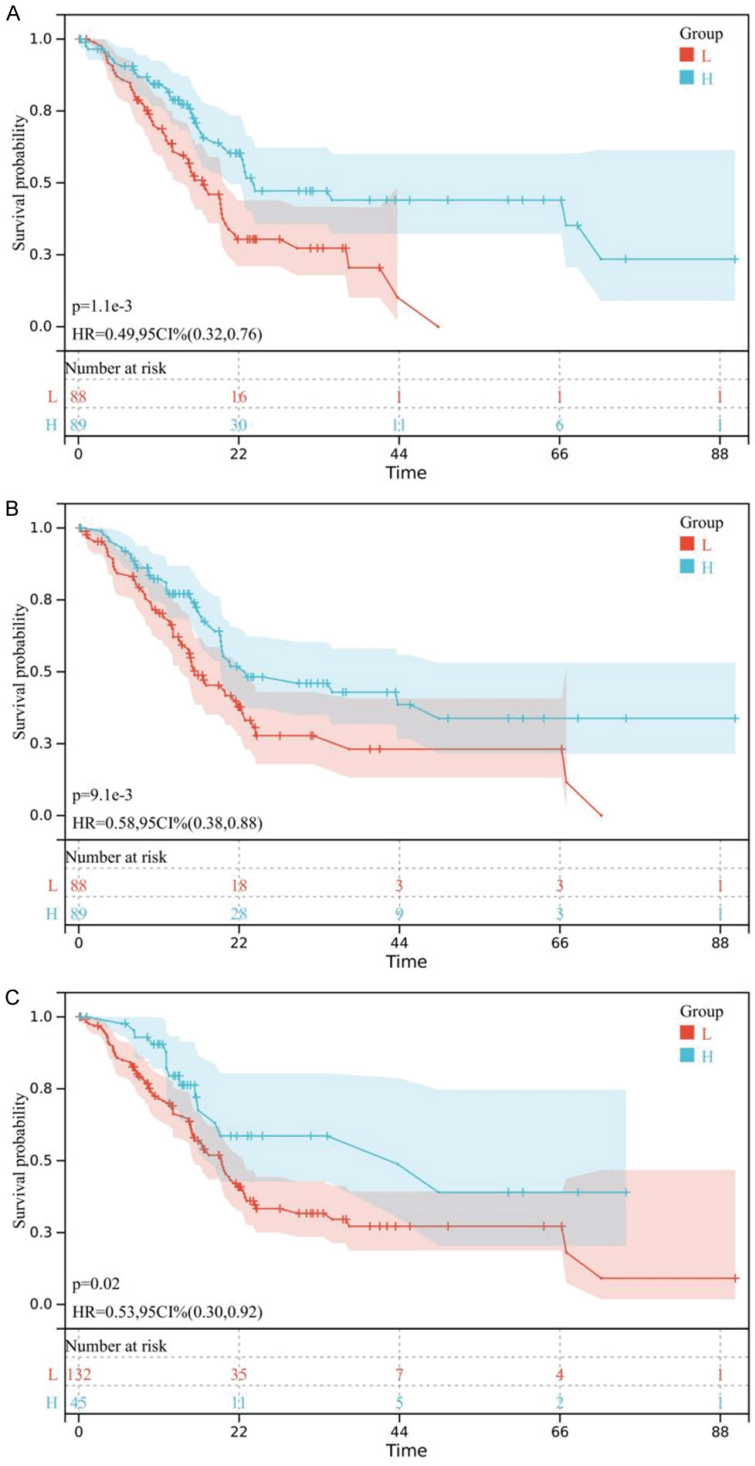


Figure 3. The prognostic value of NCCGs in PDAC. The overall survival of CHRNA10 (A), CHRNB2 (B), NGFR (C) in PDAC patients in the high/low-expression groups.

clinical stage of pancreatic cancer (**Figure 7A-C**). Compared with Stage II, Stage III and Stage IV, the CHRNB2 gene expression was higher in Stage I of pancreatic cancer.

The single cell dataset PAAD_CRA001160 was used to visualize the distribution, proportion and expression of CHRNA10, CHRNB2 and NGFR genes in pancreatic cancer. Cells in the pancreatic cancer microenvironment include acinar cells, B cells, CD8Tex cells, dendritic cells, catheter cells, endocrine cells, endothelial cells, fibroblasts, malignant tumor cells, plasma cells, stellate cells, etc. (**Figure 8A**). Among them, malignant tumor cells, catheter cells, endothelial cells, and fibroblasts occupy a relatively high proportion in the pancreatic cancer tumor microenvironment (**Figure 8B**). In addition, we also analyzed the expression levels of CHRNA10, CHRNB2 and NGFR genes in various pancreatic cancer cells, and the results showed that the expression of NGFR genes was higher in various pancreatic cancer cells (**Figure 8C**).

In order to investigate the roles of CHRNA10, CHRNB2, and NGFR genes in pancreatic cancer, GSEA enrichment analysis was employed to explore their biological functions and signaling pathways. The results of the enrichment analysis revealed that the CHRNA10 gene is enriched in pancreatic cancer core pathways, primary immune adhesion efficiency, B cell receptor signaling pathways, vascular smooth muscle contraction, hematopoietic cells, intestinal immune network gonadal generation, and ECM receptor interactions (**Figure 9A**). CHRNB2 showed enrichment in pancreatic cancer as well as systemic lupus erythematosus, ma-

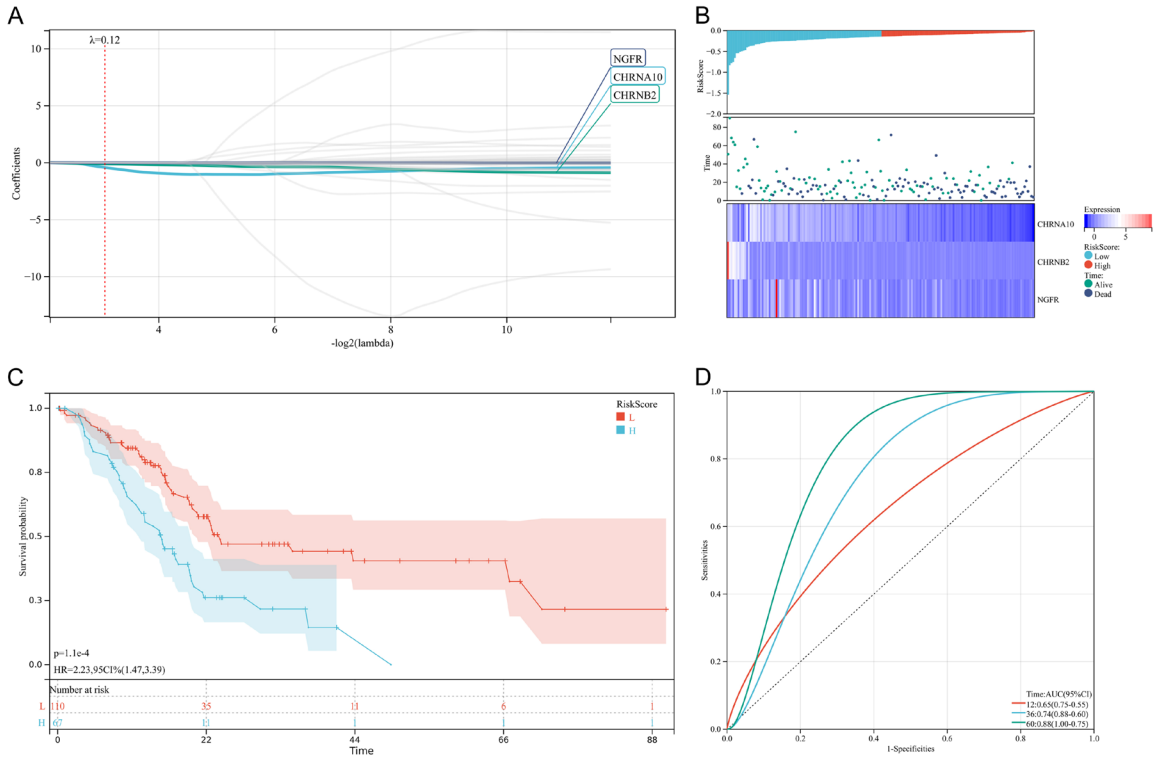


Figure 4. Construction and verification of prognostic gene model. A. Lasso coefficient profiles of the three NCCGs; B. Destruction of risk score, survival status, and the expression of the three NCCGs in PDAC; C, D. Overall survival curves for PDAC patients in the high/low-risk score group and the ROC curve of measuring the predictive value.

turity-onset diabetes of the young, long-term potentiation, type 2 diabetes, taste conduction pathway, cell cycle regulation, natural killer cell-mediated cytotoxicity, chronic myeloid leukemia, and others (**Figure 9B**). NGFR genes were found to be enriched in primary immunodeficiency, hematopoietic cell lineage, cytokine-cytokine receptor interaction, adipokine signaling pathway, Huntington's disease, Alzheimer's disease, NOTCH signaling pathway, taste transduction, and others in pancreatic cancer (**Figure 9C**).

Discussion

Nerve-cancer crosstalk is an underappreciated area of cancer research, but nerves are now gaining attention for their role in cancer, as researchers have discovered their connection to cancer metastasis and poor prognosis [16]. Evidence indicates that neurogenesis (increased number of neurons) and axonogenesis (tumor-induced neural sprouting toward the tumor microenvironment) also play vital roles in tumorigenesis and cancer progression [17]. A previous review provided perspectives and insights regarding the rational and strategies of targeting the neurotransmitter system for cancer treatment [18].

In previous studies, we defined 42 NCCGs, but the function of NCCGs in PDAC has not been elucidated. We screened valuable genes for prognosis by differential expression and K-M plotter. Then, we constructed an effective prognostic gene model. Finally, we found a correlation between the content of neurons and immune cells. By RNA sequencing data, functional enrichment analysis showed the expression of key genes in different types of cells and the possible pathways in the high-expression cells.

First, among the 9 up- or downregulated genes, three genes were associated with prognosis. In PDAC, upregulation of CHRNB2 indicates poor prognosis. Blockade of CHRNB2 expression using specific Abs shows promise for controlling metastasis in gastric cancer [19]. According to the results of the K-M plot, we believe that the neuronal content deeply affects the prognosis of PDAC patients.

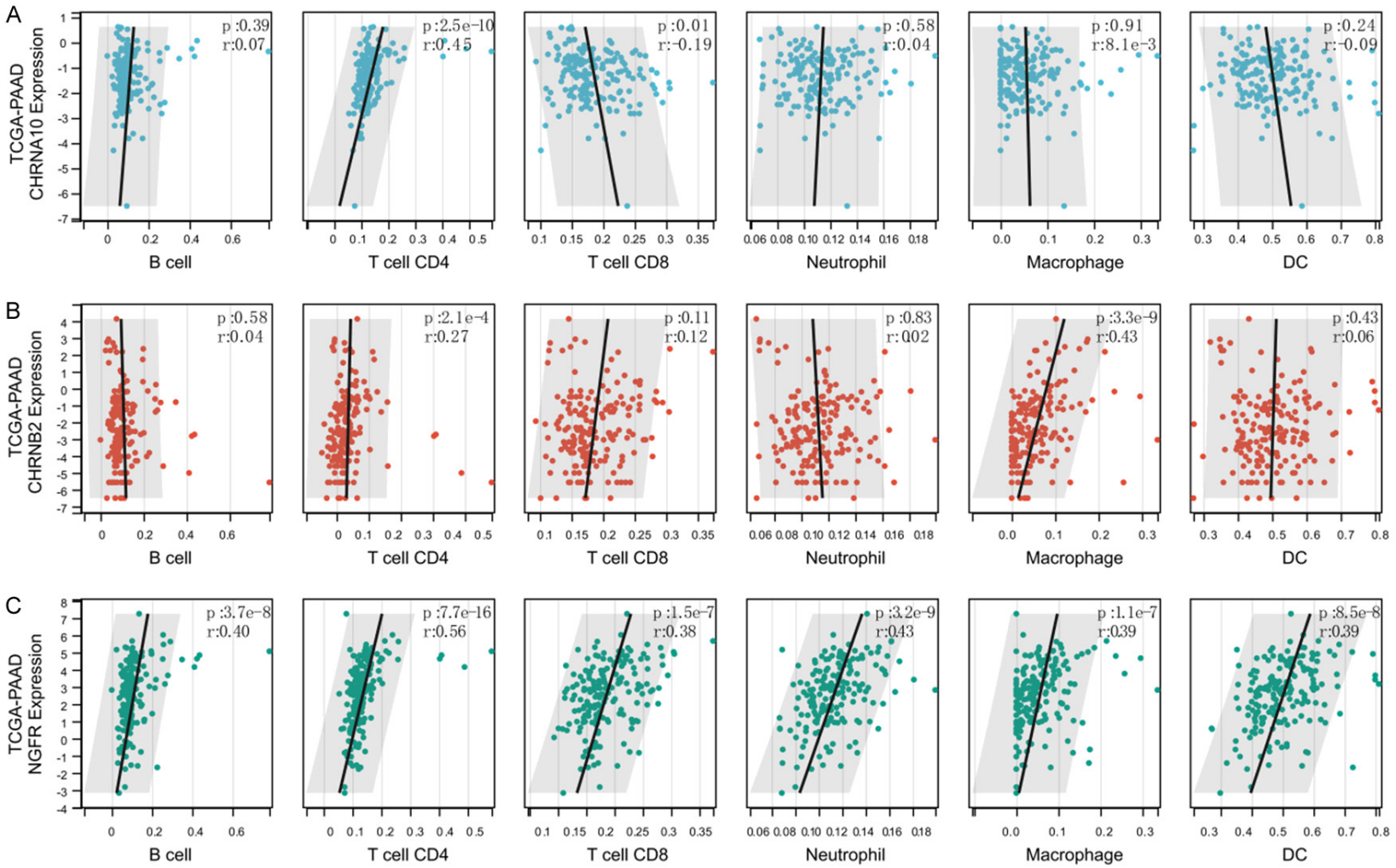


Figure 5. A. Association of CHRNA10 gene with immune cells in pancreatic cancer; B. Association of CHRNB2 gene with immune cells in pancreatic cancer; C. Relationship between NGFR gene and immune cells in pancreatic cancer.

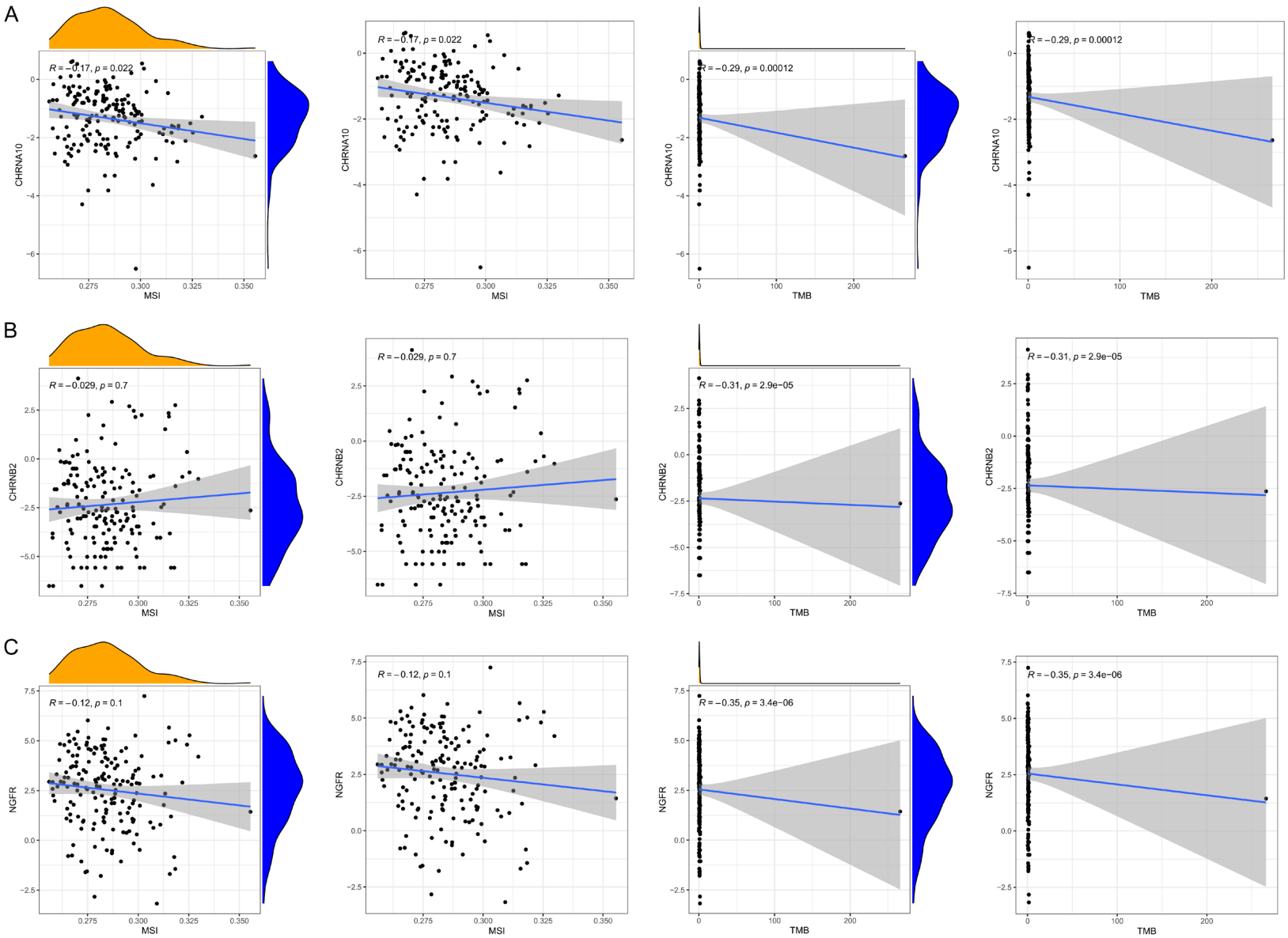


Figure 6. A. Correlation between CHRNA10 gene and tumor mutation load of microsatellite instability in pancreatic cancer; B. Correlation between CHRNB2 gene and tumor mutation load of microsatellite instability in pancreatic cancer; C. Relationship between NGFR gene and tumor mutation load of microsatellite instability in pancreatic cancer.

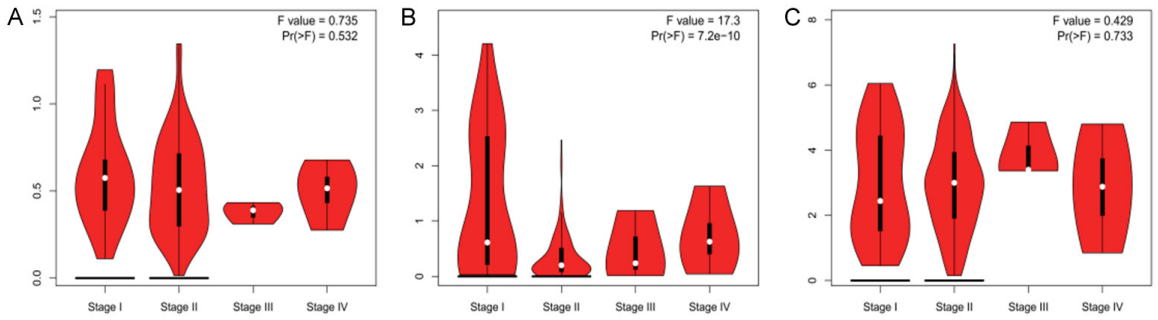


Figure 7. A. Relationship between CHRNA10 gene and clinical stage of pancreatic cancer; B. Relationship between CHRNB2 gene and Stage staging of pancreatic carcinoma; C. Relationship between NGFR gene and clinical stage of pancreatic cancer.

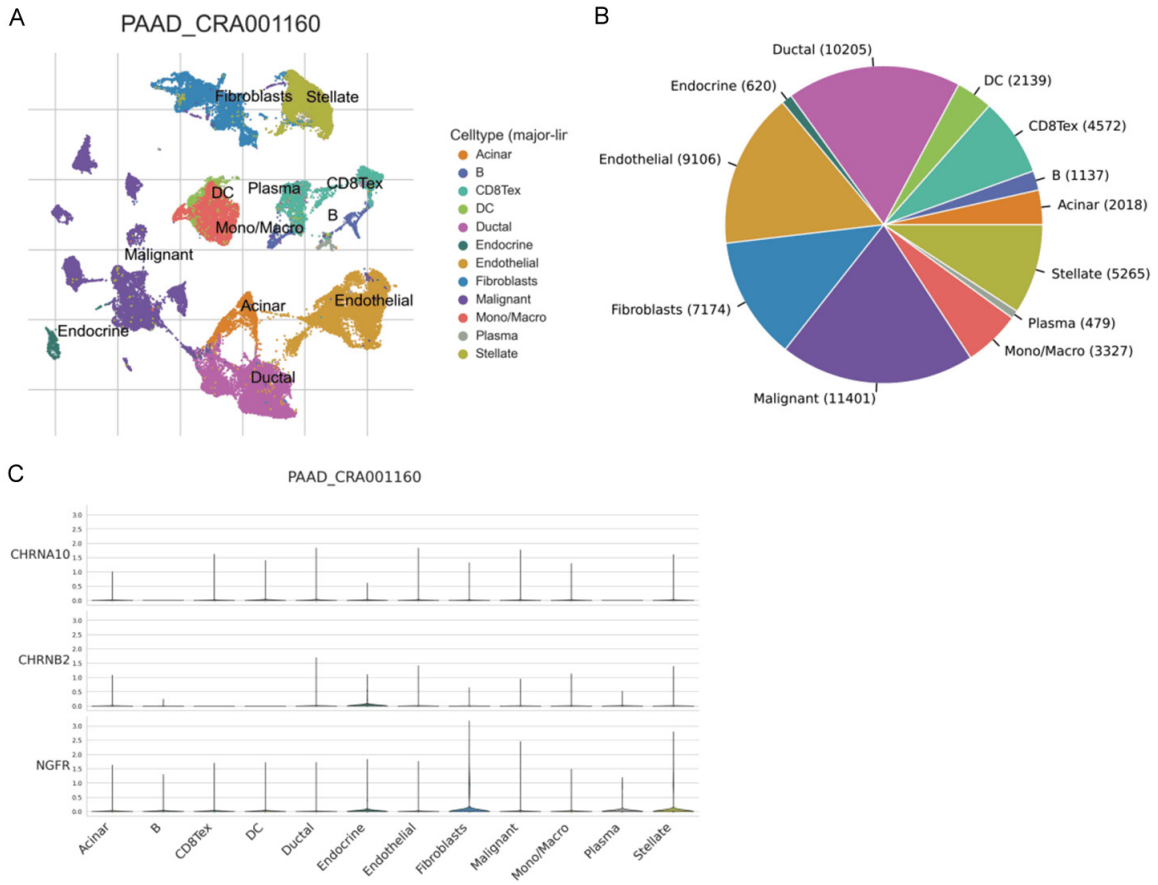


Figure 8. A. UMAP diagram showing cell distribution in pancreatic cancer single cell data set PAAD_CRA001160; B. The pie chart shows the proportion of cells in the pancreatic cancer single cell data set PAAD_CRA001160; C. Expression of CHRNA10, CHRNB2, NGFR gene in pancreatic cancer single cell data set PAAD_CRA001160.

Then, we analyzed the GO function enrichment and Gene Set Enrichment Analysis (GSEA). We clearly found that NCCGs were mainly enriched in chemical synapses, transmembrane movement, neuroreceptor activation and so on. In a recent study, a transmembrane receptor glycoprotein that is upregulated on transformed cells, cancer-associated fibroblasts and inflammatory macrophages was shown to contribute to cancer progression [20]. The construction of a prognostic gene model allowed us to classify the patients into two groups: high-risk and low-

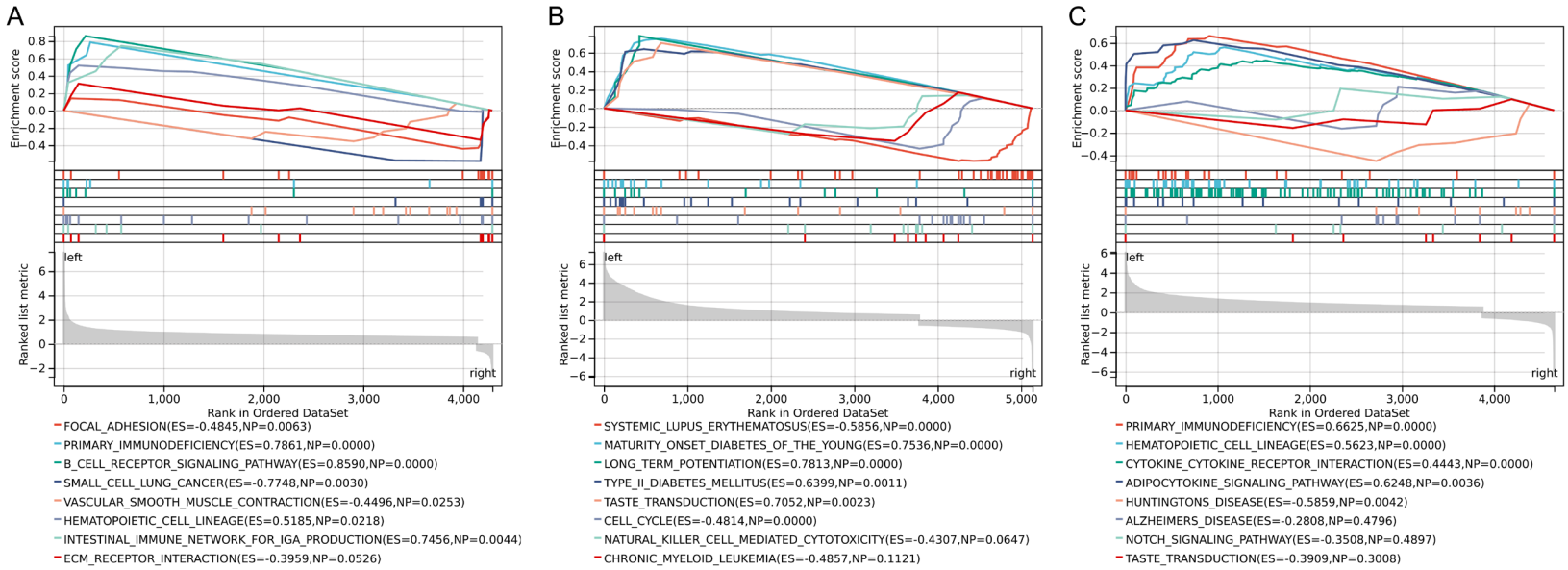


Figure 9. A. GSEA enrichment of CHRNA10 gene in pancreatic cancer; B. GSEA enrichment of CHRNB2 gene in pancreatic cancer; C. GSEA enrichment of NGFR gene in pancreatic cancer.

risk. After internal and external data verification, the effectiveness of this prognostic model was confirmed. Compared with other studies, the AUC of our prognostic model is not bad. The receiver operating characteristic curve (ROC) is a widely accepted criterion. An area under the curve (AUC) below 0.6 indicates low discrimination, 0.6 to 0.75 indicates medium discrimination, and above 0.75 indicates high discrimination. A high AUC represents high model accuracy [21].

About 10% of PDAC susceptibility genes have pathogenic germline mutations, among which BRCA2 and ATM are the two most common gene loci, followed by BRCA1, PALB2, CDKN2A/p16 and LKB1/STK11. Mismatch repair genes (hMLH1, hMSH2, hPMS6). Of note, only half of patients have pathogenic mutations, and the recently updated guidelines from ASCO and NCCN recommend that all patients diagnosed with PDAC should undergo germline mutation surveillance (rather than just those with a suspected family history), which increases the potential benefit of BRCA1/BRCA2 mutated patients from PARP inhibitor therapy. The first-degree relatives of patients with positive mutations should be tested for susceptible pathogenic mutations, which can be greatly simplified by expensive multigene chips in blood and saliva. The presence of asymptomatic germline mutation carriers indicates a large high-risk population with potential disease [22].

There are some limitations to this study. First, the predictive ability of prognostic models still needs to be supported by a large amount of multicenter clinical evidence. Furthermore, only public databases were included in this study. There is a lack of experimental support. In vivo and in vitro experiments, and RNA or DNA sequencing of nerve samples are needed for further study.

In conclusion, in the prognostic model we constructed, the nerve-cancer cross-talk genes have the potential to serve as specific molecular markers of PDAC. They may also be used as an indicator of prognosis and as a new target for the study of pathogenesis and immunotherapy in the future. In addition, we believe that nerve fibers and PDAC are inseparable, and there is some interaction, which still needs further research and evidence.

Acknowledgements

The authors would like to thank Lingdi Yin of the Pancreas Institute of Nanjing Medical University for helpful discussions on topics related to this work. This research was partially supported by National Natural Science Foundation of China (Nos. 81972323 to JW, 8220161478 to LY) and the Development Program of Jiangsu Province Hospital (JSPH-511B-2018-7 to JW).

Disclosure of conflict of interest

None.

Abbreviations

TCGA, The Cancer Genome Atlas; AJCC, American Joint Committee on Cancer; NCCGs, Cancer-nerve crosstalk-related genes; PPI, Protein-protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; KM, Kaplan-Meier; AUC, Area Under Curve; GEO, Gene Expression Omnibus; ROC, Receiver Operating Characteristic Curve; HGF, Hepatocyte growth factor; NT, Neurotrophins; TAMs, Tumor-associated macrophages; CNS, Central nervous system; PC, Pancreatic cancer; NGF, Nerve growth factor; PDAC, Pancreatic ductal adenocarcinoma; PNI, Perineural neural invasion; TME, Tumor microenvironment.

Address correspondence to: Drs. Jishu Wei and Lingdi Yin, Pancreas Center, The First Affiliated Hospital of Nanjing Medical University, No. 300 Guangzhou Road, Nanjing 210029, Jiangsu, China. E-mail: weijishu@njmu.edu.cn (JSW); yinlingdi@njmu.edu.cn (LDY)

References

[1] Siegel RL, Giaquinto AN and Jemal A. Cancer statistics, 2024. CA Cancer J Clin 2024; 74: 12-49.
[2] Khalaf N, El-Serag HB, Abrams HR and Thrift AP. Burden of pancreatic cancer: from epidemiology to practice. Clin Gastroenterol Hepatol 2021; 19: 876-884.
[3] Park W, Chawla A and O'Reilly EM. Pancreatic cancer: a review. JAMA 2021; 326: 851-862.
[4] Anderson EM, Thomassian S, Gong J, Hendifar A and Osipov A. Advances in pancreatic ductal adenocarcinoma treatment. Cancers (Basel) 2021; 13: 5510.
[5] Sarantis P, Koustas E, Papadimitropoulou A, Papavassiliou AG and Karamouzis MV. Pancreatic ductal adenocarcinoma: treatment hur-

dles, tumor microenvironment and immunotherapy. World J Gastrointest Oncol 2020; 12: 173-181.
[6] Qian Y, Gong Y, Fan Z, Luo G, Huang Q, Deng S, Cheng H, Jin K, Ni Q, Yu X and Liu C. Molecular alterations and targeted therapy in pancreatic ductal adenocarcinoma. J Hematol Oncol 2020; 13: 130.
[7] Silverman DA, Martinez VK, Dougherty PM, Myers JN, Calin GA and Amit M. Cancer-associated neurogenesis and nerve-cancer cross-talk. Cancer Res 2021; 81: 1431-1440.
[8] Jobling P, Pundavela J, Oliveira SM, Roselli S, Walker MM and Hondermarck H. Nerve-cancer cell cross-talk: a novel promoter of tumor progression. Cancer Res 2015; 75: 1777-81.
[9] Melgarejo da Rosa M, Clara Sampaio M, Virgínia Cavalcanti Santos R, Sharjeel M, Araújo C, Galdino da Rocha Pitta M, Cristiny Pereira M and Jesus Barreto de Melo Rego M. Unveiling the pathogenesis of perineural invasion from the perspective of neuroactive molecules. Biochem Pharmacol 2021; 188: 114547.
[10] Crippa S, Pergolini I, Javed AA, Honselmann KC, Weiss MJ, Di Salvo F, Burkhart R, Zamboni G, Belfiori G, Ferrone CR, Rubini C, Yu J, Gasparini G, Qadan M, He J, Lillemoe KD, Castillo CF, Wolfgang CL and Falconi M. Implications of perineural invasion on disease recurrence and survival after pancreatectomy for pancreatic head ductal adenocarcinoma. Ann Surg 2022; 276: 378-385.
[11] Zahalka AH and Frenette PS. Nerves in cancer. Nat Rev Cancer 2020; 20: 143-157.
[12] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C and Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013; 6: pl1.
[13] Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, Doncheva NT, Legeay M, Fang T, Bork P, Jensen LJ and von Mering C. The STRING database in 2021: customizable protein-protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res 2021; 49: D605-D612.
[14] Xing Z, Chu C, Chen L and Kong X. The use of Gene Ontology terms and KEGG pathways for analysis and prediction of oncogenes. Biochim Biophys Acta 2016; 1860: 2725-34.
[15] Luo W and Brouwer C. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. Bioinformatics 2013; 29: 1830-1.
[16] Gregory E, Dugan R, David G and Song YH. The biology and engineered modeling strategies of cancer-nerve crosstalk. Biochim Biophys Acta Rev Cancer 2020; 1874: 188406.
[17] Wang W, Li L, Chen N, Niu C, Li Z, Hu J and Cui J. Nerves in the tumor microenvironment: origin and effects. Front Cell Dev Biol 2020; 8: 601738.
[18] Jiang SH, Hu LP, Wang X, Li J and Zhang ZG. Neurotransmitters: emerging targets in cancer. Oncogene 2020; 39: 503-515.
[19] Kanda M, Shimizu D, Nakamura S, Sawaki K, Umeda S, Miwa T, Tanaka H, Inokawa Y, Hattori N, Hayashi M, Tanaka C, Nakayama G, Iguchi Y, Katsuno M and Kodera Y. Blockade of CHRNB2 signaling with a therapeutic monoclonal antibody attenuates the aggressiveness of gastric cancer cells. Oncogene 2021; 40: 5495-5504.
[20] Krishnan H, Rayes J, Miyashita T, Ishii G, Retzbach EP, Sheehan SA, Takemoto A, Chang YW, Yoneda K, Asai J, Jensen L, Chalise L, Natsume A and Goldberg GS. Podoplanin: an emerging cancer biomarker and therapeutic target. Cancer Sci 2018; 109: 1292-1299.
[21] Shen C, Ge B, Liu X, Chen H, Qin Y and Shen H. Predicting the occurrence of venous thromboembolism: construction and verification of risk warning model. BMC Cardiovasc Disord 2020; 20: 249.
[22] Grossberg AJ, Chu LC, Deig CR, Fishman EK, Hwang WL, Maitra A, Marks DL, Mehta A, Nabavizadeh N, Simeone DM, Weekes CD and Thomas CR Jr. Multidisciplinary standards of care and recent progress in pancreatic ductal adenocarcinoma. CA Cancer J Clin 2020; 70: 375-403.