RESEARCH ARTICLE

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To Establish a Nomogram Prediction of Prostate Cancer Based on Pyroptosis-Related Genes that Affect the Immune Microenvironment

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Abstract

Background: Prostate cancer is the most common tumor in men worldwide with a poor prognosis. In recent years, studies have revealed that pyroptosis can affect the tumor immune microenvironment. However, the relationship between the immune microenvironment regulated by pyroptosis-related genes and the prognosis of prostate cancer is still unclear. **Methods:** Thirty-three cell death-associated genes were selected from a literature review. The "DESeq2" R package was used to identify differentially expressed cell death-associated genes between normal prostate tissue (GTEx) and prostate cancer tissue (TCGA) samples. Biological functional enrichment analysis of differentially expressed cell death genes was performed using R statistical software packages, such as "clusterProfiler," "org.Hs.eg.db," "enrichplot," "ggplot2," and "GOplot." Univariate Cox and LASSO Cox regression analyses were conducted to identify prognostic genes associated with the immune microenvironment using the "survival" package. Finally, a predictive model was established based on Gleason score, T stage, and cell death-associated genes.odel was established based on Gleason score, T stage, and cell death-associated genes. **Results:** Seventeen differentially expressed genes related to pyroptosis were screened out. Based on these differentially expressed genes, biological function enrichment analysis showed that they were related to pyroptosis of prostate cells. Based on univariate Cox and (LASSO) Cox regression analysis, four pyroptosis-related genes (CASP3, PLCG1, GSDMB, GPX4) were determined to be related to the prognosis of prostate cancer, and the immune correlation analysis of the four pyroptosis-related genes was performed. The expression of CASP3, PLCG1 and GSDMB was positively correlated with the proportion of immune cells, and the expression of GPX4 was negatively correlated with the proportion of immune cells. A predictive nomogram was established by combining Gleason score, T and pyroptosis genes. The nomogram was accompanied by a calibration curve and used to predict 1 -, 2 -, and 5-year survival in PAAD patients. **Conclusion:** Cell death-associated genes (CASP3, PLCG1, GSDMB, GPX4) play crucial roles in modulating the immune microenvironment and can be used to predict the prognosis of prostate cancer.

Keywords: Prostate cancer- immune microenvironment- pyroptosis- prognostic model

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Introduction

Prostate cancer (PCa) is the most common cancer in men, accounting for 20% of all male cancers, and it is responsible for 6.8% (1 in 5) of all cancer-related deaths globally [1]. According to global cancer survey statistics, there were approximately 1.41 million new cases of prostate cancer and an estimated 375,000 deaths in 2020. It is projected that by 2040, due to population expansion and aging, the global burden of prostate cancer will increase to 2.43 million new cases and 740,000 deaths [2, 3]. Prostate cancer is considered a "cold" tumor, developing in a slow immunosuppressive environment. Current research efforts are focused on transforming the immune microenvironment of prostate cancer from cold to hot. The tumor microenvironment of prostate cancer plays a crucial role in tumor progression, metastasis, and treatment resistance in the context of endocrine therapy and immunotherapy, and a comprehensive understanding of the biological basis of the prostate tumor microenvironment can help identify new therapeutic targets [4].

Within the tumor immune microenvironment (TIME), there are various immune infiltrating cells. CD8+ cytotoxic T lymphocytes play a role in killing tumor cells, while regulatory T cells suppress T cell activity and promote immunosuppression within the TIME. Studies in mouse models have shown that radiotherapy can upregulate the expression of CD8+ T cells, dendritic cells, and regulatory T cell genes in prostate cancer cells [5]. Typically, M1-type macrophages exhibit pro-inflammatory and anti-tumor effects, but tumor-associated macrophages in

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the TIME are of the M2 type, promoting angiogenesis and tumor invasion through the secretion of Th2 cytokines. B cells, regulatory T cells, M1, and M2 macrophages are selectively enriched in the prostate cancer epithelium. Elevated levels of infiltrating T cells, M1, and M2 macrophages in the stroma and/or epithelium are associated with biochemical recurrence [6, 7] .NK cells are known to release granzyme and perforin to kill target cells, but their killing activity is inhibited by TGF-β enriched in the TIME. In prostate cancer, prostate cancer cells induce the expression of inhibitory receptors and downregulate the expression of the NK cell-activating receptor NKp46 (NCR1). The enrichment of TGF-β in cancer cells inhibits the expression of NKG2D (KLRK1) and CD16 (FCGR3), thereby preventing their recognition of tumor cells [8, 9].

Tumor resistance to apoptosis and immunosuppressed tumor microenvironment are the two main reasons of poor response to tumor treatment, pyroptosis is a lytic and inflammatory programmed cell death pathway different from apoptosis, the latest evidence shows that pyroptosis induction in tumor cells leads to strong inflammatory response and significant tumor regression. As the basis of its antitumor effects, pyroptosis is mediated by the gasdermin protein that promotes pore formation, which promotes immune cell activation and infiltration through the release of proinflammatory cytokines and immunogenic substances after cell breakdown [10]. Current research suggests that cell pyroptosis in tumor has a dual role, can explain that, on the one hand, long-term chronic inflammation can promote the development of tumor, because the inflammation caused by pyroptosis promotes the production and maintenance of inflammatory microenvironment, on the other hand, the acute activation of cell pyroptosis leads to the infiltration of various immune cells, to inhibit the development of tumor [11]. At present, basic research experiments have revealed the relationship between GSDMC, GSDMD, GSDME and TIME, but the association between GSDMA and GSDMB and TIME can only be explored by bioinformatics analysis [12, 13]. Shao Feng et al. [14] also revealed through a novel bioorthogonal system that inflammation caused by pyroptosis triggered a strong antitumor immune function and could act synergistically with checkpoint blockade. Further research found that cytotoxic T lymphocytes and NK cell lymphocytes release serine protease Granzyme A, can enter tumor cells by perforin on the surface of tumor cells, specific and efficient cutting GSDMB protein, causing tumor cell coke, toxic lymphocytes can kill target cells mediated by GSDM family protein, is an important effector mechanism of cellular immunity [15].

More and more studies show that cell pyroptosis by regulating the tumor microenvironment affect cancer progression, the prostate cancer TIME regulation mode no unified research conclusion, need to continue to explore a higher specificity and lower side effects TIME, to ensure memory T cells sustained response, deep understanding of TIME in the development of prostate cancer, find out the break immune suppression microenvironment, help to develop targeted treatment strategy of prostate cancer. Pyroptosis may be a potential way to regulate TIME, based on the influence of TIME to deeply study the role of pyroptosis in the development of prostate cancer and establish a related prognostic model, which is important for the treatment of prostate cancer. To our knowledge, there has been no prognostic model related to PCcancer based on the immune microenvironment to predict the prognosis of prostate cancer patients. In this study, we aimed to establish a prognostic model of prostate cancer patients with a pyroptosis gene associated based on the impact of pyroptosis on the prostate cancer immune microenvironment.

Datasets

The Cancer Genome Atlas (TCGA, https: / / portal. gdc.cancer. Gov) and Genotype-Tissue Expression (GTEx, https: / / www.gtexportal. The org / home /) database.

Materials and Methods

Identification of the differentially expressed cellular pyroptosis-related genes

We extracted 33 genes associated with apoptosis from the reviewed literature, which are listed in Table 1. Due to the lack of normal prostate cancer tissue data in the TCGA cohort, we also considered GTEx data from 100 normal prostate tissue samples to identify DEG between normal and tumor tissue. Prior to comparison, expression data in both datasets were normalized to million fragments per kilobase (FPKM) values. The "limma" software package was used to identify a DEG with a P-value <0.05. The DEG is represented as follows: * if P <0.05, then * * if P <0.01, then * * *, if P <0.001. The PPI network for DEG was performed using Search Interaction Gene Search (STRING) version 11.0 (https: / / string-db. The org /) construct.

Enrichment analysis of genes involved with pyroptosis in differentially expressed cells

By using the "clus-terProfiler", " org.Hs.eg. R statistical software like db "and" enrichplot "analyzed the biological process enrichment of differentially expressed genes", "ggplot2" and "GOplot" packages using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). In addition, gene set enrichment analysis (GSEA) was performed to identify different biological processes and signaling pathways between the high-risk and low-risk groups in prostate cancer. Our reference gene set is derived from the C2 subset (c2. cp.kegg.v7.5.1.symbols.gmt) The dominance threshold was determined by 1000 permutation analysis, and we considered the results significant when the p-value was less than 0.05.

Identification of prognostic genes associated with the immune microenvironment

Our training set consists of 496 prostate cancer tissue samples from TCGA and GTEx samples and 100 normal prostate tissue samples from the database. To investigate the relationship between the expression levels of pyroptosis-related genes and overall survival (PFI) in prostate cancer patients, we performed a univariate Cox regression analysis using the 'survival' package.

Table 1. The Quick Brown fox Jumps Over the Lazy Dog. The quick brown fox jumps over the lazy dog. The quick brown fox jumps over the lazy dog.

	Domains of physical activity			
Age groups	Fox	dog	Leisure time	Total physical activity
15-24	7.5	8.4	6	13
25-34	6.6	6.7	6.7	12.2
35-44	6	6.7	7.5	11.2
45-54	7.5	10.1	5.5	14.5
55-64	6.6	6.7	7.4	14.7
64<	4.4	5	6	11.8
Total	6	6.7	6.2	13.1
P.value†	0.001	0.001	0.001	0.001

A significant filtering criterion was set at p <0.05 for further analysis. Next, we used LASSO Cox regression to eliminate gene collinearity and reduce the number of genes. Finally, we performed a multivariate Cox regression analysis based on the univariate Cox regression.

Construction of the prognostic models

X represents the coefficient of the pyroptosis-related genes in the LASSO Cox regression analysis, and Y represents the expression of the pyroptosis-related genes. Prostate cancer patients were divided into high risk and low risk groups according to the median risk score, and the overall survival (PFI) between these two groups was analyzed. The time ROC package generates receiver operating characteristics (ROC) curves to assess the prognostic efficiency of the model. To make the model more convincing, we performed internal sampling validation using the TCGA database. The expression of the pyroptosis-related genes for each cell was also normalized and the risk score was then calculated by the above formula. Prostate cancer patients in the validation cohort were also divided into high risk and low risk groups based on the median risk score, and OS was compared between the two groups. Next, to determine whether the risk score was an independent prognostic factor for OS in prostate cancer patients in the training set, univariate and multivariate Cox regression analyses were performed.

Construction of the nomogram and the calibration curves

Nomograms were constructed using the'RMS " package of the R software to predict individual survival probabilities and calibration curves were plotted to predict 1-, 2-, and 3-year survival of patients with prostate cancer.

Results

Identification of cellular pyroptosis-related genes differentially expressed in prostate cancer

The detailed workflow of our study is shown in Figure 1. We obtained 496 prostate cancer tissues from TCGA and 100 normal tissues from GTEx. A total of 26 pyroptosis differentially expressed genes were identified using the R package DESeq2 from 33 pyroptosis-related genes based on the cutoff criterion of | log2 (fold change) |> 1.2 and false discovery rate (FDR) <0.05. In prostate cancer, volcano, heat, and boxplots showed that 5 pyroptosis-related genes were significantly downregulated and 12 pyroptosis-related genes were upregulated (Figure 2A, C, D). The protein – protein interaction network of these differentially expressed cellular pyroptosis-related genes is shown in Figure 2B. In addition, many mutations were observed in prostate cancer patients in these differentially expressed pyroptosis-related genes (Figure 2B).

Functional enrichment analysis

To better understand the function of the differentially expressed cellular pyroptosis-related genes, GO and KEGG pathway enrichment analyses were performed. Analysis of GO enrichment showed that these differentially expressed pyroptosis-related genes were mainly related to the formal regulation of cytokine production and defense responses to bacteria (Figure 3A). Furthermore, analysis of KEGG

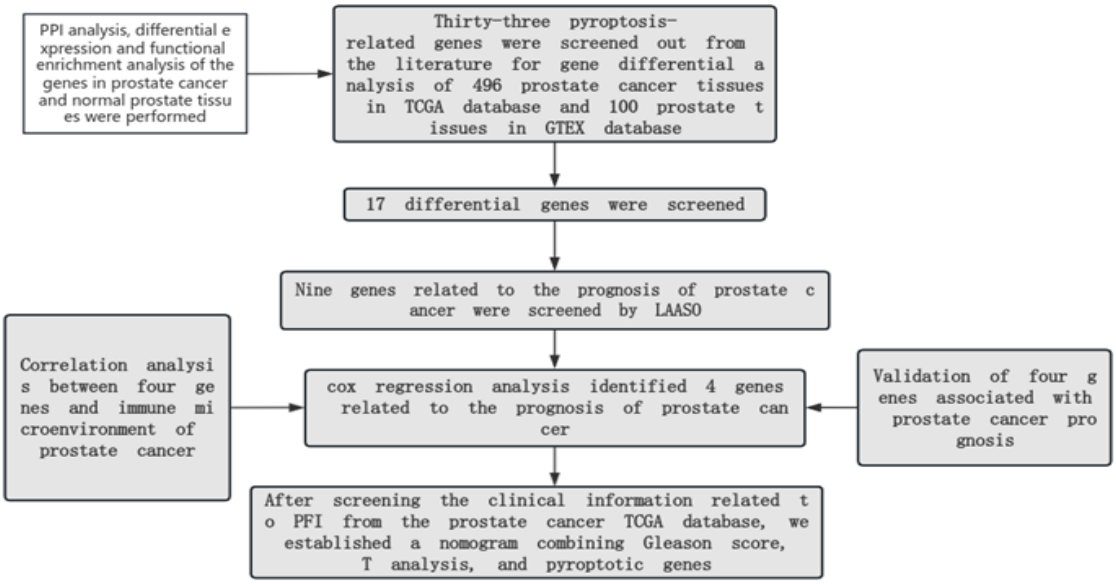

Figure1. Flow Chart of Data Analysis

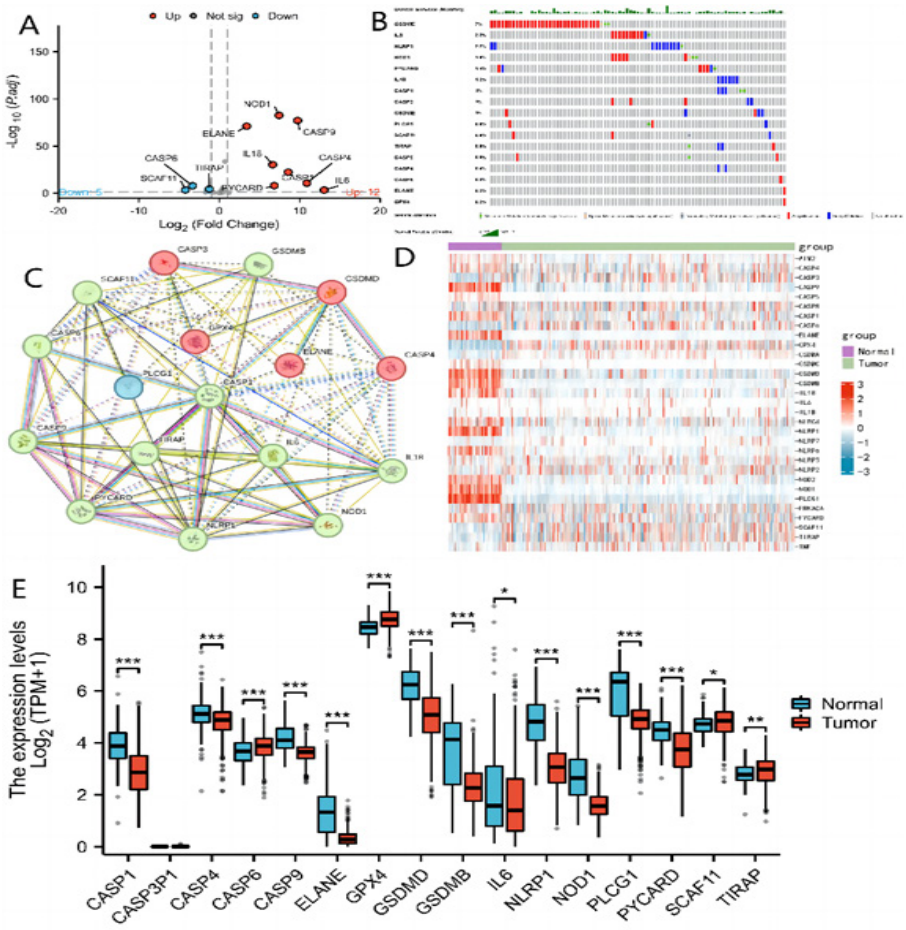

Figure 2. Diferentially Expressed Pyroptosis-Related Genes between Prostate Tissues and Normal Tissues. A.Volcano plot indicates pyroptosis-related genes, with red dots indicating high expression and blue dots indicating low expression. B. Mutation analysis of diferentially expressed pyroptosis-related genes in TCGA ;C.The protein–protein interaction (PPI) network shows the interaction of pyroptosis-related genes . D. Heatmap of diferentially expressed pyroptosis-related genes, with red indicating high expression, blue indicating low expression, n representing normal tissues, and t representing tumor tissues. E. Boxplots of diferentially expressed pyroptosis-related genes, with red boxes representing tumor groups and blue boxes representing normal groups.

showed that these differentially expressed pyroptosis-related genes are involved in platinum resistance, apoptosis multispecies, ERbB signaling pathway and apoptosis (Figure 3B). This suggests that these cellular pyroptosis-related genes are involved in other biological processes other than cellular pyroptosis.

Screening of genes related to pyroptosis and prognosis

As shown in Figure 4A, we used LASSO regression analysis to identify 9 genes, 2 protective genes (CASP 4, CASP 6) and 7 potential risk genes (GSDMD, IL 18, TIRAP, CASP 3, GSDMB, PLCG 1, GPX 4); based on lasso regression, we subsequently performed cox regression analysis and identified four potential risk genes (Figure 4B). On the basis of the univariate Cox regression, we subsequently performed a LASSO regression analysis (Figure 4C, D). Next, we constructed a prognostic cell pyroptosis correlation model using four genes by LASSO

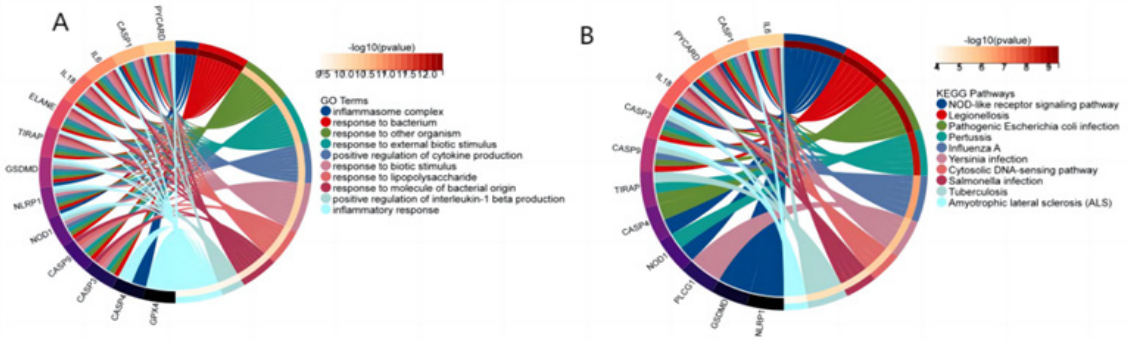

Figure 3. Biological Function Enrichment Analysis of Pyroptosis-related Genes. A,Gene Ontology Enrichment Analysis; B, Kyoto Encyclopedia of Genes and Genomes

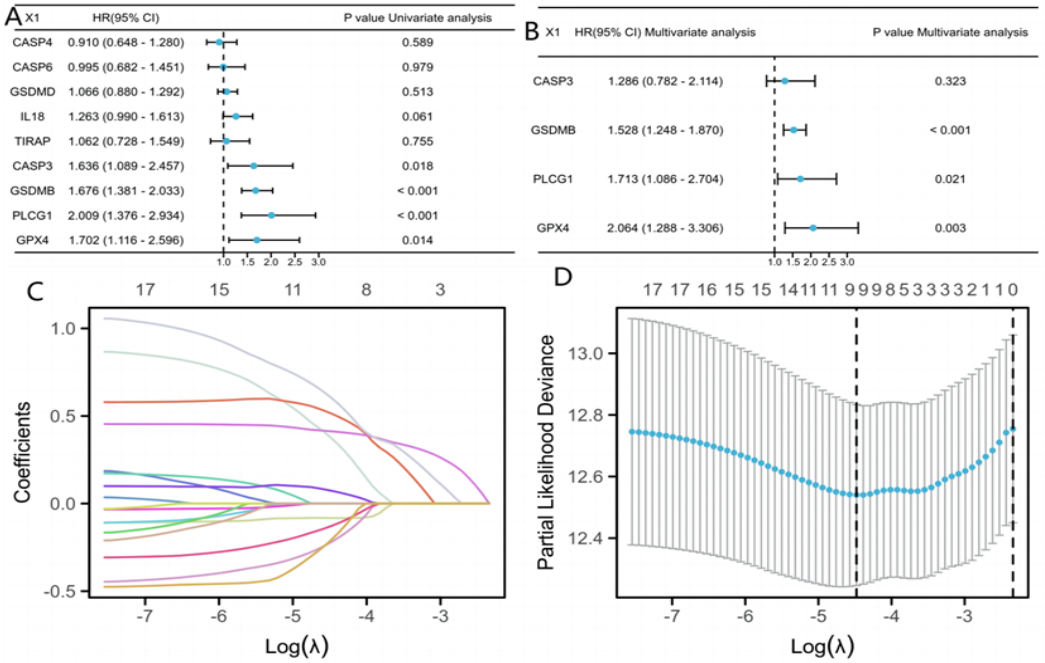

Figure 4. Screening of Prognostic Genes in Prostate Cancer. A.Results of univariate analysis and multivariate analysis.B.Results of multivariate analysis.C.LASS0 analysis coefficient screening plot.D.LASS0 analysis variable trajectory diagram.

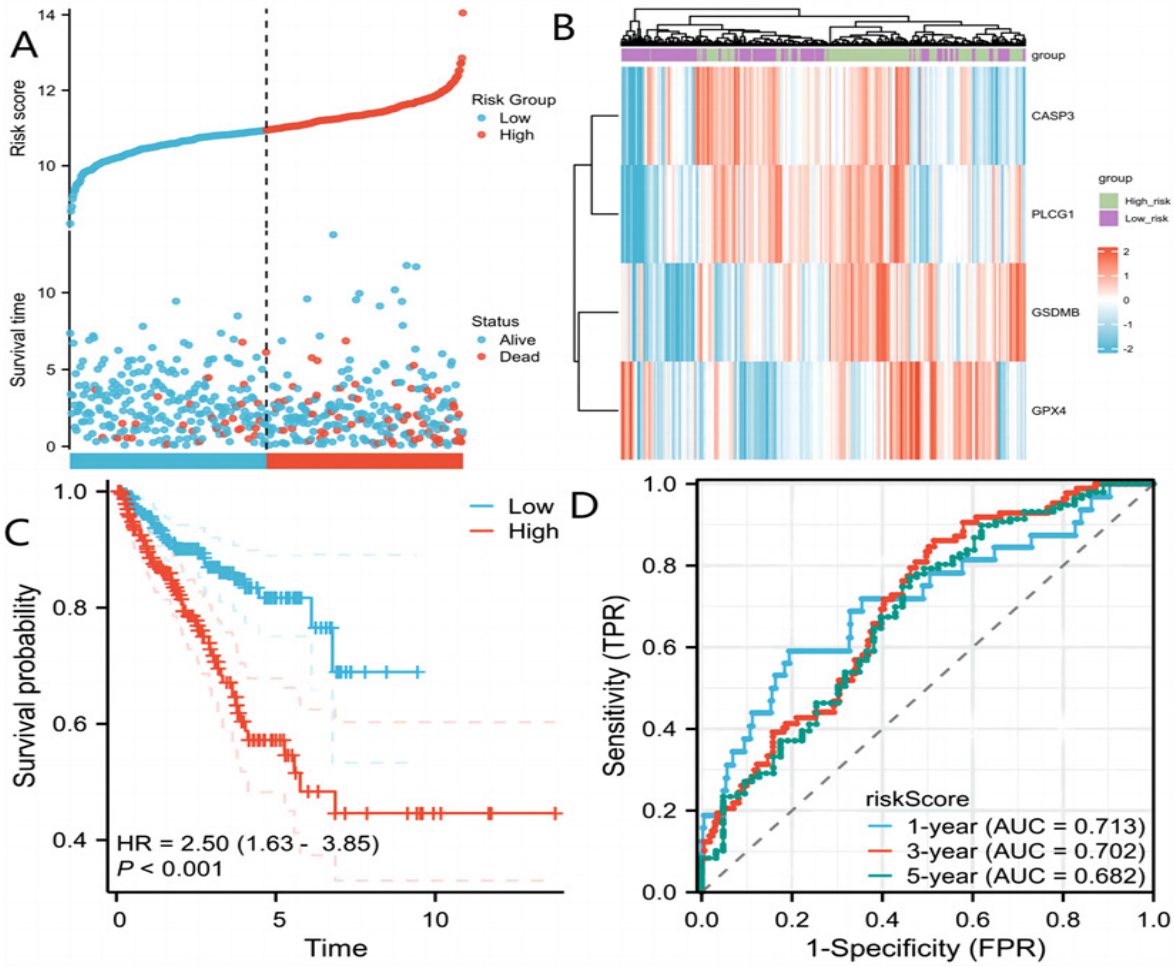

Figure 5. A.Grouping is associated with poor prognosis of prostate cancer patients.B.Heat map of CASP3, PLCG1, GSDMB, and GPX4 in normal and cancer tissues, with high expression in red and low expression in blue. C.The relationship between high-risk group and low-risk group and the survival time of prostate cancer was different. D.The ROC curve of the prediction model for the prognosis of prostate cancer was plotted, and the higher the AUC, the more correct the prediction rate

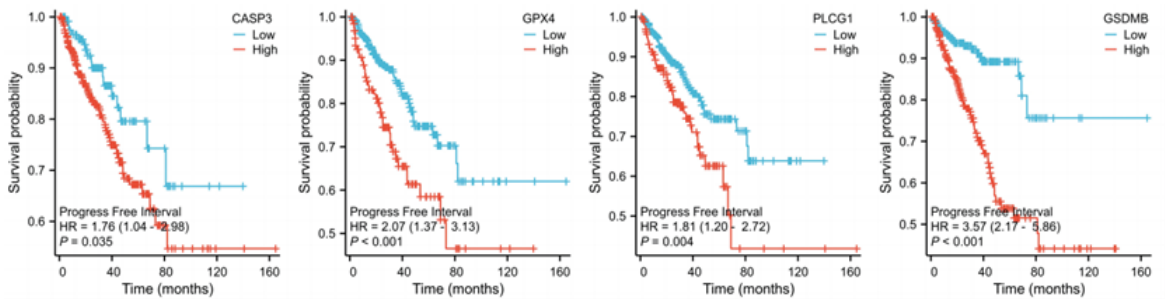

Figure 6. The Effects of CASP3, PLCG1, GSDMB and GPX4 on Survival Time in High-Risk Group and Low-Risk Group were Analyzed

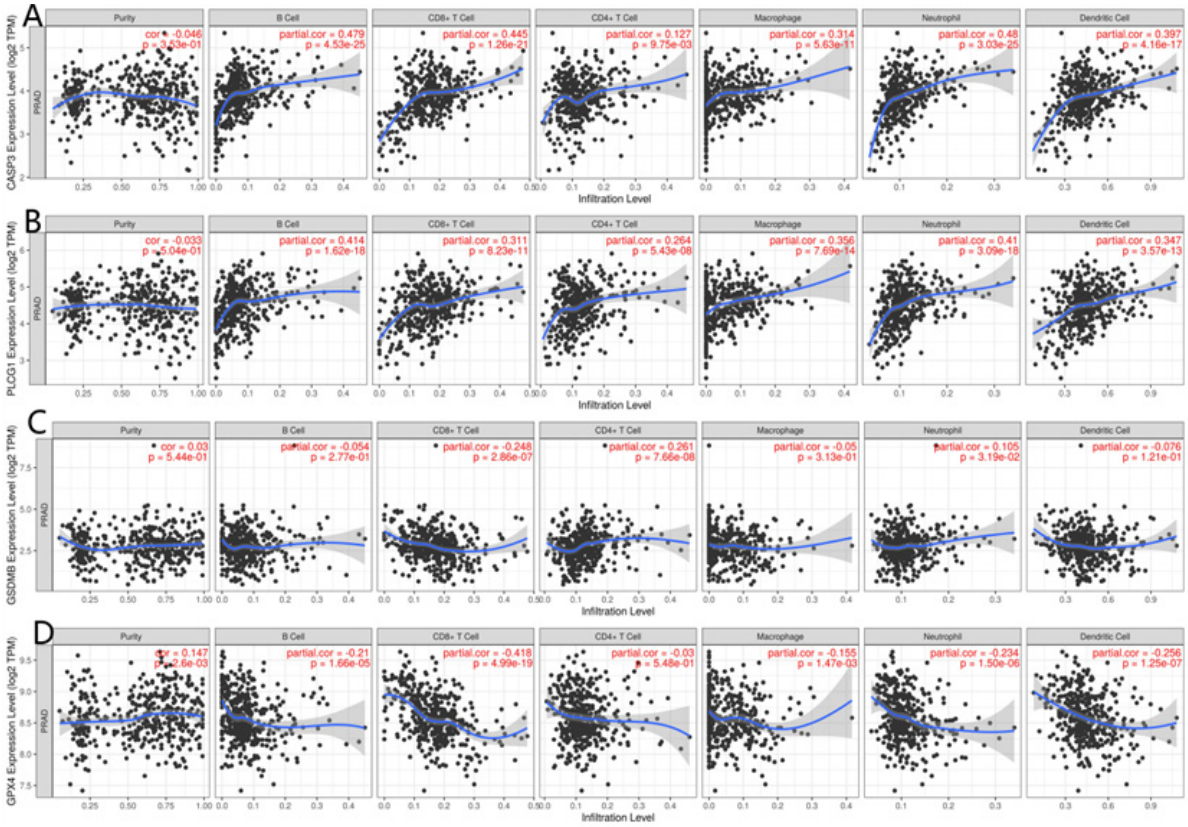

Figure 7. Relationship between Prognostic Genes and the Immune Microenvironment of Prostate Cancer. A. CASP3 relationship with immune cells. B. PLCG1 relationship with immune cells. C. GSDMB relationship with immune cells. D.GPX4relationship with immune cells

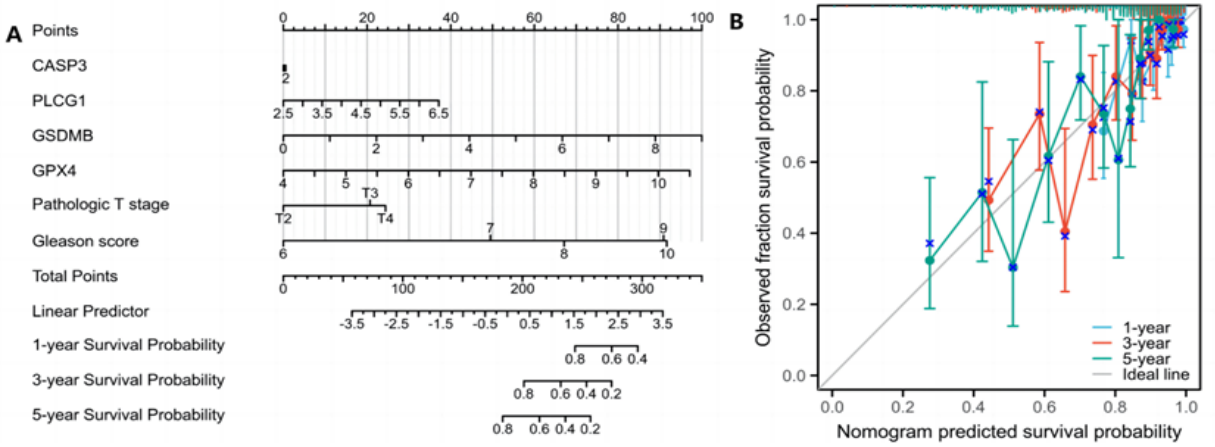

Figure 8. Prognostic Model of Prostate Cancer was Established by Combining Pyroptosis Gene, Gleason Score and Pathologic T stage. A.Prediction model nomogram.B.Predicted fitting lines for different survival times

regression.

To confirm whether this pyroptosis-related model could predict the prognosis of prostate cancer patients, we divided the TCGA database patients into high-risk and low-risk groups according to the threshold. The high-risk group had higher mortality and shorter longevity, and survival time compared with the low-risk group. Higher scores were associated with worse outcomes in prostate cancer patients (Figure 5A). CASP 3, PLCG 1, GSDMB, and GPX 4 were highly expressed in the high-risk group (Figure 5B). The Kaplan Meier curve showed a poor prognosis of patients in the high-risk group ($P < 0.05$, Figure 5C). Time-dependent ROC analysis showed that the prognostic accuracy for PFI was 0.713 at 1 year, 0.702 at 3 years, and 0.682 at 5 years (Figure 5D).

The Kaplan Meier plot of the prognostic genes

We plotted Kaplan-Meier plots to test whether the expression of the screened prognostic cell pyroptosis-related genes was correlated with PFI in prostate cancer. The results showed that the high expression of CASP 3, PLCG 1, GSDMB, and GPX 4 showed a poor prognosis Figure 6.

Analysis of the correlation with the immune cells

Immune cells are thought to play a crucial role in tumor development, metastasis, recurrence, and drug resistance. Thus, the correlation of individual genes with immune cells analyzed the association between cell-related pyroptosis genes and prostate cancer immune cells. The results showed that the expression of CASP 3, PLCG 1 and GSDMB were positively correlated with the proportion of immune cells (Figure 7A-C), and the expression of GPX 4 was negatively correlated with the proportion of immune cells (Figure 7D). Therefore, we speculate that genes related to pyroptosis can regulate the immune microenvironment of prostate cancer cells.

Construction of the nomogram and the calibration curves

Based on the results of the above studies show that the pyroptosis-related gene characteristics in our model may help to predict the prognosis of prostate cancer patients. In order to provide clinicians with better quantitative methods to predict the PFI of prostate cancer patients, after screening the clinical information related to PFI from the database, we established a nomogram combining Gleason score, T and pyroptosis genes (Figure 8A). In addition, we constructed calibration curves showing that the nomogram closely matched the recurrence rate of patients with prostate cancer (Figure 8 B). Based on these findings, we found that the nomogram containing the results of our risk score could be used to accurately predict the PFI in patients with prostate cancer.

Discussion

Numerous studies have shown that cell pyroptosis is closely associated with the occurrence and metastasis of many cancers, and that long-term exposure to the inflammatory environment increases the risk of cancer formation in cells and tissues. Specifically, pyroptosis-induced cytokine release, such as IL-1 and IL-18, can promote tumor infiltration, thereby increasing the probability of tumorigenesis and metastasis [16]. pyroptosis is a double-edged sword for cancer, because it can promote or inhibit tumor development, promote cancer has been widely studied, but the relationship between pyroptosis and anticancer immunity is not completely clear, pyroptosis death can promote tumor cell death, make cell pyroptosis death potential prognosis and therapeutic target for cancer [17-19]. Currently, we know that pyroptosis occurs in almost all types of cancer. Therefore, an in-depth investigation of the relationship between pyroptosis and cancer will broaden our understanding of cancer and inform innovation in cancer prevention and treatment. The role of cellular pyroptosis genes in prostate cancer is still unknown. This study aimed to construct a prognostic model of pyroptosis-related genes for predicting the prognosis of prostate cancer patients. Due to the longer survival of prostate cancer, here we choose PFI as the prognosis study index, mainly because the PFI statistics disease after treatment without further worse survival (from further deterioration, progression or death), in the definition of PFI, the event is tumor death, but not including death from other causes, this is more meaningful for cancer research. Recently, the research on biomarkers, prognostic markers and prognostic models of cancer have received increasing attention. Cancer patients may benefit from these models because of their strong ability to predict prognosis. In stent with previous studies, the prognostic model we constructed still has good performance in predicting the prognosis of prostate cancer patients. We constructed a prognostic model using four genes (CASP 3, PLCG 1, GSDMB, GPX 4) combined with clinical features by univariable Cox and Lasso Cox regression analysis. The Gasdermin protein activates caspase-3 to induce pyroptosis, which is associated with tumorigenesis, development, and response to therapy. These proteins can be used as therapeutic biomarkers for cancer detection, and their antagonists may be a novel target. Caspase-3 is a key protein in pyroptosis and apoptosis that controls tumor cytotoxicity upon activation, and GSDME expression regulates this. Once active caspase-3 cleaved GSDME, its N-terminal domain was punched in the cell membrane, resulting in cell expansion, rupture, and death [20]. In 2017, Wang et al. [21] found that GSDME was specifically cleaved by CASP 3 activated by chemotherapeutic drugs, producing a membrane-permeable GSDME-N fragment, which induced cell pyroptosis. PLCG 1 is involved in receptor tyrosine kinase (RTK) -mediated signal transduction pathways, which affecting cell growth, differentiation and apoptosis [22]. Recently, Kang et al. [23] demonstrated that knockdown of PLCG 1 inhibited GSDMD-N-induced cell death and showed that PLCG 1 can mediate GSDMD activity and apoptosis. However, the relationship between PLCG 1-mediated apoptosis and tumorigenesis remains unknown, and we found that high PLCG 1 expression is associated with poor survival outcome, which may be a result of its negative regulatory effect on apoptosis [24]. GSDMB is more widely expressed, mainly expressed in airway and gastrointestinal epithelial cells, liver cells,

neuroendocrine cells and immune cells. GSDMB has been shown to be activated by granzyme A shear derived from cytotoxic T cells or natural killer cells and subsequently induce pyroptosis [25-28]. GSDMB is associated with tumor progression, with increased expression in gastric, cervical, breast, and liver cancers [29-32]. Increased GSDMB gene expression in tumor cells of HER 2-positive breast cancer patients was associated with poor prognosis, decreased survival and increased metastasis, and also with adverse therapeutic response to HER 2-targeted therapy, and GSDMB was found to be co-expressed with HER 2 [31].

Kang et al. [23] revealed that GPX 4 negatively regulates the pyroptotic cell death pathway [23]. Some studies showed that GPX 4 expression is associated with metastasis and trend resistance of prostate cancer, and inhibition of GPX 4 expression helps to improve the curative effect of prostate cancer, suggesting that it may also be a useful biomarker for prostate cancer [23, 33, 34].

In conclusion, in this study, we developed a prognostic model based on the CASP 3, PLCG 1, GSDMB, and GPX 4 genes, which effectively predicted the prognosis of patients with prostate cancer. The results suggest that these genes may be potential biomarkers for predicting PFI in prostate cancer patients.

Author Contribution Statement

All authors contributed equally in this study.

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