A new prognostic model for pancreatic cancer based on pyroptosis-related genes

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ABSTRACT

Pancreatic cancer is one of the most common malignant tumors of the digestive tract. It is known as the "king of cancer" in the field of cancer, and is one of the worst prognosis malignant tumors. Pyroptosis is a kind of programmed cell death, which can promote the inflammatory response of cells. Studies have shown that the effect of pyroptosis-related genes in cancer is significant. However, the role of pyroptosis in pancreatic cancer is not clear. The aim of this study is to establish a prognostic model based on pyroptosis. The gene expression and clinical data of pancreatic cancer patients were obtained from TCGA and verified in GEO. The differential expression of 33 pyroptosis-related genes in pancreatic cancer and normal tissues was analyzed, of which 6 genes were up-regulated and 12 genes were down regulated. Then, it was analyzed that pyroptosis-related genes were mainly enriched in the defense against bacteria and pyroptosis pathways. A concise and reliable model is established by lasso-cox regression analysis. Km curve shows that there are differences between high-risk group and low-risk group. And the nomogram has reliable prediction ability. In conclusion, pyroptosis an important role in pancreatic cancer, which can be used for the prediction of pancreatic cancer and provide a new perspective for the treatment of pancreatic cancer.

AUTHOR APPROVALS

The authors have seen and approved the manuscript, and that it hasn't been accepted or published elsewhere.

COMPETING INTERESTS

The authors declare no competing interests.

INTRODUCTION

Pancreatic cancer remains one of the deadliest malignancies, recording 432,242 new deaths in 2018, with 458,918 new pancreatic cancer cases reported globally[1]. Pancreatic cancer incidence and mortality rate are nearly equivalent and the 5-year survival rate is estimated at only 1%[2]. Most patients already have advanced disease at the time of diagnosis. Currently, treatment of pancreatic cancer is dependent on surgical resection. However, up to 80% of pancreatic cancers are unresectable for their highly malignant and early metastasis[3]. Even after resection of pancreatic cancer, the prognosis of pancreatic cancer is poor[4]. Therefore, it is important to establish a good prognostic model.

Pyroptosis, also known as cellular inflammatory necrosis, is a novel form of programmed cell death[5]. Studies have shown that the serine protease granzyme A released by lymphocytes such as cytotoxic T lymphocytes (CTLs) and NK cells can

enter tumor cells through perforin on the surface of tumor cells, specifically and efficiently cut GSDMB protein, resulting in tumor cell scorch[6]. Caspase-1–deficient mice demonstration increased colonic epithelial cell proliferation in early stages of injury-induced tumor formation and reduced apoptosis in advanced tumors[7]. These studies have proved that pyroptosis plays a dual role in tumors.

To further improve the effectiveness of pancreatic cancer treatment and formulate accurate treatment strategies, oncologists need to identify the prognosis of patients with pancreatic cancer. At present, there is no prognostic model of pyroptosis gene in pancreatic cancer. Therefore, exploring the related molecular biomarkers of pyroptosis would have an attractive value in estimating and treating pancreatic cancer, which may be an important therapeutic trend of pancreatic cancer.

METHODS

Data acquisition and pretreatment of pancreatic cancer

We downloaded the RNA sequencing data and clinical data of 178 pancreatic cancer patients from TCGA in August 19, 2021. In addition, two sets of pancreatic cancer RNA-Seq data and corresponding clinical information were downloaded in GEO as external validation cohort (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi> , ID:GSE71729; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi>, ID: GSE57495) . Two sets of data in GEO are extracted, annotated and normalized by ((<https://www.omicstudio.cn/tool>)). The gene expression matrix after the combination of GSE71729 and GSE57495 was obtained.

Identification of Differential Expression pyroptosis-related genes and Enrichment Analysis

We analyzed differential genes in 178 patients in the TCGA cohort and 167 normal tissues in the GTEX cohort using the *deseq2* package. The thresholds were adjusted $P < 0.05$ and $|\log FC| > 1.2$. The volcano map and heat map of differentially expressed genes were drawn by LiancHuan cloud tool, and the protein-protein interaction (PPI) was drawn by string database (<https://string-db.org/>) , the box diagram is drawn by package R (*ggpubr*). The differentially expressed genes were enriched by GO and KEGG by R software. The R packages used include *go*: *goplot*, KEGG: *clusterProfiler*, *org.hs.eg.db*, *enrich plot* and *ggplot2*. In addition, we also did GASE enrichment analysis.

Construction of pyroptosis-related genes risk score and prognosis model

We first made a single factor COX regression analysis of genes related to focal burn. The significant filtering standard was $p\text{Filter}=0.05$ (R package: *survival*). After preliminary screening, we excluded genes associated with pancreatic cancer prognosis. After that, the prognosis model was constructed by lasso regression analysis, and through scoring formula Risk score = $\sum_{i=1}^n x_i y_i$ (x is the coef value obtained by lasso regression analysis and Y is the gene expression), and the risk score was given to the patients. According to the median of risk, the patients in the TCGA cohort were divided into high risk group and low risk group. Similarly, the patients in the GEO cohort were divided into high-risk group and low risk group according to the median TCGA queue risk. The “*survival*” package of R software was used for Kaplan–Meier survival analysis. The predictive accuracy of each gene and the risk score were evaluated by performing time receiver-operating characteristic (ROC) analysis. We extracted the clinical information (age, grade, rade, stage, T and N) of patients in the TCGA cohort .These variables were analysed in combination with the risk score in our regression model. Univariate and multivariable Cox regression models were employed for the analysis.

The Construction of Nomogram and Calibration Curves

A clinical practical nomogram was established to predict individual survival probability by the “rms” package of R software. To assess the consistency between actual and predicted survival in the nomogram, calibration curves for predicting 1-, and 3-year survival rate were also drawn. The prediction ability of signature was assessed by area under ROC curve.

RESULTS

Screening and identification of differentially expressed genes related to PAAD

We obtained 178 PAAD patients from TCGA and 167 normal tissues from GTEx. The differential expression of 33 pyroptosis-related genes was analyzed by r-packet (deseq2). It was found that 18 pyroptosis-related genes were differentially expressed ($P < 0.05$). Obtain volcano map ($|\log_2(\text{fold change})| > 1.2$, $\text{FDR } p\text{-value} < 0.05$) on LianChuan biological cloud platform (Figure.1A). Six genes were up-regulated and 12 genes were down regulate. The RNA expression levels of these genes are shown in the figure. To further explore the interactions of these pyroptosis-related genes, we conducted a protein–protein interaction (PPI) analysis, and the results are shown. The minimum required interaction score for the PPI analysis was set at 0.4 (medium confidence) (Figure.1B). At the same time, the heat map also proves that 6 genes are adjusted, and 12 genes are lowered (Figure.1C). At the same time, we also made a mutation of the focal pyroptosis-related genes related to PAAD. The results showed that the expression of the pyroptosis-related genes was more valuable than the mutation (Figure.1E).

GO and KEGG pathway analysis

In order to further understand the function of genes related to pyroptosis, we analyzed these genes by go and KEGG. We found that these 33 cases were mainly related to the formal regulation of cytokine production and the defense response to bacteria (Figure.2A). In addition, KEGG pathway analysis showed that pyroptosis-related genes were involved in platinum drug resistance, apoptosis-multiple species, ErbB signaling pathway and apoptosis (Figure.2B).

Construction of differential gene prediction model for pancreatic cancer

A total of 170 patients with pancreatic cancer who had survival data were analyzed by univariate regression analysis. We screened 9 genes related to blepharoptosis based on P value less than 0.05 (IL18, GSDMC, NLRP2, PLCG1, GPX4, CASP8, PRKACA, NLRP1 and CASP4). Among them, and higher expressions of IL18, GSDMC, NLRP2, CASP8, CASP4 were associated with increased risk ($\text{HR} > 1$), while upregulated expressions of PLCG1, GPX4, PRKACA, NLRP1 were correlated with lower risk ($\text{HR} < 1$) (Figure.3A). Subsequently, LASSO Cox regression analysis retrieved 5 genes for prognostic model construction based on the optimum λ value (Figure.3C, 3D).

Establishment of prognostic model in TCGA cohort

In order to better construct the prognostic model related to pyroptosis genes. We calculated the risk score for each patient according to the following formula and divide 170 patients into high-risk group($n = 85$) and low-risk group ($n = 85$) according to the median value of risk score (Figure.4A). Compared with the low-risk group, the high-risk group had more deaths and shorter survival time. And risk of death was significantly increased in the high-risk group (Figure. 4C). IL18, CASP4, GSDMC and NLRP2 were up-regulated in high-risk group and NLRP1 was down regulated in high-risk group (Figure. 4D). The Kaplan–Meier curve

demonstrated that patients in the high-risk group have a poorer prognosis ($P < 0.05$, Figure.4B).

External validation of the risk signature

To verify the accuracy of the prognostic model established by TCGA, we obtained 186 pancreatic cancer patients from GEO and made a risk score with the same formula. Based on the median risk score in the TCGA cohort, 99 patients in the GEO cohort were classified into the low-risk group, while the other 87 patients were classified into the high-risk group (Figure. 5A). Patients in the low-risk subgroup (Figure. 5C, on the left side of the dotted line) were found to have longer survival times and lower death rates than those in the high-risk subgroup. IL18, CASP4, GSDMC and NLRP2 were up-regulated in high-risk group and NLRP1 was down regulated in high-risk group (Figure. 5D). In addition, Kaplan–Meier analysis also indicated a significant difference in the survival rate between the low-risk and high-risk groups ($P < 0.05$, Figure. 5B). The results are the same as those in the TCGA cohort.

Independent prognostic analysis of TCGA

In order to verify whether the prognostic model we constructed can be independent of other clinical traits as an independent prognostic factor, univariate and multivariate Cox regression analyses were performed. Univariate Cox results showed that age, N stage and risk score were correlated with survival ($P < 0.05$), and risk score was significantly correlated with survival status ($P < 0.001$, Figure. 6A). Multivariate Cox regression analysis showed that the risk score was significantly correlated with overall survival ($P < 0.001$, Figure. 6B). The results showed that risk score was an independent prognostic index.

Relationship between pyroptosis-related genes expression and clinical information

Nlrp1 (Figure. 7A, $p=0.022$) was negatively correlated with grade, N (Figure. 7B, $p=0.022$) and T stage (Figure. 7C, $p=0.046$). Nlrp2 was positively correlated with grade (Figure. 7D, $p=0.022$). GSDMC was negatively correlated with stage (Figure. 7E, $p=0.010$). CASP4 was positively correlated with grade (Figure. 7F, $p=0.012$). Risk scores were positively correlated with grade (Figure. 7G, $p=5.002e-04$).

Construction and Validation of the Nomogram

In order to predict the prognosis of pancreatic cancer more accurately, we used clinical information and risk scores to make a nomogram. The survival probability of pancreatic cancer patients in 1 and 3 years is evaluated. The nomogram showed that our risk score was the most important factor among the various clinical parameters (Figure. 8A). In order to determine the accuracy of the constructed nomogram, we constructed the calibration curve, and the calibration curve showed that the prediction of the line map was consistent with the actual survival of the patients of pancreatic cancer (Figure. 8B, C). Finally, the predictive abilities of the nomogram were analyzed by the AUC values (AUC of 1-year OS=0.664, Figure 8D).

Gene Set Enrichment Analysis

Finally, we performed gene enrichment analysis between high-risk and low-risk groups. The results showed that the main enrichment pathway of high-risk group was apoptosis, tight junction, spliceosome, axon guidance, natural killer cell mediated cytotoxicity. The main enrichment pathways of low risk are neuroactive

ligand receptor interaction , taste transduction , autoimmune thyroid disease , chemokine signaling pathway, focal adhesion (Figure 9A) .

Single gene Kaplan Meier curve

In addition, we also constructed a single gene model. The results showed that there was a significant difference between NLRP1 and NLRP2 ($P < 0.05$, Figure 10C,D).

There was no significant difference in GSDMC, IL18 and CASP4 ($P > 0.05$, Figure 10A, B, E).

DISCUSSION

Pyroptosis is a kind of caspase-1 or caspase-11-dependent programmed cell death[8, 9]. In recent years, there are more and more studies on cell pyroptosis, and the role of pyroptosis in cancer cells is gradually clear. Studies have shown that pyroptosis genes play different roles in different cancers. Cell death releases inflammatory factors, which provides a suitable living environment for tumor cells[10]. Pyroptosis can promote tumour cell death, making pyrolysis a potential prognostic and therapeutic target for cancer[11]. However, the role of pyroptosis gene in pancreatic cancer is not clear. The purpose of this study is to elucidate the role of pyroptosis gene in pancreatic cancer.

In recent years, with the rapid development of cancer biology knowledge, there are many researches on pancreatic cancer metastasis, new biomarkers, and new prognostic markers[12-16]. These provide good prognostic models for pancreatic cancer patients. Compared with these studies, our research mainly demonstrates the effect of differential expression of pyroptosis genes on pancreatic cancer patients. Although there is a certain gap with other prognostic models, the results suggest that the pyroptosis gene has a good prognosis in pancreatic cancer patients.

We constructed a prognostic risk model of 5 genes (IL18, CASP4, NLRP1, NLRP2, GSDMC) through lasso Cox regression analysis, and different risk groups have different survival results. This result has also been verified in the validation group, which proves the effectiveness of the model. The nomogram also well predicted the 1-year and 3-year overall survival rates.

We found that the expression of IL18, CASP4, GSDMC and NLRP2 in high-risk group was higher than that in low-risk group, but the expression of these genes was different in other diseases. For example, the lack of IL18 can lead to seizures in patients with Alzheimer's disease [17]. High expression of CASP4 will lead to poor prognosis in patients with renal cell carcinoma[18], but low expression of CASP4 will lead to poor prognosis in patients with esophageal squamous cell carcinoma[19]. Overexpression of GSDMC inhibits TGFB β 2 (transforming growth factor β receptor type ii) promotes the proliferation of colorectal cancer cells and leads to poor prognosis in patients with lung adenocarcinoma[20, 21]. This shows that the same gene plays different roles in different diseases.

It is worth mentioning that in the study, we found that NLRP1 is one of the important genes. NLRP1 is a key regulator of the innate immune system. Patients with vitiligo

increase interleukin-1 through NLRP 1 inflammatory corpuscles β the high expression of [22]. NLRP1 can aggravate inflammatory bowel disease by limiting the production of butyric acid [23]. The high expression of NLRP1 can cause psoriasis [24]. In addition, NLRP1 is also closely related to type 1 diabetes [25]. These studies indicate that high expression of NLRP1 aggravates the disease, but in our study, high expression of NLRP1 is more beneficial to patients. This shows that NLRP1 plays different roles in different cancers. Although NLRP1 has been widely studied, the role of NLRP1 in pancreatic cancer is limited. In our research, NLRP1 is one of the biomarkers of prognosis in pancreatic cancer.

We analyzed the effect of single gene on the survival rate of pancreatic cancer patients. We found that NLRP1 gene had a significant effect on pancreatic cancer patients. Moreover, the prognosis of pancreatic cancer is better when NLRP1 is overexpressed. This is consistent with the results of figure 4D and figure 5D, indicating that the role of NLRP1 in pancreatic cancer deserves further study.

However, our study has potential limitations. First, we do not have enough clinical data to verify the expression of M stage in the prognostic model. Secondly, the model we established needs to be further verified by in vitro and in vivo experiments to increase the reliability of the prognostic model.

In conclusion, we constructed a prognostic risk model for pancreatic cancer using 5 differentially expressed pyroptosis genes, which can accurately predict the prognosis of patients with pancreatic cancer. It is demonstrated that pyroptosis gene is one of the biomarkers of survival rate in pancreatic cancer patients.

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Figure.1 The differentially expressed pyroptosis-related genes between lung adenocarcinoma and normal tissues. (A) Volcanic map representation differentially expressed pyroptosis-related genes, with red dots indicating high expression and blue dots indicating low expression. (B) Protein-protein interactions (PPI) network showing the interactions of the pyroptosis-related genes (interaction score=0.4). (C) Heatmap of differentially expressed pyroptosis-related genes, with red indicating high expression and green indicating low expression. (D) Box plot of DEAGs, with the red boxes representing the tumor group and blue boxes representing the normal group. Differential expression of the 18 selected genes between normal and pancreatic cancer tissues. (E) Differential somatic mutation analysis between high-risk group and low-risk group in The Cancer Genome Atlas (TCGA) cohort.

Figure.2 Functional enrichment analyses of gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). (A) Bubble graph for GO enrichment (the bigger bubble means the more genes enriched, and the increasing depth of red means the differences were more obvious; q-value: the adjusted p-value). (B) The circular scatter plot of enriched KEGG terms.

Figure.3 Forest map of heat sag related genes in patients with pancreatic cancer. And, construction of pyroptosis-related gene risk signature in TCGA cohort. (A) Univariate Cox regression analysis. (C) Cross-validation for tuning the parameter selection in the LASSO regression. (D) LASSO regression of the 9 OS-related genes.

Figure.4 Construction of risk signature in the TCGA cohort. (A) Distribution of patients based on the risk score. (B) Kaplan–Meier curves for the overall survival of patients in the high- and low-risk groups. (C) Survival status of patients with pancreatic cancer. (D) Expression of five pyroptosis-related risk genes.

Figure.5 Validation of the risk model in the GEO cohort. (A) Distribution of risk score. (B) Kaplan–Meier curves for the overall survival of patients in the high- and low-risk groups. (C) Survival status of pancreatic cancer patients in GEO cohort. (D) Expression heat map of 5 differential expression of pyroptosis-related genes.

Figure.6 Independent prognostic analysis. (A) Univariate independent prognostic analysis. (B) Multivariate independent prognostic analysis.

Figure.7 Clinicopathological significance of pyroptosis-related genes and risk

score in pancreatic cancer. (A,B,C) Clinicopathological correlation of NLRP1 in pancreatic cancer. (D) Clinicopathological correlation of NLRP2 in pancreatic cancer. (E) Clinicopathological correlation of GSDMC in pancreatic cancer. (F) Clinicopathological correlation of CASP4 in pancreatic cancer. (E) Clinicopathological correlation of risk score in pancreatic cancer.

Figure.8 Nomogram to predict the probability of patients with pancreatic cancer. (A) A nomogram integrating the signature risk score with the pathologic characteristics. (B, C) The calibration plots for predicting patient 1-, 3-year OS. (D) The ROC curve of OS for risk score, age, gender, stage, N stage and T stage at 1- year.

Figure.9 Multi GSEA enrichment map. (A) GSEA analysis of the differentially expressed genes between high- and low-risk groups.

Figure.10 Single gene Kaplan Meier curve. (A,B,C,D,E,) Single gene Kaplan Meier curve of GSDMC, IL18, NLRP 1, NLRP 2 and CASP4.

A

C