Molecular Biotechnology
https://doi.org/10.1007/s12033-025-01388-7

ORIGINAL PAPER

Genome Mining for Hub Genes Related to Endoplasmic Reticulum Stress in Pancreatitis: A Perspective from In Silico Characterization

Huiwei Ye[1] · **Laifa Kong**[2]

Received: 9 September 2024 / Accepted: 25 January 2025

Abstract

Pancreatitis, as a common exocrine pancreatic disease, poses a daunting challenge to patients' health and the medical system. Endoplasmic reticulum stress (ERS) plays an essential role in the pathologic process of pancreatitis. However, its mechanism is not fully understood. Therefore, this study was designed to deepen the understanding of the pathogenic mechanism of the disease by screening key ERS-related genes (ERSRGs) associated with pancreatitis. Pancreatitis mRNA data for GSE194331 (Normal: 32, Pancreatitis: 87) and pancreatitis GSE143754 (Normal: 9, Pancreatitis: 6) were downloaded from the GEO database and were used as a training and validation set, respectively. First, the training set GSE194331 was differentially expressed and intersected with the ERSRGs (n = 265) obtained from the MSigDB database to result in 42 differentially expressed ERSRGs (DE-ERSRGs). Subsequently, five candidate genes were further screened by PPI network and MCC and MCODE algorithms. However, according to the ROC curve results, AUC values of CCND1, BCL2, PIK3R1, and BCL2L1 were all greater than 0.6, indicating that they had good diagnostic performance, which was verified by the GSE143754 data set. Based on the GeneMANIA network, it was found that hub genes BCL2 and BCL2L1 may be the key factors in the regulation of mitochondrial metabolism. 24 differentially expressed pancreatitis-related genes (DE-PRGs) were found by difference analysis and Venn analysis. Hub genes BCL2 and PIK3R1 that were significantly correlated with 24 DE-PRGs were determined by Spearman analysis. ssGSEA algorithm was further used to reveal the significant correlation between these hub genes and the immune microenvironment of pancreatitis. The miRNA and lncRNA targeting hub genes were predicted using miRWalk, TargetScan, miRDB, and ENCORI databases, providing research directions for the mechanism of pancreatitis. Finally, the Network Analyst website was used to predict potential compounds associated with the hub gene. In conclusion, this study not only further supports the role of ERS in the development of pancreatitis but also provides a new perspective and direction for the development of biomarkers and mechanism of pancreatitis. At the same time, the results of this study provide a reliable research direction for the targeted treatment of pancreatitis.

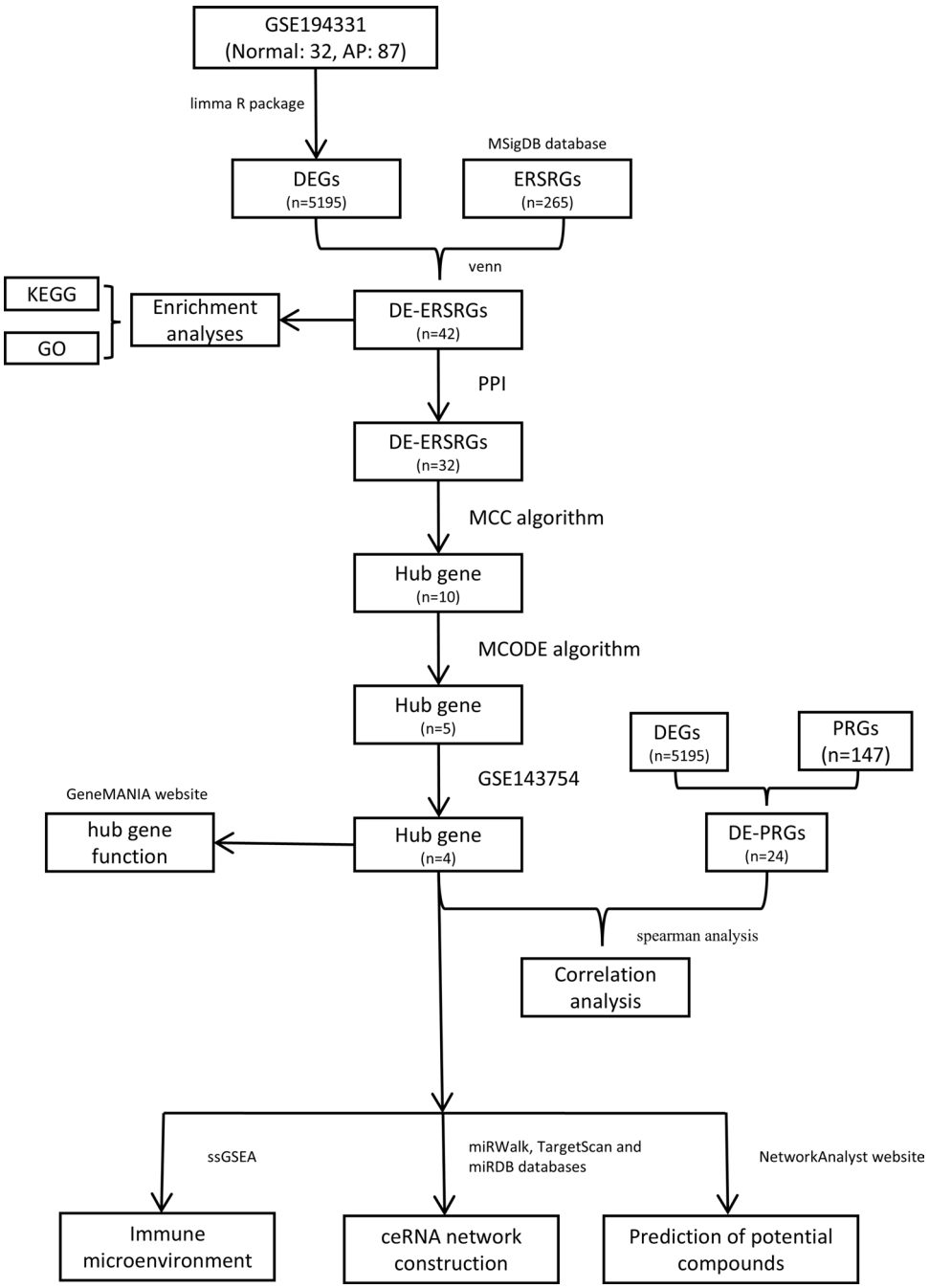
✉ Laifa Kong
Konglaifa@163.com

[1] Department of Emergency Surgery, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, No. 365, Renmin East Road, Jinhua 321000, Zhejiang, China

[2] Department of Emergency, Affiliated Jinhua Hospital, Zhejiang University School of Medicine, Jinhua 321000, Zhejiang, China

Published online: 12 March 2025

Graphical Abstract



Keywords Drug prediction · Endoplasmic reticulum stress · Hub gene · Immune microenvironment · Pancreatitis

Introduction

Pancreatitis is the most common exocrine pancreatic disease and a primary cause of consultation and hospitalization for gastrointestinal diseases [1, 2]. Pancreatitis can be divided into acute pancreatitis (AP) and chronic pancreatitis (CP), with AP being the most common type [3]. According to statistics from 2017, there are approximately 1.6 million new cases of AP globally, with 100,000 deaths [4]. Even if AP can be cured, the life quality of patients after surgery will be substantially reduced [5]. Additionally, about 17% of AP patients will relapse and 8% of AP patients will progress to

CP, imposing a serious economic burden on the healthcare system [6, 7]. Furthermore, CP is a chronic inflammatory disease that can result in irreversible changes in pancreatic tissue and function. Factors such as persistent inflammation and pancreatic injury, genetic susceptibility, autoimmune reactions, ductal changes, smoking, or alcohol abuse may contribute to the progression from AP to CP [8–10]. Moreover, CP dramatically elevates the risk of developing pancreatic cancer (PC), threatening human health [11]. Currently, although considerable progress has been achieved in the treatment of pancreatitis, including the application of techniques, such as surgery, endoscopic ultrasound examination, and immunotherapy, the exact mechanism of pancreatitis remains unclear, which hinders in-depth research on the disease [12–14]. Therefore, there is an urgent need to figure out the mechanism of pancreatitis development.

Former studies have suggested that the occurrence of pancreatitis is tightly linked to autophagy and inflammatory responses, and endoplasmic reticulum stress (ERS) plays a pivotal role in autophagy and inflammatory responses [15]. ERS is a pathologic process induced by the excessive aggregation of misfolded proteins in the endoplasmic reticulum (ER) triggered by external stimuli (such as oxidative stress, calcium homeostasis imbalance, and phospholipid synthesis disorders), which initiates the unfolded protein response (UPR) to correct this error [16]. A study has shown that ERS/UPR can activate the NLRP3 inflammasome, inducing inflammatory responses through oxidative stress, calcium homeostasis, and NF-κB activation [17]. Recently, He et al. [18] uncovered that the suppression of SRXN1 expression activates ERS, leading to the accumulation of M1 phenotype macrophages and neutrophils, facilitating inflammation and exacerbating the progression of AP. Additionally, Zhang et al. [19] pointed out that the accumulation of GSDMD in the ER induces ERS in acinar cells, further exacerbating the local pancreatic symptoms and systemic inflammation in AP. In recent years, many studies have revealed that ERS-related genes can be used as prognostic and diagnostic markers for cancers or diseases, such as pancreatic cancer [20], diabetic nephrosis [21], and non-alcoholic fatty liver disease [22]. However, the role of ERS-related genes in pancreatitis has not been studied.

In this study, we identified the ERS-related genes (ERSRGs) that play a key regulatory role in pancreatitis through analysis of public databases, which were also hub genes. After evaluating the diagnostic ability of these hub genes, we further investigated their linkage with pancreatitis-related genes (PRGs) and their roles in the immune microenvironment. Our investigation further confirmed the potential value of these genes in pancreatitis diagnosis. Finally, potential miRNAs, lncRNAs, and drugs targeting these hub genes were predicted. In conclusion, these findings function as a solid theoretical basis for future in-depth research on the clinical diagnosis and treatment of pancreatitis and offer important reference directions for further mechanistic studies and clinical applications.

Materials and Methods

Data Acquisition

Since there are few pancreatitis datasets from human samples, two mRNA datasets, GSE194331 and GSE143754, were downloaded from the GEO database as training and validation sets, respectively. Dataset GSE194331 contained peripheral blood RNA-Seq data from 32 healthy controls and 87 AP patients with varying degrees of severity (mild = 57, moderate = 20, severe = 10). Dataset GSE143754 contained mRNA data from 6 CP tissue samples and 9 adjacent normal tissue samples. Genes associated with pancreatitis disease were obtained from the Genecards database (https://www.genecards.org/), with genes greater than the Relevance score median retained. We referenced the method by Chen et al. [23] to download 265 ERSRGs (Table S1) from the MSigDB database (https://www.gsea-msigdb.org/gsea/msigdb/index.jsp) with the search term as GOBP_RESPONSE_TO_ENDOPLASMIC_RETICULUM_STRESS.

Screening and Enrichment Analysis of Differentially Expressed ERSRGs (DE-ERSRGs) in Pancreatitis

The *limma* R package [24] was employed to analyze the GSE194331 training set from differential expression analysis, yielding differentially expressed genes (DEGs) with thresholds of P value < 0.05 and |logFC|> 0.5. The intersection of integrated DEGs and ERSRGs with the Venn diagram was used to obtain DE-ERSRGs. To clarify the related functional pathways, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses were carried out on the DEGs using the *clusterProfiler* R package, with final results visualized using the *enrichplot* R package [25].

Identification of Hub Genes in DE-ERSRGs and Evaluation of Their Clinical Diagnostic Performance

We input DE-ERSRGs into the Search Tool for the Retrieval of Interacting Genes (STRING) database (https://string-db.org/) to construct a protein–protein interaction (PPI) related to DE-ERSRGs, with a confidence level of 0.4. Based on the PPI network results, the top 10 genes were calculated using the MCC method in the CytoHubba plugin of Cytoscape 3.10.0 and integrated with the results of MCODE algorithm screening to identify hub genes [26, 27]. The differential

Table 1 Primer sequences for qRT-PCR

Genes	Primer sequence (5'-3')	
CCND1	CATCTACACCGACAACTCCATC	TCTGGCATTTTGGAGAGGAAG
BCL2	GTGGATGACTGAGTACCTGAAC	GCCAGGAGAAATCAAACAGAGG
PIK3R1	GATGGCACTTTTCTTGTCCG	CTGTACAAGTTATAGGGCTCGG
BCL2L1	GACATCCCAGCTCCACATC	GTTCCCATAGAGTTCCACAAAAG
GAPDH	CAATGACCCCTTCATTGACC	GACAAGCTTCCCGTTCTCAG

expression of hub genes in normal samples and pancreatitis samples in the GSE194331 training set was analyzed. Subsequently, the receiver operation characteristic (ROC) curves of hub genes in the training set were plotted using the *pROC* R package, with the area under the curve (AUCs) calculated to evaluate the clinical diagnostic performance of hub genes [28]. The higher the AUC value indicated better the diagnostic performance. Finally, the ROC curve was used to verify the diagnostic performance of the hub gene in the validation cohort GSE143754 dataset.

Prediction of Hub Gene Function and Correlation Assessment of Hub Genes and PRGs

The GeneMANIA website (http://genemania.org) was used to predict genes with similar functions to the hub gene and their interaction network diagram was constructed [29]. From Genecards database (https://www.genecards.org/) [30], genes associated with pancreatitis (Relevance score > 10) were screened, with genes having Relevance scores higher than the median value retained. The screened PRGs were intersected with DEGs to obtain the differentially expressed PRGs (DE-PRGs). Finally, the "*spearman*" was used to calculate the correlation between the hub gene and these genes in pancreatitis samples, and the correlation heat map was drawn using *ggcorrplot* package.

The Role of the Hub Genes in the Immune Microenvironment

With the use of the single sample gene set enrichment analysis (ssGSEA) algorithm, we evaluated the immune microenvironment differences between normal samples and pancreatitis samples in the GSE194331 training set. Furthermore, the correlation between hub genes and immune cells in pancreatitis samples was assessed, with *ggcorrplot* R package [31] utilized for result visualization.

The Construction of the ceRNA Network

First, the miRNAs interacting with hub genes were predicted using the miRWalk database (http://mirwalk.umm.uni-heidelberg.de/). In order to ensure the accuracy of the results, we further integrated the results of TargetScan (https://www.targetscan.org/vert_80/) and miRDB database (https://mirdb.org/) prediction [32]. The TargetScan database was used to predict the potential sites of hub gene binding to miRNA. The miRDB database was used for miRNA target prediction and functional annotation. At the same time, these databases were used to analyze the correlation between hub genes and predicted miRNAs. Then, we further predicted lncRNAs targeting miRNAs in the ENCORI database (https://rnasysu.com/encori/) and used ENCORI online database analysis to express the relationship between the two. We selected negative correlation lncRNAs as target lncRNAs. Finally, the ceRNA network was visualized [33] via Cytoscape 3.10.0.

Prediction of Potential Compounds Targeting Hub Genes

Network Analyst (https://www.networkanalyst.ca/), as an integrated online platform for gene expression visualization, can help find regulation Networks of drug interaction compounds [34]. The data are based on data from the Comparative Toxicogenomics Database (CTD). Therefore, in this study, hub genes were imported into the Network Analyst website, so as to locate the compounds interacting with hub genes and build the corresponding hub gene.

Collection of Samples

About 10 whole blood samples of pancreatitis patients and 10 normal control samples were collected. The research was executed under the Helsinki Declaration and approval from the Affiliated Jinhua Hospital, Zhejiang University School of Medicine Ethics Committee.

qRT-PCR

Total cellular RNA was extracted using TRIzol reagent (Invitrogen, USA). RNA concentration and purity were

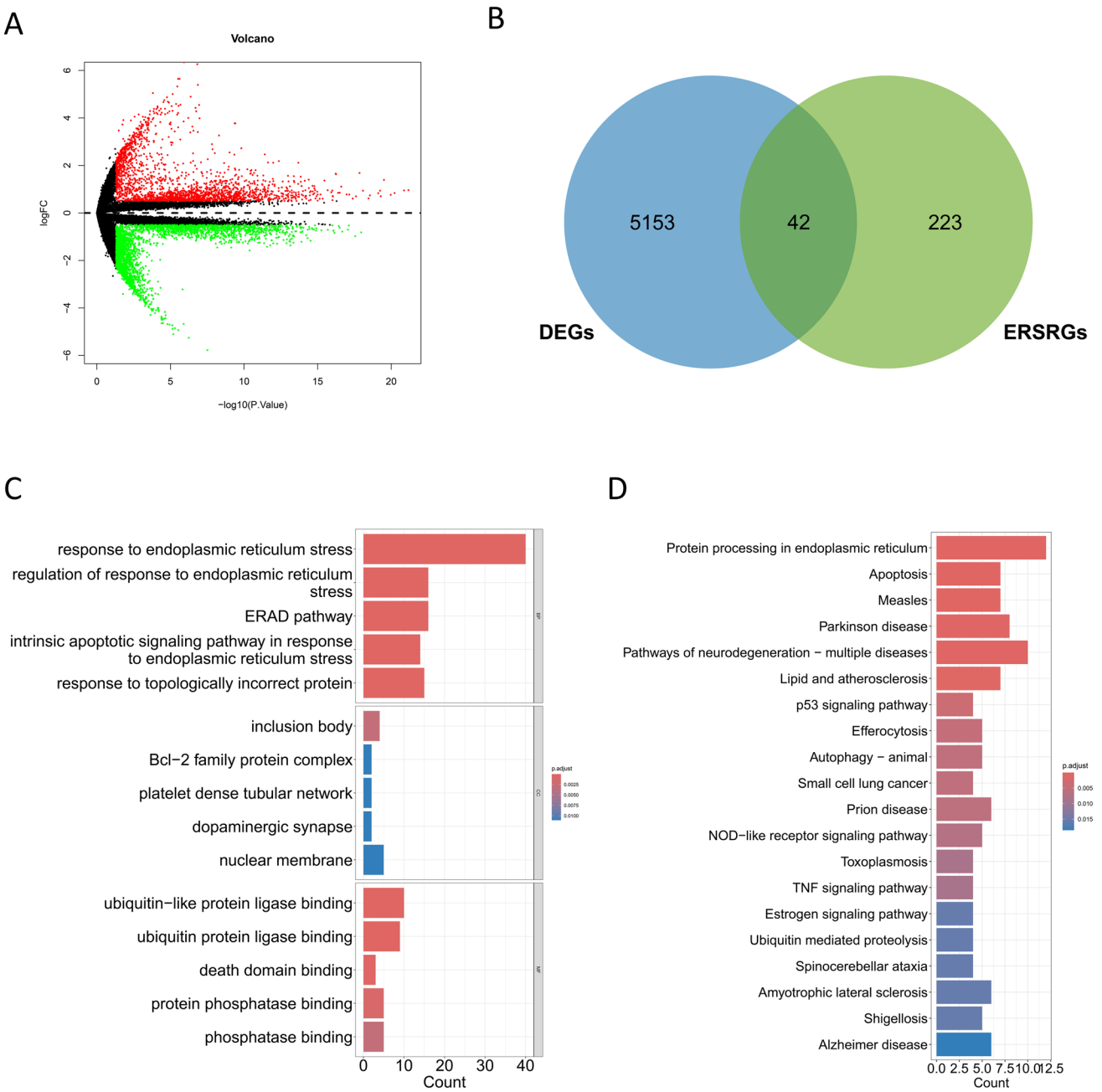
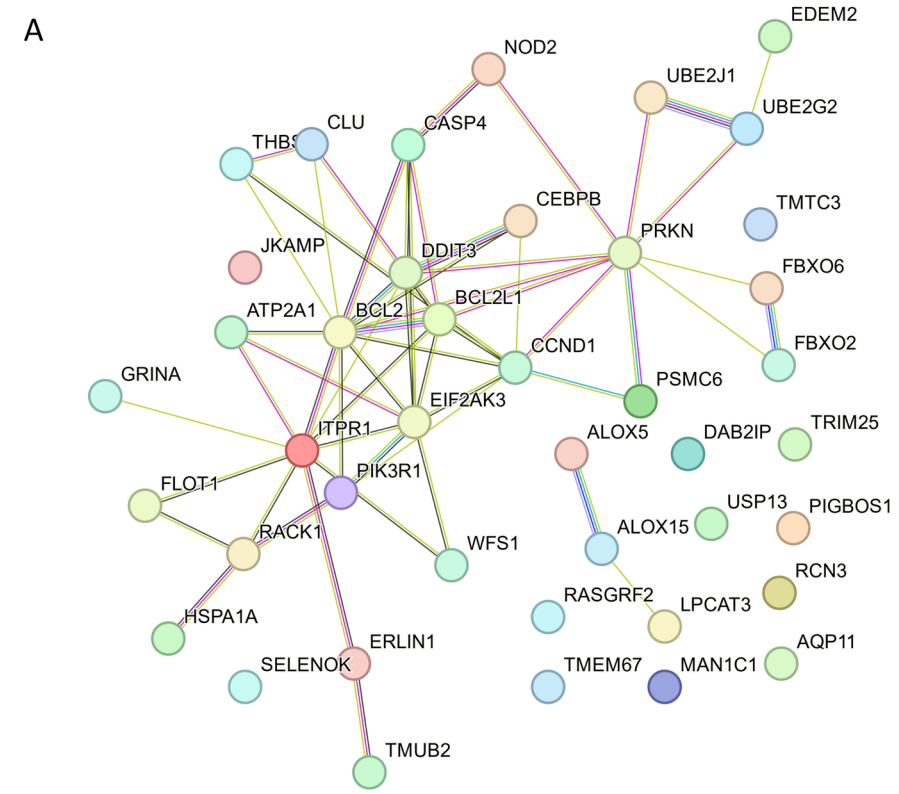


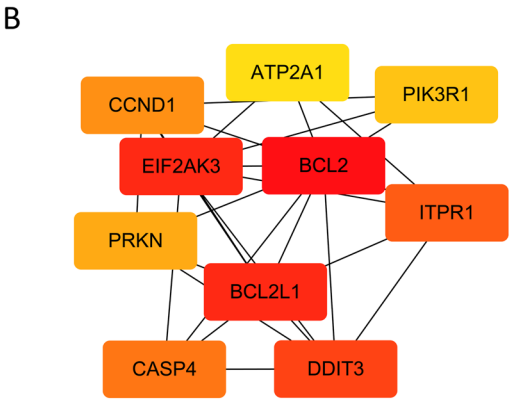
Fig. 1 Identification and potential pathway analysis of DE-ERSRGs. **A** Volcano plot of mRNA expression in the training set GSE194331, with red representing upregulated mRNA and green representing downregulated mRNA. **B** Venn diagram analysis of identified DE-ERSRGs. **C** GO enrichment analysis of DE-ERSRGs. **D** KEGG enrichment analysis of DE-ERSRGs

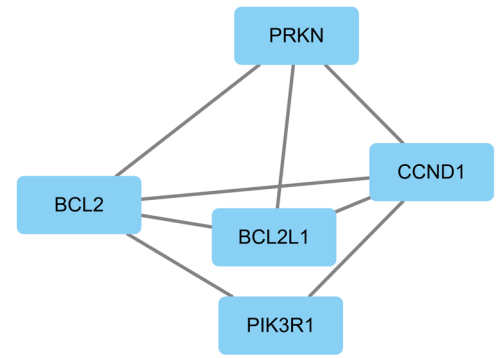
determined using a NanoDrop One microUV visible spectrophotometer. SuperScript II reverse transcriptase (Invitrogen, USA) was employed for reverse transcription of RNA to cDNA. qRT-PCR was performed using TB Green® Premix Ex Taq™ II (Takara, Japan) on an ABI 7500 PCR system (Applied Biosystems, USA). GAPDH was used as the reference gene. The $2^{-\Delta\Delta CT}$ method was applied to calculate relative mRNA expression. Primer sequences are shown in Table 1.

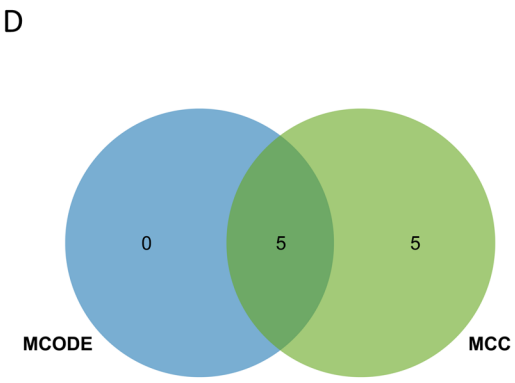
Statistical Analysis

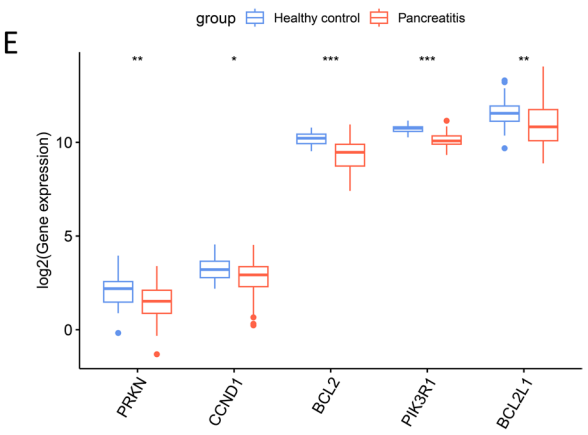
Statistical analysis of bioinformatics-related data was performed using R software (version 4.2.2). Experimental data were analyzed using GraphPad Prism 8.0 software. The Student's *t* test was used to compare two sets of normally distributed data, while the Wilcoxon rank-sum test was used to compare non-normally distributed data. The data


A
EDEM2
NOD2
UBE2J1
UBE2G2
CLU
CASP4
THBS
CEBPB
TMTC3
PRKN
JKAMP
DDIT3
FBXO6
ATP2A1
BCL2
BCL2L1
FBXO2
CCND1
GRINA
PSMC6
EIF2AK3
ITPR1
ALOX5
DAB2IP
TRIM25
PIK3R1
FLOT1
USP13
PIGBOS1
ALOX15
RACK1
WFS1
RCN3
RASGRF2
LPCAT3
HSPA1A
ERLIN1
AQP11
SELENOK
TMEM67
MAN1C1
TMUB2


B
ATP2A1
CCND1
PIK3R1
EIF2AK3
BCL2
ITPR1
PRKN
BCL2L1
CASP4
DDIT3


C
PRKN
CCND1
BCL2
BCL2L1
PIK3R1


D
0
5
5
MCODE
MCC


E
group
Healthy control
Pancreatitis
log2(Gene expression)
10
5
0
PRKN
CCND1
BCL2
PIK3R1
BCL2L1

◂**Fig. 2** Identification of hub genes in DE-ERSRGs. **A** PPI network of DE-ERSRGs. **B** The MCC method identified 10 hub genes with interacting relationships. **C** 5 hub genes with interaction relationships identified by MCODE algorithm. **D** Venn analysis of MCC and MCODE algorithms. **E** Differences in hub gene expression between normal healthy samples and samples with pancreatitis in the GSE194331 training set

of categorical variables between the two groups were tested by the Chi-square test, and Spearman was used to calculate the correlation. Each experiment for qRT-PCR was repeated at least three times. $p < 0.05$ was considered a significant difference.

Results

Identification of DE-ERSRGs and Potential Pathway Analysis

We performed differential analysis on 32 normal samples and 87 pancreatitis samples from the training set GSE194331, obtaining a total of 5195 DEGs (including 2202 upregulated genes and 2993 downregulated genes) (Fig. 1A, Table S2). By Venn analysis, we intersected the obtained DEGs with ERSRGs to obtain 42 DE-ERSRGs (Fig. 1B). Subsequently, enrichment analyses were conducted to dig out the potential biologic processes and signaling pathways affected by the DE-ERSRGs. The enrichment analyses revealed that these DE-ERSRGs were mainly enriched in response to ERS, regulation of response to ERS, intrinsic apoptotic signaling pathway in response to ERS, dopaminergic synapse, ubiquitin-like protein ligase binding, and other Gene Ontology (GO) entries (Fig. 1C), as well as important KEGG pathways, such as Protein processing in ER, Pathways of neurodegeneration-multiple diseases, Autophagy-animal, and TNF signaling pathway (Fig. 1D). This implied that the occurrence and progression of pancreatitis are closely linked to the modulation of ERSRGs, which may function through ERSRGs to mediate these biologic processes or signaling pathways.

Identification of Hub Genes in DE-ERSRGs and Analysis of Diagnostic Value

In order to further obtain the key genes in DE-ERSRGs, we first constructed the PPI network of DE-ERSRGs (confidence 0.4), and we analyzed the intrinsic connections among DE-ERSRGs. The results uncovered that among the 42 DE-ERSRGs, 32 DE-ERSRGs (72.6% (32/42)) had interactions with each other (Fig. 2A). Furthermore, we identified the top 10 hub genes that play an instrumental part by utilizing the MCC algorithm (Fig. 2B). To ensure the reliability of the identification results, we re-identified hub genes using the MCODE algorithm (Fig. 2C). Subsequently, the intersection of the results from the two algorithms yielded 5 hub genes, namely PRKN, CCND1, BCL2, PIK3R1, and BCL2L (Fig. 2D). At the same time, compared with normal samples, all five hub genes were significantly under-expressed in patients with pancreatitis ($P < 0.05$, Fig. 2E).

Further, to evaluate the diagnostic performance of the hub gene, we plotted the ROC curve and calculated the AUC value. The ROC curves of the GSE194331 training set demonstrated that the AUC values of these 5 hub genes were 0.724, 0.665, 0.833, 0.919, and 0.689, all greater than 0.6 (Fig. 3A). Further validation was performed on the verification set GSE143754. The ROC curves also showed that the AUC values of CCND1, BCL2, PIK3R1, and BCL2L were all greater than 0.6, but the AUC value of the PRKN homologous gene PARK2 was only 0.519 (Fig. 3B), indicating that the diagnostic ability of PRKN may be poor. Therefore, we finally concluded that the four hub genes CCND1, BCL2, PIK3R1, and BCL2L have good diagnostic performance and may play a pivotal role in regulating pancreatitis treatment.

Functions of Hub Genes and their Potential Roles in Pancreatitis

To understand the main functions of hub genes, we presented the interaction network diagram of hub genes at the gene level, finding a strong correlation between the genes BCL2 and BCL2L1 and pathways, such as apoptotic mitochondrial changes, regulation of membrane permeability, and regulation of mitochondrial organization (Fig. 4A). Therefore, BCL2 and BCL2L1 may be key factors in the metabolic regulation of mitochondria. Unfortunately, we identified no similar genes with CCND1 or PIK3R1. Furthermore, to elucidate the functions of hub genes in pancreatitis, we first intersected 5195 DEGs with 147 PRGs (Table S3) and obtained a total of 24 intersecting genes, namely DE-PRGs (Fig. 4B, Table S4). The hub gene CCND1 was included, indicating that it was both a DE-PRG and a key hub gene identified in our study. This finding further strengthened the credibility of hub gene screening. Subsequently, we assessed the intrinsic relationship between hub genes and the expression of these genes (Fig. 4C), revealing that when the 4 hub genes interacted with DEGs associated with pancreatitis, they all exhibited a consistent trend of upregulation or downregulation. At the same time, BCL2 and PIK3R1 have a strong correlation with most of the DE-PRGs, but further experimental verification is still needed. All in all, we believe that the identified hub gene may be of great value to the progression of pancreatitis and can provide a reference direction for the subsequent investigation of the mechanism of pancreatitis.

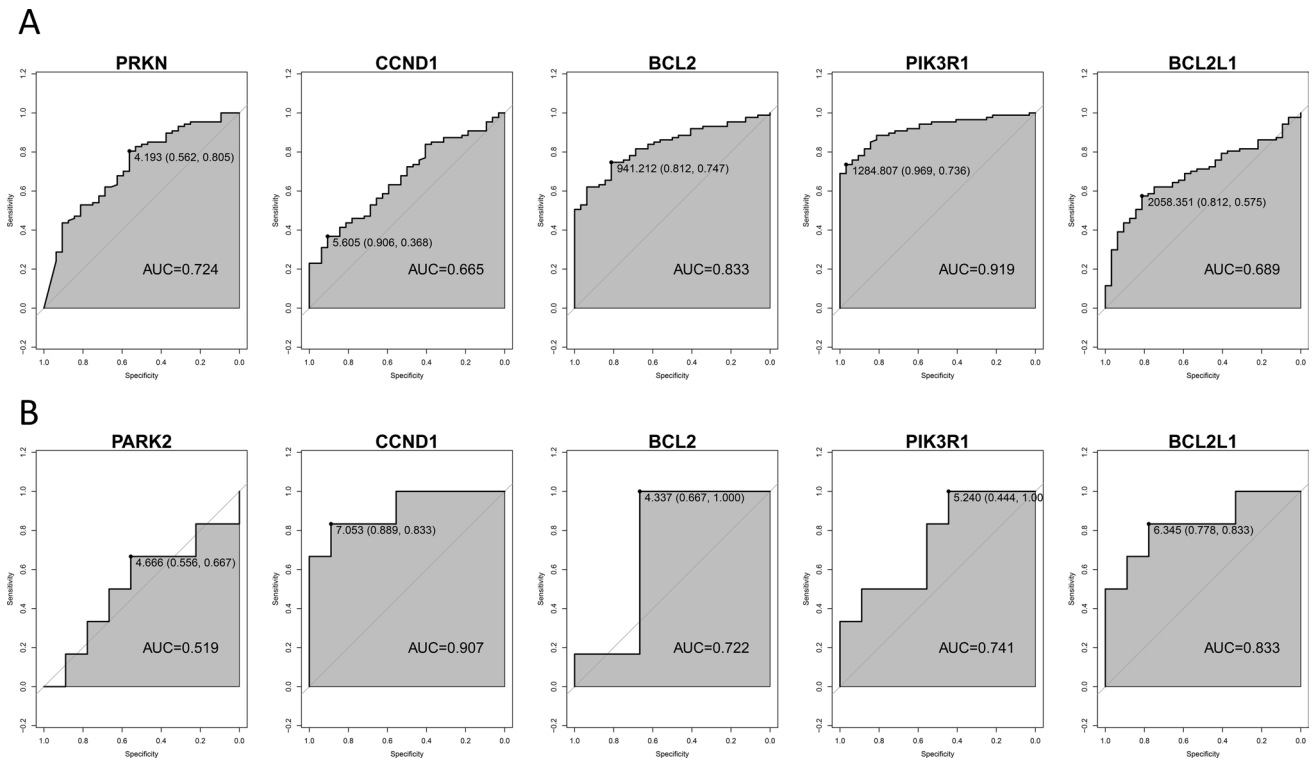


Fig. 3 Diagnostic value of hub genes in patients with pancreatitis. **A** ROC curves of hub genes in the training set GSE194331. **B** ROC curves of hub genes in the validation set GSE143754

The Role of Hub Genes in Pancreatitis Immune Microenvironment

To evaluate the role of the hub gene in the immune microenvironment of pancreatitis, we used the ssGSEA algorithm to analyze the level of immune cell infiltration in patients with pancreatitis. Only DCs, Mast cells, NK cells, and Treg showed no significant difference between patients with pancreatitis and normal samples. However, compared with the normal sample, Macrophage and Neutrophils in the patient with pancreatitis had a higher invasion level, while B cells, $CD8^+$ T cells, and Tfh had a lower invasion level ($P < 0.001$) (Fig. 5A). Further, we analyzed the degree of enrichment of immune function. The results showed that the enrichment level of Check-point, HLA, Inflammation promoting, T-cell co-inhibition, and T-cell co-stimulation in patients with pancreatitis was significantly lower than that in normal samples (Fig. 5B). We then evaluated the association of the hub gene with immune function. The results showed that the hub gene was negatively correlated with Macrophage and Neutrophils, but positively correlated with B cells, $CD8^+$ T cells, and Tfh with low invasion (Fig. 5C). Moreover, hub genes affected the enrichment of most immune functions, such as Checkpoint, HLA, and Inflammation promoting. This indicated that the hub gene plays an important role in the immune microenvironment of patients with pancreatitis, which may have reference value for pancreatitis-related immunotherapy.

The Construction of the ceRNA Network

In order to analyze the potential regulatory mechanism of the hub gene in pancreatitis, we used miRWalk, TargetScan, and miRDB databases to predict the miRNAs and lncRNAs that may target the hub gene. Prediction results showed that a total of 26 miRNAs targeting hub genes were obtained. Subsequently, analysis in the ENCORI database yielded 1095 lncRNAs interacting with miRNAs. Finally, the ceRNA network was mapped using Cytoscape 3.10.0 software to demonstrate the predicted complex interactions between lncRNA–miRNA–mRNA (Fig. 6, Table S5). Therefore, the regulatory network of the hub gene in pancreatitis can be obtained through the ceRNA network, which provides a new research direction for the subsequent experimental investigation of the pathogenesis of pancreatitis.

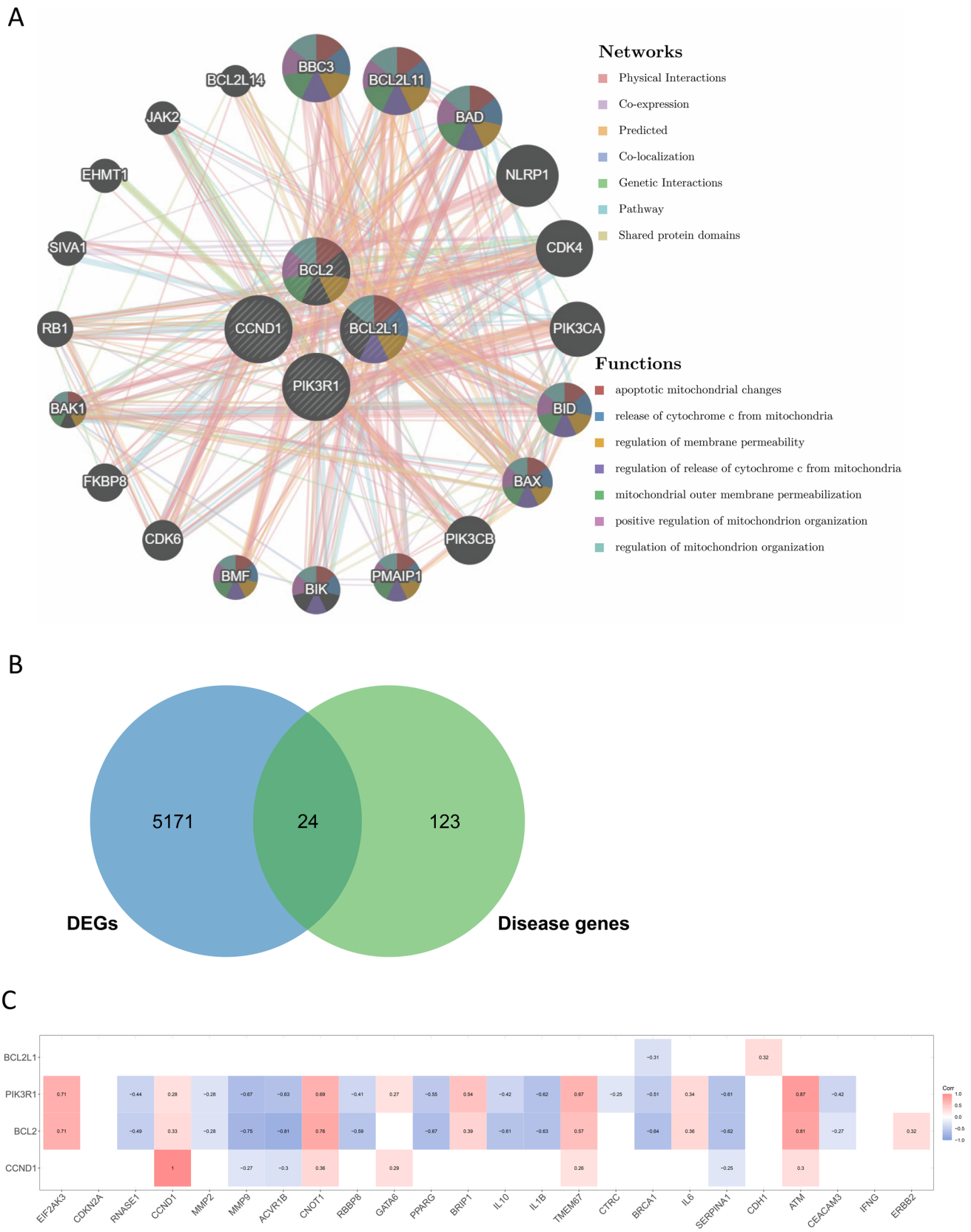


Fig. 4 Functional analysis of hub genes and their potential roles in pancreatitis. **A** PPI network diagram for hub gene function prediction. **B**: Venn diagram of hub genes and genes related to pancreatitis. **C** Heatmap of the correlation between hub genes and genes related to pancreatitis

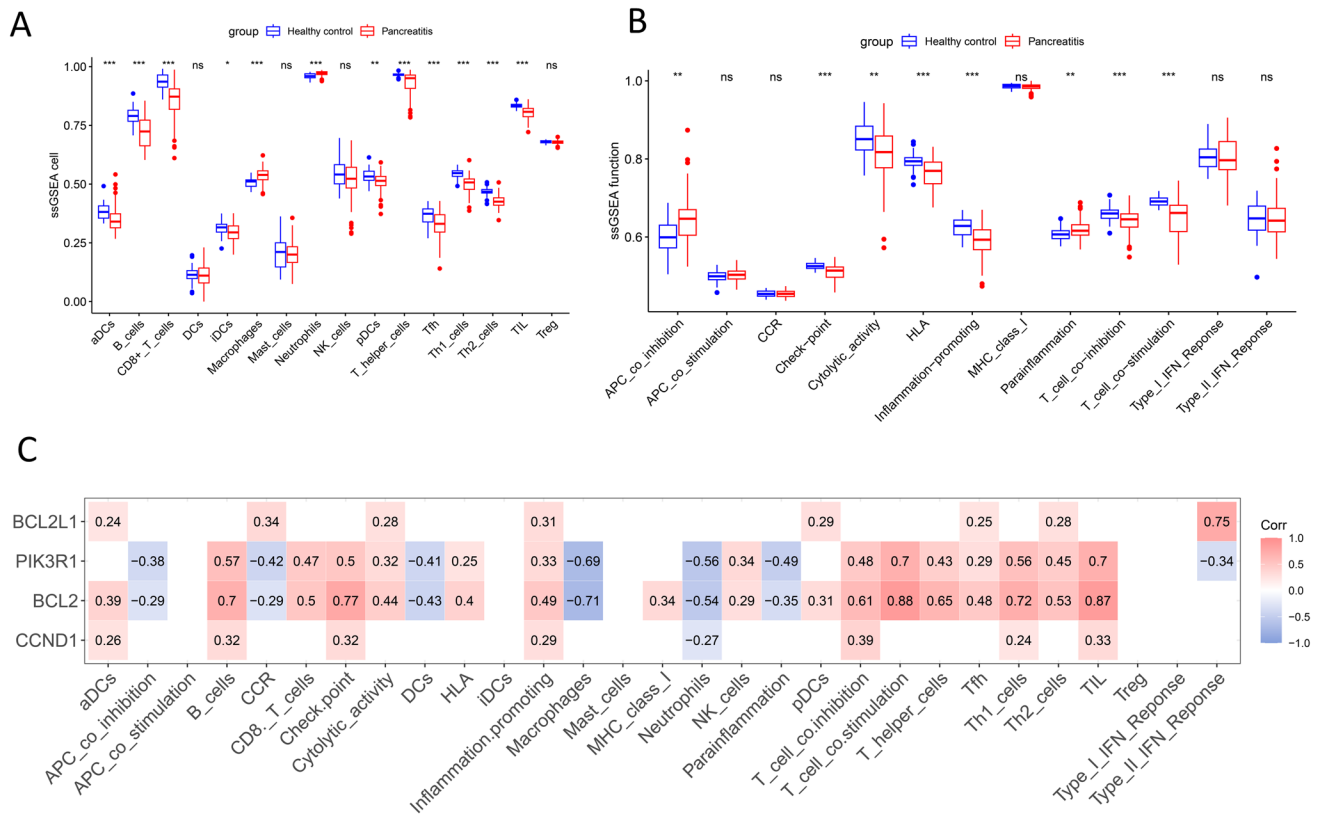


Fig. 5 The role of hub genes in the immune microenvironment of pancreatitis patients. **A** Infiltration analysis of immune cells in normal samples and pancreatitis patient samples in the training set GSE194331. **B** Enrichment analysis of immune function in normal samples and pancreatitis patients in the training set GSE194331. $*P<0.05$; $**P<0.01$; $***P<0.001$. **C** Correlation analysis of hub genes with immune cells and immune functions in pancreatitis patients

Prediction of Potential Compounds Targeting Hub Genes

Lastly, we obtained 154 potential protein compounds targeting hub genes through the NetworkAnalyst website (https://www.networkanalyst.ca/), including 24 compounds with degree = 4 and 120 compounds with degree = 3, through the NetworkAnalyst website (https://www.networkanalyst.ca/) (Table S6). Simultaneously, we also displayed the compounds with degree ≥ 3 for visualization (Fig. 7). These potential compounds could provide new strategies for the subsequent treatment of pancreatitis. Of course, the efficacy of these compounds needs to be verified in further experiments.

Validation of the Expression Levels of Hub Genes in Pancreatitis Patients using qPCR

We further validated the expression levels of 4 hub genes (CCND1, BCL2, PIK3R1, and BCL2L) using qRT-PCR. These genes were significantly down-regulated in the blood of the pancreatitis group. The validation results were consistent with the previous analysis of gene expression in the database (Fig. 8).

Discussion

ERS and its induced UPR can modulate many physiologic processes such as gene expression, protein secretion, cell growth, differentiation, and metabolism, which are key mechanisms determining cell fate [35]. ERS is linked to the pathogenesis of inflammatory diseases in various tissues or organ systems, including hepatitis B virus, inflammatory bowel disease, and pancreatitis [36–38]. However, the mechanism and function of ERS in inflammatory diseases, especially in pancreatitis, are still elusive. Therefore, this study collected data from pancreatitis patients through the GEO database and used bioinformatics analysis to identify 4 hub genes closely related to pancreatitis in ERSRGs, namely CCND1, BCL2, PIK3R1, and BCL2L1. Moreover, the ROC curve proved that the diagnostic performance of these four hub genes was good. At the same time, immune infiltration analysis showed that the level of immune cell infiltration in

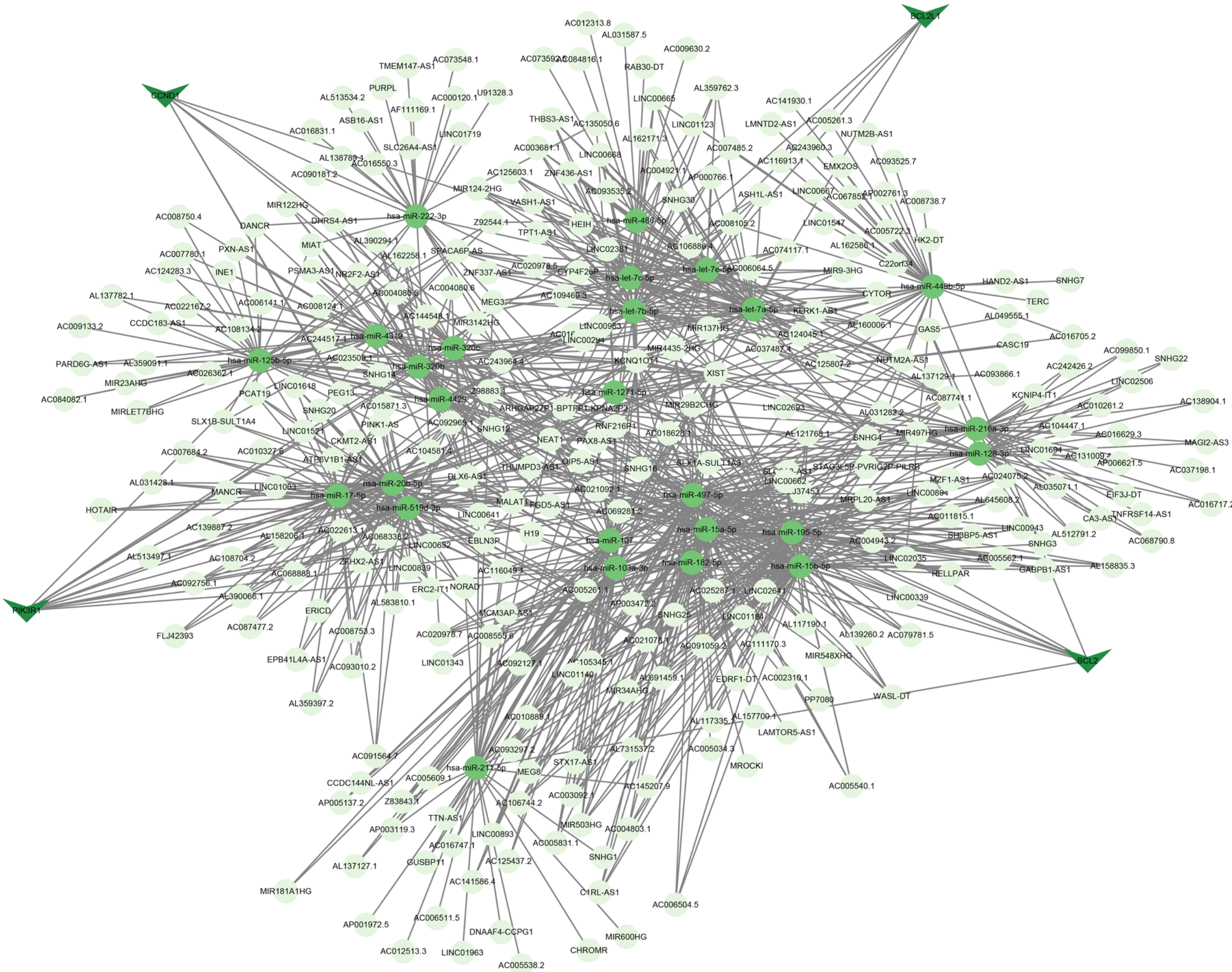


Fig. 6 ceRNA network targeting hub genes. Dark green square: hub genes. Green circle: miRNA. Light green circle: lncRNA

patients with pancreatitis was significantly different from that in the normal group, and the hub gene was significantly correlated with most immune functions. In addition, the construction of ceRNA networks and the analysis of compounds also provide new directions for investigating the mechanism of hub genes in pancreatitis and potential drugs.

CCND1 is a key protein for integrating extracellular mitotic signaling [39]. Wang et al. [40] also found through bioinformatics analysis that CCND1 is a potential miRNA target in pancreatitis. Furthermore, Biliran et al. [41] demonstrated that overexpression of CCND1 can enhance NF-κB activity, dampen drug-induced apoptosis, and boost chemoresistance in PC cells. NF-κB is a key factor in inducing inflammation, suggesting that CCND1 also exerts a key regulatory influence in pancreatitis. Studies have shown that when ER stress is severe or prolonged, the UPR changes from a pro-survival signal to a pro-apoptotic signal, causing cell death [42]. The hub gene was obtained in this study. BCL2 and BCL2L1 (also known as BCL-X) are important members of the BCL2 family, located in mitochondria to exert anti-apoptosis effects, reinforcing cell survival [43]. Wang et al. [44] identified key biomarkers related to immunogenic cell death (ICD) in patients with severe AP (SAP) through WGCNA and machine learning, and found that BCL2 is also a hub gene and is under-expressed in SAP. The Wang's study also showed that BCL2 is closely related to the infiltration of SAP-specific immune cells and activation of immune pathways, which is similar to our results. Xu et al. [45] induced AP in vitro and in vivo and found that the expression of Bax increased, while the expression of BCL2 decreased. This is consistent with our predicted functions of BCL2 and BCL2L1. Feng et al. [46] reported that BCL2 and BCL2L1 can be induced by IL-22, which then binds to Beclin-1 to repress autophagosome formation, thereby improving pancreatitis. As a key regulatory subunit of PI3K, PIK3R1 hinders the catalytic activity of the P110α kinase

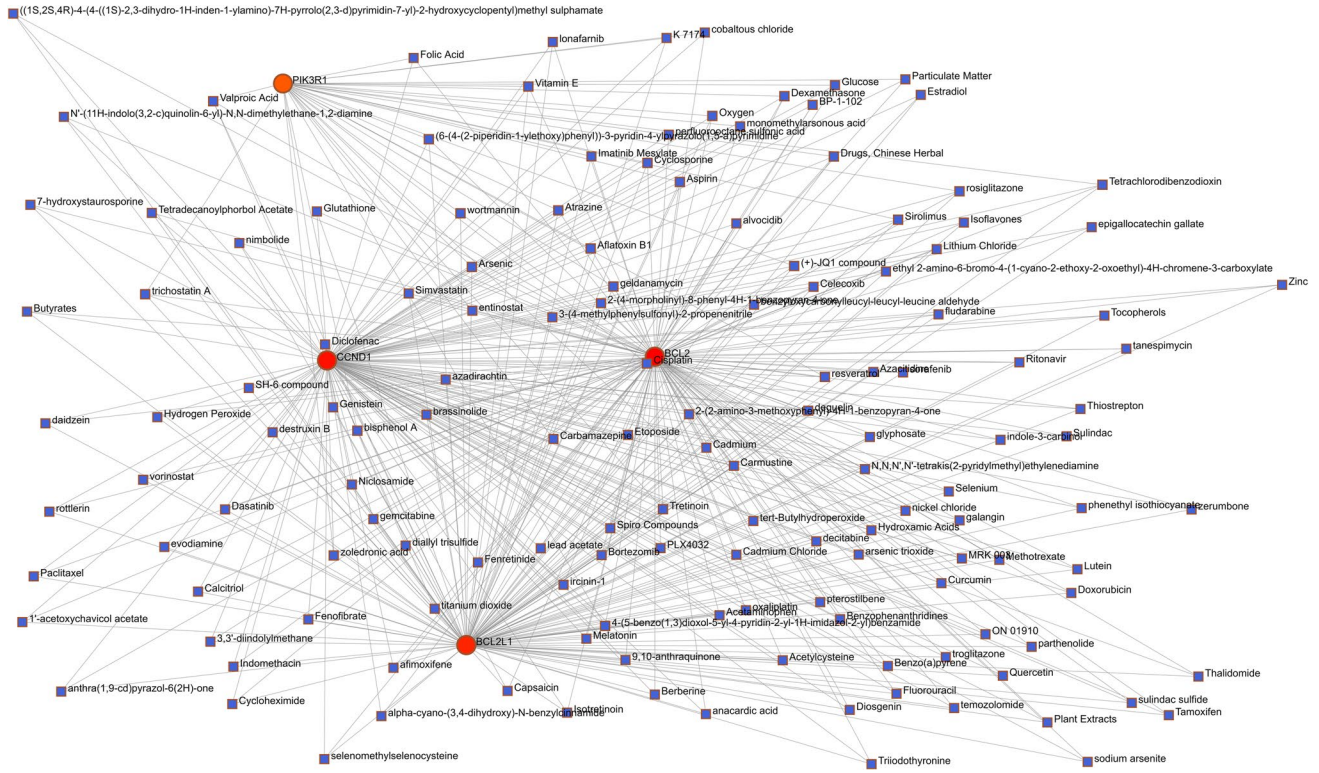


Fig. 7 Compound network interacting with hub genes. Red circle: hub gene. Blue square: predicted potential compound

affecting PI3K signaling [47]. The PI3K signaling pathway is essential in inflammatory diseases, and the development of PI3K inhibitors may be an effective approach for treating inflammation [48]. Therefore, we speculate that the 4 hub genes identified in this study may play major roles in pancreatitis progression.

The immune microenvironment is crucial in different stages of pancreatitis, proffering potential therapeutic targets for AP and CP diseases [49, 50]. In this study, we analyzed the immune microenvironment in pancreatitis and explored the function of hub genes in it. The upregulation of macrophages and neutrophils is a great manifestation of the occurrence and progression of pancreatitis [51]. Macrophages mainly differentiate into pro-inflammatory M1 phenotype in AP, causing local pancreatic inflammation, while in CP, they typically exhibit an M2 phenotype, interacting with pancreatic stellate cells to accelerate pancreatic fibrosis formation, further damaging the pancreas [52]. Neutrophils can result in premature activation of trypsin, reinforcing the release of inflammatory factors and exacerbating AP [53]. The repression of the activation of macrophages and neutrophils can aid in alleviating pancreatic inflammation [54, 55]. Therefore, the negative correlation of the 4 hub genes with macrophages and neutrophils suggested that these genes may be potential therapeutic targets for pancreatitis. B lymphocytes and T lymphocytes are the main immune defense of the human body against diseases, especially cancer [56]. Pietruczuk et al. [57] detected a substantial decrease in peripheral blood B lymphocytes and T lymphocytes in AP patients, possibly due to excessive apoptosis and migration to inflammatory sites. This is in line with our findings of low infiltration of B cells, $CD8^+$ T cells, and Tfh in patients with pancreatitis. Furthermore, correlation analysis of immune cells manifested that hub genes were positively correlated with B lymphocytes and T lymphocytes, indicating that hub genes may potentiate the activity of these immune cells to alleviate pancreatic inflammation. Taken together, we believe that the identified 4 hub genes play a positive regulatory role in the progression of pancreatitis, potentially serving as therapeutic targets for pancreatitis patients.

Next, this study predicted miRNAs and lncRNAs targeting hub genes and dissected their complex interactions with hub genes through the construction of the ceRNA network. Xu et al. [58] found through in vitro experiments that silting hsa-circ-0032449 could inhibit pancreatic differentiation of human embryonic stem cells by regulating the hsa-miR-195-5p/CCND1/PI3K/AKT signaling pathway. Meanwhile, Wang et al. [40] identified key miRNAs in pancreatitis through bioinformatics analysis and found that

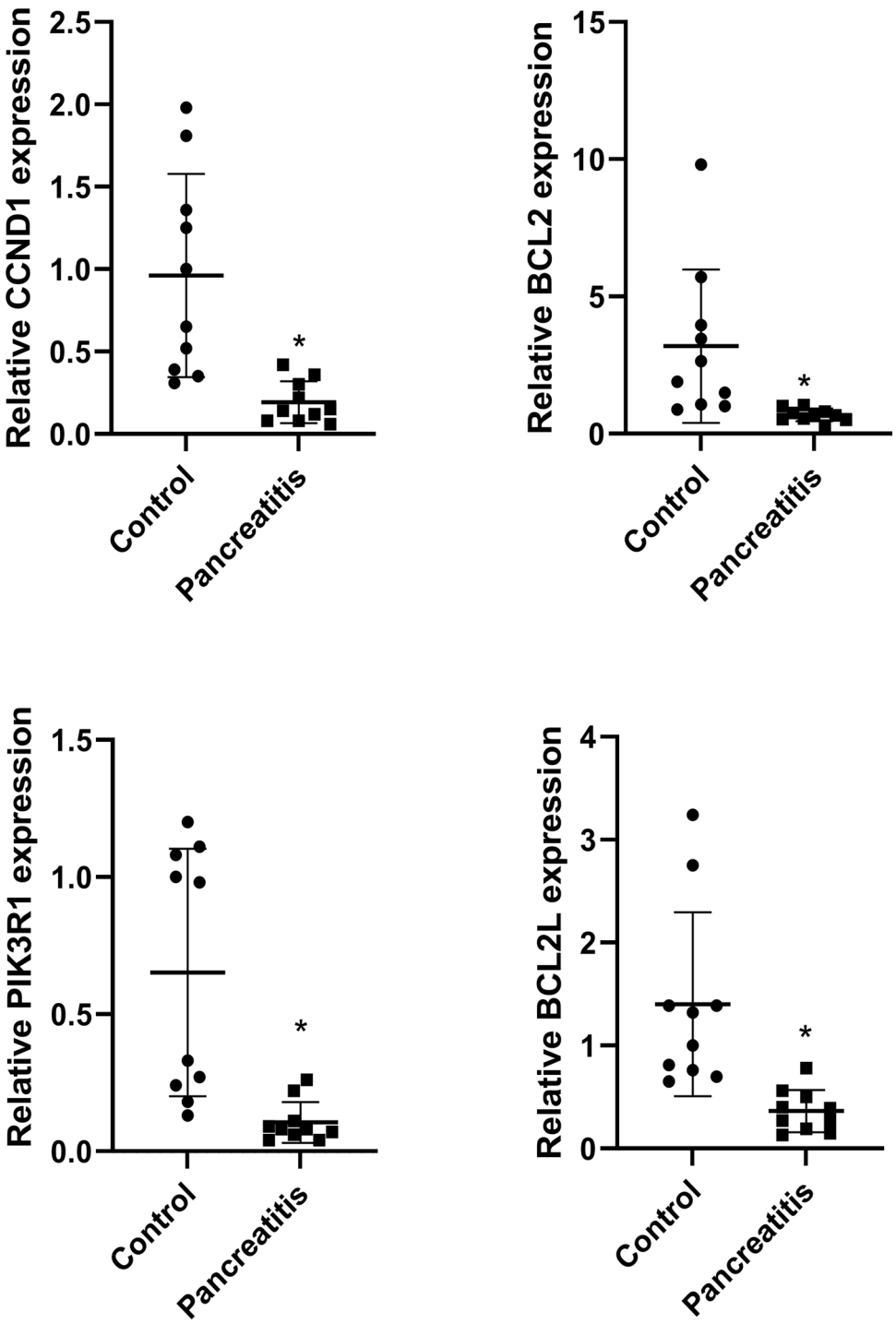


Fig. 8 Validation of the expression levels of hub genes in pancreatitis patients using qPCR. $^{*}P < 0.05$

hsa-miR-15a may participate in the pathogenesis of pancreatitis by targeting CCND1. In the ceRNA network in this study, it was also found that hsa-miR-195-5p and hsa-miR-15a-5p may participate in the occurrence and development of pancreatitis through the regulation of ERS-related hub genes. Therefore, our results can provide ideas for future research on the specific pathogenesis of pancreatitis. Moreover, we predicted 154 potential compounds most likely to target hub genes through the NetworkAnalyst website. However, further extensive research is warranted to illuminate the relationship and potential effects of their targeting actions.

Conclusion

In conclusion, this study identified four hub genes related to ERS and concluded that these hub genes have good diagnostic performance for pancreatitis. In addition, hub genes BCL2 and PIK3R1 were significantly correlated with PRGs and immune-related functions. Finally, the ceRNA network demonstrated a potential mechanism of the hub gene in pancreatitis. The prediction of potential protein compounds provides potential drug applications for the clinical treatment of pancreatitis. These findings are of utmost help in gaining a deeper interpretation of the role of ERS in the development of pancreatitis. At the same time, they are expected to offer effective guidance for innovating novel biomarkers for pancreatitis. However, certain limitations persist in this study. Firstly, the data collected from pancreatitis patients and ERSRGs is limited, which restricts the screening range of hub genes, awaiting an expanded scope of data in future to further validate the reliability and universality of these genes. Secondly, the sample types in the dataset selected in this study are inconsistent, which may have a certain impact on the result analysis. Therefore, it is necessary to expand the amount of data collected and unify the types of samples to further verify the reliability and universality of these genes. Thirdly, this study mainly employed the bioinformatics method for analysis and prediction, failing to conduct a large number of wet experiments to verify the accuracy and credibility of these results. In future, we will continue to dig out the pathways of these hub genes to reveal their potential mechanisms in pancreatitis. Fourth, future studies may need to take different pathologic factors into consideration. For example, the effects of ERS in pancreatitis may vary with different severity. Fifth, hub genes BCL2 and PIK3R1 show excellent diagnostic and immunologic relevance.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12033-025-01388-7.

Author Contributions Huiwei Ye participated in the design and interpretation of the data and drafting the manuscript. Laifa Kong performed the statistical analysis and revised the manuscript critically. All the authors read and approved the final manuscript.

Funding This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data Availability The data and materials in the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interests The authors have no conflicts of interest to declare.

Ethical Approval The research was executed under the Helsinki Declaration and approval from the Affiliated Jinhua Hospital, Zhejiang University School of Medicine Ethics Committee.

References

1. Xiao, A. Y., Tan, M. L., Wu, L. M., Asrani, V. M., Windsor, J. A., Yadav, D., & Petrov, M. S. (2016). Global incidence and mortality of pancreatic diseases: A systematic review, meta-analysis, and meta-regression of population-based cohort studies. *Lancet Gastroenterol Hepatol, 1*, 45–55.
2. Peery, A. F., Crockett, S. D., Murphy, C. C., Jensen, E. T., Kim, H. P., Egberg, M. D., Lund, J. L., Moon, A. M., Pate, V., Barnes, E. L., Schlusser, C. L., Baron, T. H., Shaheen, N. J., & Sandler, R. S. (2022). Burden and Cost of Gastrointestinal, Liver, and Pancreatic Diseases in the United States: Update 2021. *Gastroenterology, 162*, 621–644.
3. (2021) Incorrect Positive Predictive Values Reported. JAMA 325, 2405.
4. Collaborators, G. B. D. C., & o. D. (2018). Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet, 392*, 1736–1788.
5. Machicado, J. D., Gougol, A., Stello, K., Tang, G., Park, Y., Slivka, A., Whitcomb, D. C., Yadav, D., & Papachristou, G. I. (2017). Acute Pancreatitis Has a Long-term Deleterious Effect on Physical Health Related Quality of Life. *Clinical Gastroenterology and Hepatology, 15*(1435–1443), e1432.
6. Vipperla, K., Papachristou, G. I., Easler, J., Muddana, V., Slivka, A., Whitcomb, D. C., & Yadav, D. (2014). Risk of and factors associated with readmission after a sentinel attack of acute pancreatitis. *Clinical Gastroenterology and Hepatology, 12*, 1911–1919.
7. Ahmed Ali, U., Issa, Y., Hagenaars, J. C., Bakker, O. J., van Goor, H., Nieuwenhuijs, V. B., Bollen, T. L., van Ramshorst, B., Witteman, B. J., Brink, M. A., Schaapherder, A. F., Dejong, C. H., Spanier, B. W., Heisterkamp, J., van der Harst, E., van Eijck, C. H., Besselink, M. G., Gooszen, H. G., van Santvoort, H. C., Boermeester, M. A. and Dutch Pancreatitis Study, G. (2016). Risk of Recurrent Pancreatitis and Progression to Chronic Pancreatitis After a First Episode of Acute Pancreatitis. *Clinical Gastroenterology and Hepatology, 14*, 738–746.
8. Liu, J., Gao, M., Nipper, M., Deng, J., Sharkey, F. E., Johnson, R. L., Crawford, H. C., Chen, Y., & Wang, P. (2019). Activation of the intrinsic fibroinflammatory program in adult pancreatic acinar cells triggered by Hippo signaling disruption. *PLoS Biology, 17*, e3000418.
9. Strum, W. B., & Boland, C. R. (2023). Advances in acute and chronic pancreatitis. *World Journal of Gastroenterology, 29*, 1194–1201.
10. Yadav, D., & Whitcomb, D. C. (2010). The role of alcohol and smoking in pancreatitis. *Nature Reviews. Gastroenterology & Hepatology, 7*, 131–145.
11. Hart, P. A., & Conwell, D. L. (2020). Chronic Pancreatitis: Managing a Difficult Disease. *American Journal of Gastroenterology, 115*, 49–55.
12. Tandan, M., Reddy, D. N., Talukdar, R., Vinod, K., Santosh, D., Lakhtakia, S., Gupta, R., Ramchandani, M. J., Banerjee, R., Rakesh, K., Varadaraj, G., & Rao, G. V. (2013). Long-term clinical outcomes of extracorporeal shockwave lithotripsy in painful chronic calcific pancreatitis. *Gastrointestinal Endoscopy, 78*, 726–733.
13. Yin, M., Liu, L., Gao, J., Lin, J., Qu, S., Xu, W., Liu, X., Xu, C., & Zhu, J. (2023). Deep learning for pancreatic diseases based on

endoscopic ultrasound: A systematic review. *International Journal of Medical Informatics, 174*, 105044.
14. Xiang, H., Yu, H., Zhou, Q., Wu, Y., Ren, J., Zhao, Z., Tao, X., & Dong, D. (2022). Macrophages: A rising star in immunotherapy for chronic pancreatitis. *Pharmacological Research, 185*, 106508.
15. Gukovskaya, A. S., Gukovsky, I., Algul, H., & Habtezion, A. (2017). Autophagy, Inflammation, and Immune Dysfunction in the Pathogenesis of Pancreatitis. *Gastroenterology, 153*, 1212–1226.
16. Oakes, S. A., & Papa, F. R. (2015). The role of endoplasmic reticulum stress in human pathology. *Annual Review of Pathology: Mechanisms of Disease, 10*, 173–194.
17. Li, W., Cao, T., Luo, C., Cai, J., Zhou, X., Xiao, X., & Liu, S. (2020). Crosstalk between ER stress, NLRP3 inflammasome, and inflammation. *Applied Microbiology and Biotechnology, 104*, 6129–6140.
18. He, J., Ma, M., Li, D., Wang, K., Wang, Q., Li, Q., He, H., Zhou, Y., Li, Q., Hou, X., & Yang, L. (2021). Sulfiredoxin-1 attenuates injury and inflammation in acute pancreatitis through the ROS/ER stress/Cathepsin B axis. *Cell Death & Disease, 12*, 626.
19. Zhang, C., Niu, H., Wan, C., Yu, X., Xin, G., Zhu, Y., Wei, Z., Li, F., Wang, Y., Zhang, K., Li, S., Dong, Y., Li, Y. and Huang, W. (2022) Drug D, a Diosgenin Derive, Inhibits L-Arginine-Induced Acute Pancreatitis through Meditating GSDMD in the Endoplasmic Reticulum via the TXNIP/HIF-1alpha Pathway. Nutrients 14.
20. Liu, X., Ren, B., Fang, Y., Ren, J., Wang, X., Gu, M., Zhou, F., Xiao, R., Luo, X., You, L., & Zhao, Y. (2024). Comprehensive analysis of bulk and single-cell transcriptomic data reveals a novel signature associated with endoplasmic reticulum stress, lipid metabolism, and liver metastasis in pancreatic cancer. *Journal of Translational Medicine, 22*, 393.
21. Su, J., Peng, J., Wang, L., Xie, H., Zhou, Y., Chen, H., Shi, Y., Guo, Y., Zheng, Y., Guo, Y., Dong, Z., Zhang, X., & Liu, H. (2023). Identification of endoplasmic reticulum stress-related biomarkers of diabetes nephropathy based on bioinformatics and machine learning. *Front Endocrinol (Lausanne), 14*, 1206154.
22. Guo, Z., Yu, X., Fang, Z., Yang, K., Liu, C., Dong, Z., & Liu, C. (2024). The role of endoplasmic reticulum stress-related genes in the diagnosis and subtyping of non-alcoholic fatty liver disease. *General Physiology and Biophysics, 43*, 85–102.
23. Chen, W., Liao, Y., Sun, P., Tu, J., Zou, Y., Fang, J., Chen, Z., Li, H., Chen, J., Peng, Y., Wen, L., & Xie, X. (2024). Construction of an ER stress-related prognostic signature for predicting prognosis and screening the effective anti-tumor drug in osteosarcoma. *Journal of Translational Medicine, 22*, 66.
24. Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research, 43*, e47.
25. Yu, G., Wang, L. G., Han, Y., & He, Q. Y. (2012). clusterProfiler: An R package for comparing biological themes among gene clusters. *OMICS: A Journal of Integrative Biology, 16*, 284–287.
26. Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T., Ramage, D., Amin, N., Schwikowski, B., & Ideker, T. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Research, 13*, 2498–2504.
27. Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., Huerta-Cepas, J., Simonovic, M., Doncheva, N. T., Morris, J. H., Bork, P., Jensen, L. J., & Mering, C. V. (2019). STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research, 47*, D607–D613.
28. Wang, N., Yao, C., Luo, C., Liu, S., Wu, L., Hu, W., Zhang, Q., Rong, Y., Yuan, C., & Wang, F. (2023). Integrated plasma and exosome long noncoding RNA profiling is promising for diagnosing non-small cell lung cancer. *Clinical Chemistry and Laboratory Medicine, 61*, 2216–2228.
29. Franz, M., Rodriguez, H., Lopes, C., Zuberi, K., Montojo, J., Bader, G. D., & Morris, Q. (2018). GeneMANIA update 2018. *Nucleic Acids Research, 46*, W60–W64.
30. Stelzer, G., Rosen, N., Plaschkes, I., Zimmerman, S., Twik, M., Fishilevich, S., Stein, T. I., Nudel, R., Lieder, I., Mazor, Y., Kaplan, S., Dahary, D., Warshawsky, D., Guan-Golan, Y., Kohn, A., Rappaport, N., Safran, M. and Lancet, D. (2016) The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses. Curr Protoc Bioinformatics 54, 1 30 31–31 30 33.
31. Zhou, J., Huang, J., Li, Z., Song, Q., Yang, Z., Wang, L., & Meng, Q. (2023). Identification of aging-related biomarkers and immune infiltration characteristics in osteoarthritis based on bioinformatics analysis and machine learning. *Frontiers in Immunology, 14*, 1168780.
32. Zhu, Y., Chang, S., Liu, J., & Wang, B. (2023). Identification of a novel cuproptosis-related gene signature for multiple myeloma diagnosis. *Immun Inflamm Dis, 11*, e1058.
33. Wu, X., Sui, Z., Zhang, H., Wang, Y., & Yu, Z. (2020). Integrated Analysis of lncRNA-Mediated ceRNA Network in Lung Adenocarcinoma. *Frontiers in Oncology, 10*, 554759.
34. Zhang, Y., Yang, Y. S., Wang, C. M., Chen, W. C., Chen, X. L., Wu, F., & He, H. F. (2023). Copper metabolism-related Genes in entorhinal cortex for Alzheimer's disease. *Science and Reports, 13*, 17458.
35. Wang, M., & Kaufman, R. J. (2016). Protein misfolding in the endoplasmic reticulum as a conduit to human disease. *Nature, 529*, 326–335.
36. He, C., Qiu, Y., Han, P., Chen, Y., Zhang, L., Yuan, Q., Zhang, T., Cheng, T., Yuan, L., Huang, C., Zhang, S., Yin, Z., Peng, X. E., Liang, D., Lin, X., Lin, Y., Lin, Z., & Xia, N. (2018). ER stress regulating protein phosphatase 2A–B56gamma, targeted by hepatitis B virus X protein, induces cell cycle arrest and apoptosis of hepatocytes. *Cell Death & Disease, 9*, 762.
37. Shi, Y., Jiang, B., & Zhao, J. (2024). Induction mechanisms of autophagy and endoplasmic reticulum stress in intestinal ischemia-reperfusion injury, inflammatory bowel disease, and colorectal cancer. *Biomedicine & Pharmacotherapy, 170*, 115984.
38. Yan, C., Ma, Y., Li, H., Cui, J., Guo, X., Wang, G., & Ji, L. (2023). Endoplasmic reticulum stress promotes caspase-1-dependent acinar cell pyroptosis through the PERK pathway to aggravate acute pancreatitis. *International Immunopharmacology, 120*, 110293.
39. Musgrove, E. A. (2006). Cyclins: Roles in mitogenic signaling and oncogenic transformation. *Growth Factors, 24*, 13–19.
40. Wang, D., Zhu, Z. M., Tu, Y. L., Dou, C. Q., Xu, Y., Tan, X. L., Han, M. M., Yang, Z. J., Jin, X., Zhang, B., Cai, S., & Liu, Z. W. (2016). Identfication of key miRNAs in pancreatitis using bioinformatics analysis of microarray data. *Molecular Medicine Reports, 14*, 5451–5460.
41. Biliran, H., Jr., Wang, Y., Banerjee, S., Xu, H., Heng, H., Thakur, A., Bollig, A., Sarkar, F. H., & Liao, J. D. (2005). Overexpression of cyclin D1 promotes tumor cell growth and confers resistance to cisplatin-mediated apoptosis in an elastase-myc transgene-expressing pancreatic tumor cell line. *Clinical Cancer Research, 11*, 6075–6086.
42. Yang, X., Zhang, X., Lin, Z., Guo, J., Yang, X., Yao, L., Wang, H., Xue, P., & Xia, Q. (2020). Chaiqin chengqi decoction alleviates severe acute pancreatitis associated acute kidney injury by inhibiting endoplasmic reticulum stress and subsequent apoptosis. *Biomedicine & Pharmacotherapy, 125*, 110024.

43. Warren, C. F. A., Wong-Brown, M. W., & Bowden, N. A. (2019). BCL-2 family isoforms in apoptosis and cancer. *Cell Death & Disease, 10*, 177.
44. Wang, Z., Liu, J., Wang, Y., Guo, H., Li, F., Cao, Y., Zhao, L. and Chen, H. (2023) Identification of Key Biomarkers Associated with Immunogenic Cell Death and Their Regulatory Mechanisms in Severe Acute Pancreatitis Based on WGCNA and Machine Learning. Int J Mol Sci 24.
45. Xu, H., Yue, H., Ge, H., & Wang, F. (2024). Vitamin B6 ameliorates acute pancreatitis by suppressing the caspase3 signaling pathway. *BMC Gastroenterology, 24*, 151.
46. Feng, D., Park, O., Radaeva, S., Wang, H., Yin, S., Kong, X., Zheng, M., Zakhari, S., Kolls, J. K., & Gao, B. (2012). Interleukin-22 ameliorates cerulein-induced pancreatitis in mice by inhibiting the autophagic pathway. *International Journal of Biological Sciences, 8*, 249–257.
47. Rathinaswamy, M. K., & Burke, J. E. (2020). Class I phosphoinositide 3-kinase (PI3K) regulatory subunits and their roles in signaling and disease. *Adv Biol Regul, 75*, 100657.
48. Hawkins, P. T., & Stephens, L. R. (2015). PI3K signalling in inflammation. *Biochimica et Biophysica Acta, 1851*, 882–897.
49. Fang, Z., Li, J., Cao, F. and Li, F. (2022) Integration of scRNA-Seq and Bulk RNA-Seq Reveals Molecular Characterization of the Immune Microenvironment in Acute Pancreatitis. Biomolecules 13.
50. Lee, B., Namkoong, H., Yang, Y., Huang, H., Heller, D., Szot, G. L., Davis, M. M., Husain, S. Z., Pandol, S. J., Bellin, M. D., & Habtezion, A. (2022). Single-cell sequencing unveils distinct immune microenvironments with CCR6-CCL20 crosstalk in human chronic pancreatitis. *Gut, 71*, 1831–1842.
51. Xue, J., Sharma, V., & Habtezion, A. (2014). Immune cells and immune-based therapy in pancreatitis. *Immunologic Research, 58*, 378–386.
52. Hu, F., Lou, N., Jiao, J., Guo, F., Xiang, H., & Shang, D. (2020). Macrophages in pancreatitis: Mechanisms and therapeutic potential. *Biomedicine & Pharmacotherapy, 131*, 110693.
53. Ishqi, H. M., Ali, M., & Dawra, R. (2023). Recent advances in the role of neutrophils and neutrophil extracellular traps in acute pancreatitis. *Clinical and Experimental Medicine, 23*, 4107–4122.
54. Irie, Y., Tsubota, M., Ishikura, H., Sekiguchi, F., Terada, Y., Tsujiuchi, T., Liu, K., Nishibori, M., & Kawabata, A. (2017). Macrophage-derived HMGB1 as a Pain Mediator in the Early Stage of Acute Pancreatitis in Mice: Targeting RAGE and CXCL12/CXCR4 Axis. *Journal of Neuroimmune Pharmacology, 12*, 693–707.
55. Chen, G., Xu, F., Li, J., & Lu, S. (2015). Depletion of neutrophils protects against L-arginine-induced acute pancreatitis in mice. *Cellular Physiology and Biochemistry, 35*, 2111–2120.
56. Cyster, J. G., & Allen, C. D. C. (2019). B Cell Responses: Cell Interaction Dynamics and Decisions. *Cell, 177*, 524–540.
57. Pietruczuk, M., Dabrowska, M. I., Wereszczynska-Siemiatkowska, U., & Dabrowski, A. (2006). Alteration of peripheral blood lymphocyte subsets in acute pancreatitis. *World Journal of Gastroenterology, 12*, 5344–5351.
58. Xu, Y., Xu, T., Huang, Y., Wan, J., & Jiang, Z. (2024). Silencing hsa_circ_0032449 inhibits the pancreatic differentiation of human embryonic stem cells via the hsa_miR-195-5p/CCND1/PI3K/AKT signaling pathway. *Experimental Cell Research, 434*, 113879.