A Novel Defined Pyroptosis-Related Gene Signature For Predicting the Prognosis of Prostate Cancer

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Abstract

Background: Pyroptosis can not only inhibit the occurrence and development of tumors but also develop a microenvironment conducive to cancer growth. However, pyroptosis research in prostate cancer (PCa) has rarely been reported.

Methods: The expression profile and corresponding clinical data were obtained from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. Patients were divided into different clusters using consensus clustering analysis, and differential genes were obtained. We developed and validated a prognostic signature for biochemical recurrence (BCR) of PCa using univariate Cox analysis, LASSO-COX analysis, Kaplan-Meier (K-M) survival analysis, and time-dependent receiver operating characteristics (ROC) curves.

Results: The expression levels of most pyroptosis-related genes (PRGs) are different not only between normal and tumor tissues but also between different clusters. Cluster2 patients have a better prognosis than cluster1 patients, and there are significant differences in immune cell content and biological pathway between them. Based on the classification of different clusters, we constructed an eight genes signature that can independently predict the progression-free survival (PFS) rate of a patient, and this signature was validated using a GEO data set (GSE70769). Finally, we established a nomogram model with good usability.

Conclusions: In this study, PRGs were used as the starting point and based on the expression profile and clinical data, a prognostic signature with a high predictive value for biochemical recurrence (BCR) following radical prostatectomy (RP) was finally constructed, and the relationship between pyroptosis, immune microenvironment, and PCa was explored, providing important clues for future research on pyroptosis and immunity.

Background

Prostate cancer (PCa) is the most common malignant tumor in male genitourinary system. According to Cancer Statistics, there were about 248,530 new cases and 34,130 deaths in the United States in 2021, accounting for 26% of the total incidence of malignant tumors and 11% of the total mortality (1). Most patients with localized cancers receive standard treatments, such as radical prostatectomy (RP) or radiation therapy (2). However, biochemical recurrence (BCR) occurs in approximately 20–30% of patients (3). BCR patients develop clinical relapses and metastases that ultimately result in death. While numerous indicators exist for predicting the prognosis of PCa patients, such as Gleason score and prostate-specific antigen (PSA) (4, 5), their ability to predict the BCR time of patients is limited. Therefore, developing a signature with high accuracy and strong specificity is critical for predicting the prognosis and guiding PCa patients' treatment. Pyroptosis, also known as cellular inflammatory necrosis, is a programmed cell death that manifests as constantly enlarging cells, releasing cell contents and thus activating a strong inflammatory response (6). Unlike apoptosis, pyroptosis requires inflammasomes and

gasdermin family to act as executors (7). Pyroptosis is believed to play a dual action in tumorigenesis by inhibiting the occurrence and development of tumors and developing a microenvironment that provides cancer with nutrients and accelerates its growth (8). At present, numerous studies have revealed that pyroptosis is critical for tumor cell proliferation, invasion, and metastasis. For instance, transcription factor p53 inhibits tumor growth by promoting apoptosis in non-small cell lung cancer (9). Nevertheless, the prognostic value of genes associated with pyroptosis has not been explored in PCa patients.

This study used bioinformatics methods to investigate the expression level, clinical value, and related immune process of pyroptosis-related genes (PRGs) in PCa patients to develop a good model for predicting the PCa patients' prognosis and improving the treatment effect of disease.

Methods

Data collection and collation

The workflow of this study is depicted in Figure 1. We obtained transcriptome and clinical data of 495 prostate cancer patients from the Cancer Genome Atlas (TCGA) database. Corresponding data from 92 patients in the Gene Expression Omnibus (GEO) database (GSE70769) were used for further model validation. In addition, mutation and copy number variation (CNV) data were also obtained from TCGA database. The batch effect of non-biotech deviations was removed using R package "SVA". We collected 52 PRGs from previous literature (**Supplementary Table 1**). We explored the imbalance of their expression in tumor tissues using R-package "limma" and plotted the heatmap using R-package "heatmap". We explored these PRGs interactions and mapped the protein-protein interaction (PPI) network using the online tool string (<https://string-db.org/>).

Mutation analysis of PRGs

The landscape of mutations of 52 PRGs was represented by the waterfall diagram drawn by R-package "maltools", and CNV position changes of 52 PRGs on 23 chromosomes were drawn using R-package "RCircos".

Consensus clustering

Unsupervised clustering analysis was performed using R-package "Consensus Cluster Plus" and the "k-means" method to identify different modification patterns according to the expression of these PGRs and classify the patients for further analysis. These steps were repeated 1000 times to ensure the classification's stability (10).

Gene Set Variation Analysis (GSVA)

We downloaded "c2.cp.kegg.v7.2.symbols" from MSigDB database and performed GSVA analysis on different clusters using R package "GSVA", where $|\log FC| > 0.2$ and adjusted $p < 0.05$ were considered significantly enriched and presented as a heatmap.

Differentially Expressed Genes (DEGs)

The R-package "limma" was used to obtain the differential gene expression between different clusters, with the filtering criteria of foldchange>2 or < 0.5 and false discovery rate (FDR)<0.05.

Immune microenvironment analysis

The algorithms "CIBERSORT (11)" and "ESTIMATE (12)" were used to quantify immunocytes and evaluate the purity of tumors from different clusters. Single sample gene set enrichment analysis (ssGSEA)(13) algorithm was employed to quantify immune cells, immune function, and pathways between different scoring subgroups.

Univariate and multivariate Cox regression analyses

Univariate Cox regression analysis was used to screen for prognosis-related DEGs. In addition, univariate and multivariate Cox analyses were also employed to test independent prognostic performance of signature. P<0.05 was considered significant.

Establishment and prognostic analysis of PRG-score signature

Using TCGA and GSE70769 cohorts as Training and Testing sets, respectively, a valid signature was established using Lasso-Cox analysis. Then, the most useful prediction characteristics were obtained from Training set using progression-free survival (PFS) and verified in the Testing set. The calculation formula is as follows:

$$\text{PRG - score} = \sum_{i=1}^n \text{coef}_i * \text{expri}$$

Where n, coef_i, and expri respectively represent the number, corresponding coefficient, and corresponding expression of signature genes. Two cohorts of patients were each assigned to high-and low-score groups by median PRG-score of Training set. We evaluated the signature's ability to differentiate between subgroups of patients using Kaplan-Meier survival curve and determined the model's accuracy using time-dependent receiver operating characteristics (ROC) curves.

Functional enrichment analysis of DEGs between low- and high-score groups

PCa patients in TCGA cohort were divided into two subgroups based on median PRG-score. DEGs between high- and low-score groups were screened based on filtration criteria (|log2FC| ≥ 0.585 and FDR < 0.05). These DEGs were analyzed for GO and KEGG using R-package "clusterProfiler".

Construction of a Nomogram and Performance Detection

A nomogram model was constructed using R-package "rms" based on PRG-score and other independent clinical factors to enhance the clinical utility of PRG-score signature. A score was matched for each

variable in the nomogram scoring system. The total score was obtained by summing the scores of all variables of each sample (14). The three-, five-, and seven-year progression-free survival probabilities of corresponding patients were then determined. Calibration charts were used to verify the nomogram's accuracy.

Statistical Analysis

All visualization and statistical analyses for this study were performed using R version 4.1.0 and the corresponding feature packages. Differences between groups for different data sets or different classifications of data were determined using chi-square tests. Comparisons between two groups were performed using Wilcoxon test. In addition, Kaplan Meier (K-M) survival analysis was conducted using a log-rank test. The above statistical methods were considered statistically significant when $p < 0.05$.

Results

Defining of the expression of PRGs in PCa

First, we analyzed the difference in expression levels of these 52 PRGs between tumor and precancerous tissues using TCGA cohort and plotted the heatmap. The results indicated a difference in the expression levels of 35 PRGs between PCa and precancerous tissues (Figure 2A, Wilcoxon test, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). The expression levels of IL1A, TP63, ELANE, IL6, CASP1, GSDME, NLRP1, IL18, NOD2, PYCARD, IL1B, NLRP7, CHMP3, IRF2, PRKACA, TNF, CHMP7, CASP5, CHMP2B, PJKV, NOD1, HMGB1, and GSDMD were down-regulated in tumor tissues, whereas those of BAK1, CASP6, CYCS, PLCG1, TP53, CHMP2A, CASP8, GPX4, BAX, CHMP4C, GSDMB, and GSDMA were down-regulated in normal tissues. PPI network was constructed by string (**Figure S1A**), and hub genes in it were identified using "MCODE" plugin of Cytoscape software, which were CASP5, GSDMD, IL18, CASP1, TNF, IL1A, IL6, IL1B, NLRP1, and PYCARD (**Figure S1B**). The correlation network consisting of 35 PRGs is depicted in Figure 2B (correlation coefficient > 0.4 , positive correlation is shown with red line, negative correlation is shown with blue line).

Landscape of genetic variation of PRGs in PCa

We analyzed the incidence of somatic and copy number mutations in 52 PRGs from PCa. As illustrated in Figure 2C, 83 of 495 (16.77%) PCa samples had genetic mutations. 23 of 52 PRGs were mutated, with TP53 showing the highest mutation frequency. Among these types of gene mutations, nonsense mutations are the most frequent. We also found that at the CNVs level, most of the focus was on missing copies (Figure 2E). Additionally, we identified changes in regulatory factors with CNV features on chromosomes (Figure 2D).

Identification of 52 PRGs-mediated PCa classification patterns

Based on the expression levels of 52 PRGs, two different regulatory patterns, cluster 1 (n=271) and cluster 2 (n=224) were identified using unsupervised clustering (Figure 3A and **Supplementary Table 2**). We found that the expression levels of these PRGs were significantly different between the two clusters (Figure 3C). Simultaneously, cluster 2 had a significantly higher PFS rate than cluster 1 (Figure 3B) (log-rank test, $p=0.005$). To explore the differences in the immune microenvironment between these two patterns, we performed immunocyte infiltration analysis and tumor purity evaluation using “CIBERSORT” and “ESTIMATE” algorithms (Figure 3D and S2A-D). The results indicated that the contents of Tregs and CD4+ activated memory T cells in cluster 1 were significantly increased, whereas the content of resting memory CD4 T cells was significantly decreased. Meanwhile, the tumor purity of cluster 1 was higher. PD-1, PD-L1, PD-L2, and CTLA4 are common immune checkpoints. We quantified immune checkpoints of different clusters, and the results indicated that the content of these immune checkpoints was higher in cluster 2, indicating that patients in cluster 2 were more likely to benefit from immunotherapy (Figure S3). To explore the difference in biological behavior between these two clusters, we performed a GSVA enrichment analysis (Figure 3F and **Supplementary Table 3**). The results showed that cluster 2 was mainly enriched in carcinogen pathways, such as focal adhesion, EMC receptor interaction, etc. Following that, the principal component analysis (PCA) confirmed that the two regulatory modes could be distinguished using 52 PRGs (Figure 3E).

Construction and verification of prognostic signature based on PRG clusters

To better apply these subtypes to the clinical treatment of PCa and determine the specific score for each patient, we next explored the differences between the two patterns and identified specific genetic signatures. Additionally, we quantified gene signatures for use in predicting prognosis of individual patients. First, using $\text{foldchange} > 2$ or < 0.5 and $\text{FDR} < 0.05$ as the filtering criteria, we identified 516 DEGs according to the above two patterns (Figure S4 and **Supplementary Table 4**). Following that, according to univariate Cox regression analysis results, the obtained 110 prognosis-related genes were used as candidate molecules for constructing a prognosis signature ($p < 0.05$) (Figure S5 and **Supplementary Table 5**). Through LASSO-COX analysis, a signature consisting of eight genes was finally obtained and called PRG-score signature (Figure 4A and Table 1). By the product of the expression level of each gene and its coefficient, the PRG-score of each sample can be obtained, and $\text{PRG-score} = (0.741 * \text{expression CENPA}) + (-0.134 * \text{expression LCN2}) + (0.802 * \text{expression COL7A1}) + (0.222 * \text{expression ALB}) + (-0.610 * \text{expression UBXN10}) + (0.302 * \text{expression SPZ1}) + (-0.227 * \text{expression SCNN1A}) + (-0.111 * \text{expression TFF3})$. Based on the median PRG-score (0.873), all patients were assigned to high- and low-score groups (Supplementary Table 6). We can find that BCR patients gradually increased as the score increases (Figure 4B). PFS rates were significantly lower in the high-score group than in the low-score group, as determined by K-M survival analysis (log-rank test, $p < 0.001$) (Figure 4C). ROC analysis revealed AUC values of 0.804, 0.772, and 0.794 for 3, 5, and 7 years, respectively, indicating high signature accuracy (Figure 5A). To further verify the accuracy of signature, validation was performed using GSE70769 cohort from GEO database (GSE70769 cohort was divided into high and low scoring groups using the median of TCGA cohort) as shown in Figure 4D-E and **Supplementary Table 7**. The two groups

had significantly different prognoses (log-rank test, $p<0.001$) and AUC values of 0.753, 0.763, and 0.774 at 3, 5, and 7 years respectively (Figure 5B), further demonstrating signature accuracy. Furthermore, we were surprised to identify that this signature reflected the overall survival rate of PCa patients (log-rank test, $p=0.02$) (Figure 4F-G), and AUC values of 0.753, 0.763, and 0.774 at 3, 5, and 7 years respectively (Figure 5C). However, further research found that this signature has no obvious independent prognostic value on the overall survival rate of patients (**Figure S6**). Researchers have studied prognosis models of PCa with modification conditions such as ferroptosis, m6A, and immune score, such as seven-gene signature discovered by Liu et al. (15), eleven-gene signature discovered by Zhang et al. (16), and seven-gene signature discovered by Lv et al. (17). Through comparison, we found that the accuracy of PRG-score signature was superior to other prognostic models (Figure 5D). Subsequently, we confirmed by PCA that PRG-score could be used as an independent indicator to distinguish PCa (Figure 5E-F).

Table 1
Results of multivariate Cox proportional hazard regression analysis of candidate genes in progression-free survival rate.

Gene	coefficient	HR	HR.95%CI	p-value
CENPA	0.741	2.099	1.424-3.094	<0.001
LCN2	-0.134	0.875	0.758-1.009	0.066
COL7A1	0.802	2.230	1.675-2.969	<0.001
ALB	0.222	1.249	1.056-1.478	0.009
UBXN10	-0.610	0.543	0.271-1.092	0.087
SPZ1	0.302	1.353	1.041-1.758	0.024
SCNN1A	-0.227	0.797	0.633-1.004	0.054
TFF3	-0.111	0.895	0.808-0.992	0.034
Coefficient: Weights for each gene obtained by multivariate Cox analysis.				

HR: hazard ratio. CI: confidence interval.

PRG-score signature has excellent independent prognostic value

To determine whether signature could represent its prognostic value independently of other clinical factors such as age and T-stage, we performed univariate and multivariate Cox analyses based on TCGA and GEO cohorts, respectively (Figure 6A-D). The results revealed that PRG-score and T-stage were independent factors affecting PCa patients' prognosis, and the hazard ratio (HR) of PRG-score in TCGA and GEO cohorts was 2.598 and 1.943, respectively. The heatmap containing clinical features demonstrates that signature can significantly distinguish the patient's clinical features (**Figure S7**). To further validate the clinical independence of PRG-score, we performed clinical stratification. Patients in TCGA cohort were divided into different subgroups based on their clinical characteristics (including age

(≤ 65 and > 65), T stages (T2 and T3-4) and N stages (N0 and N1-3), and K-M survival analysis was performed on each subgroup according to the grouping of high and low scores. The results indicated that PFS rates of patients in the high-score group were significantly higher than those in the low-score group in all subgroups (Figure 7A-F). Due to the lack of information on age and N stage compared to clinical data for TCGA, we included all clinical features in GSE70769 in the study, grouping them into subgroups of T stage (T1 and T2-3), PSA value ($\text{PSA} \leq 10$ and $\text{PSA} > 10$) and Gleason score (≤ 7 and > 7). The results showed that signature could be successfully applied to patients with T1 and T2-3, $\text{PSA} \leq 10$, Gleason score ≤ 7 , and Gleason score > 7 (Figure 7G-L).

Functional analyses based on PRG-score signature

To further explore differences in gene function and pathway between subgroups by PRG-score, we used R-package "limma" to obtain DEGs based on a filter criterion of $\text{FDR} < 0.05$ and $|\log_2\text{FC}| \geq 0.585$. A total of 134 DEGs were identified between high and low scoring groups in TCGA cohort (**Supplementary Table 8**). Gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were then performed based on these DEGs. The results revealed that these DEGs were mainly enriched in the muscle system process, contractile fiber, actin binding, and PPAR signaling pathway (Figure 8A-B).

High PRG-score indicates suppressed immune activity

Based on functional analysis, we further compared the enrichment scores of 16 immune cells and the activities of 13 immune-related pathways between low- and high-score groups in TCGA and GEO cohorts using Single Sample Gene Set Enrichment Analysis (ssGSEA) (Wilcoxon test, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). In TCGA cohort, most immunocytes were higher in low-scoring groups, notably mast cells, neutrophils, th1 cells, and Treg (Figure 9A). Moreover, the activities of most immune pathways in the low-score group were higher than those in the high-score group, such as APC co-stimulation, Type II IFN response, and MHC class I (Figure 9C). Similar findings were observed in GEO cohort, where most immunocytes and immune pathways were higher in the low-score group (Figure 9B, D). The above results indicated that increasing the PRG-score of tumor cells may inhibit their immunologic activity. The results of comparative analysis of immune checkpoints of different PRG-score are depicted in Figure 9E-F. It could be seen that the expression levels of many immune checkpoints, such as CTLA4 and PDCD1LG2 (PD-L2), differed between high and low score groups (Wilcoxon test, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$).

Establishment and validation of a nomogram model

To improve the clinical power of PRG-score signature, we established a nomogram model comprising T stage, and PRG-score using TCGA set, and then generated calibration charts for 3-, 5- and 7-year periods to demonstrate its accuracy (Figure 10A-D). In the nomogram scoring system, each variable was matched with a score, and the total score was obtained by adding the scores of all variables of each sample (14). Calibration charts revealed that nomogram model was highly accurate, affirming its practicability in predicting prognosis of patients.

Discussion

Pyroptosis is an inflammation-mediated, programmed cell death (18). It can not only inhibit the occurrence and development of tumors but also develop a microenvironment that provides nutrition for cancer and accelerates its growth (8). In gastric cancer, a new pyroptosis-related gene signature has been identified to predict prognosis (19). However, the role of PRGs in prostate cancer (PCa) remains unknown, and we sought to elucidate this role.

PCa is a common malignant tumor found in elderly men worldwide (1). Unfortunately, approximately 27–53% of PCa patients develop local recurrence or distant metastasis within ten years of radical prostatectomy (20, 21). Biochemical recurrence (BCR) was defined as the second elevation of prostate-specific antigen (PSA) concentration above 0.2 µg/L, confirmed by two consecutive elevations. It is a determining risk factor for distant metastasis, prostate cancer specificity, and overall mortality (22). There is evidence that approximately 30% of patients with BCR develop distant metastases with clinical presentation, and 19–27% of patients may die of prostate cancer within ten years without receiving second treatment (23, 24). Therefore, stratifying patients with post-RP localized PCa into high-risk BCR patients is highly desirable, which may provide more frequent monitoring, early intervention, and even decision-making regarding adjuvant therapy.

It is an effective method to classify samples based on a predetermined gene expression signature (25). Our classification strategy is based on this approach and classifies PCa based on 52 PRGs expression patterns. We found that the expression of these PRGs was completely different between the two clusters due to heterogeneity. Besides, the prognosis of different clusters varies significantly. Several agreements emerged from our analysis: 1. The expression level of most PRGs was higher in cluster 2; 2. Most DEG expression levels among different clusters were higher in cluster 2; 3. Cluster 1, as a separate subtype, has a worse prognosis; 4. A combination of clinical information and RNA transcriptome data is more likely to reflect cell phenotype. After quantifying immune cells of different clusters, we discovered that many of them had a higher content of cluster 1, particularly T regulatory cells (Tregs). Tregs are the key barrier for tumor immunotherapy, as they actively mediate autoimmune tolerance (26, 27). In recent years, as medicine has advanced, immune checkpoint inhibitors have become the key treatment measures for many malignant tumors (28). PD-1, PD-L1, PD-L2, and CTLA4 are common immune checkpoints, where PD-L1 and PD-L2 are the two ligands of PD-1 and belong to B7 family (29, 30). PD-L1 is widely expressed throughout the body, particularly in immune and cancer cells, whereas PD-L2 expression is relatively limited to professional antigen-presenting cells and increases in response to congenital receptor signals (31). In addition, CTLA4 antibodies have been demonstrated to reverse T-cell allergy, leading to an antitumor response (32). We quantified immune checkpoints for different clusters and found cluster 2 had higher levels of these immune checkpoints, indicating that patients with cluster 2 were more likely to benefit from immunotherapy.

Clinical trials have tested anti-tumor molecular targeting drugs in all PCa subtypes, regardless of the underlying molecular subtypes. For instance, immune checkpoint molecules are expressed differently in

different subtypes, and immunotherapy should be distinguished accordingly. To enhance clinical utility, we developed a scoring model (PRG-score) to quantify prognostic risk based on two clusters. This study provided strong evidence for clinical management of PCa. First, PRG-score considers the heterogeneity of patients, and PCA results indicate that scoring models can significantly distinguish patients from different risk subgroups. Second, the score can be associated with pyroptosis and prognosis. Specifically, PRG-score characterizes and assigns different weights to both tumor suppressor and tumor promoter genes. The signature included eight genes: CENPA, LCN2, COL7A1, ALB, UBXN10, SPZ1, SCNN1A, and TFF3. The coefficients of UBXN10, SCNN1A, LCN2, and TFF3 are negative, indicating that they can be used as protective factors for patients. Increased expression of these genes improves the prognosis of patients. The coefficients of ALB, SPZ1, CENPA, and COL7A1 are positive, indicating that increased expression increases the risk of poor prognosis in patients. After a careful review of relevant literature, we found that these genes were strongly associated with the occurrence and development of inflammation or malignant tumor. For instance, Masayuki Watanabe stated that Transcription factor SPZ1 might promote TWIST-mediated epithelial-mesenchymal transition in thoracic malignancies (33). By targeting SLPI, Xu et al. found that LCN2 mediated by IL-17 affects proliferation, migration, invasion, and cell cycle of gastric cancer cells (34). Stefan et al. found that COL7A1 editing via CRISPR/Cas9 in recessive dystrophic epidermolysis bullosa (35). Lin et al. found that TFF3 contributes to epithelial-mesenchymal transition (EMT) in papillary thyroid carcinoma cells via MAPK/ERK signaling pathway (36). Third, PRG-score can significantly distinguish the clinical characteristics of different patients, indicating that as the score increases, the proportion of PCa patients with T3, T4, and N1 increases significantly. PRG-Score predicts progression-free survival and, to a certain extent, the overall survival rate. Fourth, data on immune cell infiltration indicate that PRG-score has significant immunotherapeutic value. The results of ssGSEA reveal that the content of most immune cells in the low score group is higher than that in the high score group, which results in an overactive immune system that responds better to immunotherapy. There are significant differences in the content of immune checkpoints among different subgroups, which can provide important information for future research on immune checkpoints associated with PCa, particularly PD-L2 and CTLA4. Finally, PRG-score focuses directly on PCa cell death patterns compared to other models. Researchers have studied prognosis models of PCa with modification conditions such as ferroptosis, m6A, and immune score, such as seven-gene signature (15) discovered by Liu et al., eleven-gene signature (16) discovered by Zhang et al., and seven-gene signature (17) discovered by Lv et al. Through comparison, we found that the accuracy of PRG-score signature was superior to other prognostic models, and our research focused more on factors that directly contributed to tumor cell death and changed tumor microenvironment. As a result, our model is more valuable for facilitating treatment.

In our study, pyroptosis-related genes are used as the starting point, PCa patients are divided into different subtypes, DEGs are identified, and a prognosis model is constructed that can accurately predict tumor progression-free survival rate and overall survival rate of patients. At present, research progress on pyroptosis is limited, and the relationship between prostate cancer and pyroptosis has not been studied. Although we explored and verified it from multiple perspectives and different databases, this research still

has certain limitations. Although our findings were validated using external data sets, additional in vivo and in vitro experiments were required due to limited sample size of data set for prostate cancer. In addition, we could not confirm whether these regulatory factors also play corresponding roles in the pyroptosis pathway of PCa, which warrants further investigation.

Conclusion

In summary, the results of this study indicated that pyroptosis was strongly associated with PCa. This study provided a new genetic marker for predicting the prognosis of PCa patients and laid the groundwork for future research on the relationship between pyroptosis-related genes and PCa immunity.

Abbreviations

PCa: prostate cancer

TCGA: The Cancer Genome Atlas

GEO: Gene Expression Omnibus

BCR: biochemical recurrence

K-M: Kaplan-Meier

ROC: receiver operating characteristics

PRG: pyroptosis-related gene

PFS: progression-free survival

RP: radical prostatectomy

PSA: prostate-specific antigen

CNV: copy number variation

PPI: protein-protein interaction

GSVA: gene set variation analysis

DEG: differentially expressed gene

PCA: principal component analysis

HR: hazard ratio

CI: confidence interval

GO: gene ontology

KEGG: kyoto encyclopedia of genes and genomes

EMT: epithelial-mesenchymal transition

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analysed during the current study are available in the TCGA repository, (<https://portal.gdc.cancer.gov>) and GEO repository, (<https://www.ncbi.nlm.nih.gov/geo/>).

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

DH and MT designed the research framework and revised the manuscript. DH and QC contributed to the data analysis and wrote the manuscript. ZL, WH, YJ, GT, YW, PL and HZ provided comments during the writing. All authors reviewed and approved the final manuscript.

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Figures

Figure 1

The workflow chart of this research.

Figure 2

Characterization of pyroptosis-related genes at the biological level in tumor. (A) The heatmap revealed that expression levels of 35 of 52 PRGs in tumor tissues were imbalanced, with red representing high expression and blue representing low expression (Wilcoxon test, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). (B) Correlation between PRGs expressed by 35 abnormalities (correlation coefficient > 0.4 , with red line representing positive correlation and blue line representing negative correlation). (C) Genomic changes

from 495 PCa samples from TCGA with waterfalls representing information on different PRGs mutations. A note at the bottom of corresponding color indicates different mutation types. The histogram above represents the tumor mutation burden for each sample. The number on the right indicates the mutation frequency. (D) The location of PRGs at which CNV occurs on chromosome. (E) The frequency of copy number variation (CNV) for different PRGs indicated that deletion with copy number existed in most PRGs.

Figure 3

Subunit types based on PRGs in PCa and their immune microenvironment. (A) Consensus clustering matrix for $k = 2$. (B) The results of K-M analysis indicated that cluster 1 had a significantly lower tumor progression-free survival than cluster 2 (log-rank test, $p=0.005$). (C) Heatmap with different clinical features revealed that PRGs had differential expression in different clusters. (D) Immune cell infiltration of different clusters based on "cibersort" algorithm (Wilcoxon test, $*P < 0.05$; $**P < 0.01$; $***P < 0.001$). (E) The heatmap shows the biological pathway to pyroptosis-related clusters by gene set variation analysis (GSVA) ($|\log_2FC| > 0.2$ and adjusted $p < 0.05$). (F) Principal component analysis (PCA) showed that these PRGs could well divide TCGA cohort into two distinct clusters.

Figure 4

Eight genes-based prognostic signature was constructed using LASSO-COX regression analysis, and its efficacy was tested. (A) The distribution of partial likelihood deviation of LASSO coefficient preserves ten variables when partial likelihood deviation reaches the minimum (Log Lambda = -3.45). PFS risk profile for PRG-score-based patients in TCGA cohort (increased number of patients with biochemical relapse (BCR) as scores increased) (B), K-M surviving curve (log-rank test, $p < 0.001$) (C). PFS risk profile for PRG-

score-based patients in GSE70769 cohort (same as TCGA cohort, with increased incidence of BCR as scores increased) (D), K-M survival curve (log-rank test, $p < 0.001$) (E). Overall survival risk profile for PRG-score-based patients in TCGA cohort (increased number of patients die as the score increases) (F), K-M survivable curve (log-rank test, $p = 0.02$) (G).

Figure 5

The signature has high accuracy and applicability. 3, 5, 7-year ROC curve for PRG-score-based patients in TCGA cohort (A) and GSE70769 cohort (B). (C) 3, 5, 7-year ROC curve of overall survival risk for PRG-score-based patients in TCGA cohort. (D) The ROC curve for the 3-year progression-free survival prediction of PRG-score signature and the other four prognostic signatures. Results from PCA in TCGA cohort (E) and GSE70769 cohort (F) showed that PRG-score well divided the samples into two subgroups.

Figure 6

PRG-score is an excellent independent prognostic factor in PCa patients. Forest map based on univariate Cox analysis (A) and multivariate Cox analysis (B) of TCGA cohort. Forest map based on univariate (C) and multivariate (D) Cox analyses of GSE70769 cohort.

Figure 7

PRG-score based clinical stratification in TCGA and GSE70769 sets. Kaplan-Meier survival curves for patients under 65 years (A), age over 65 (B), T2 (C), T3-4 (D), N0 (E), N1-3 (F) in TCGA set, and T1 (G), T2-3 (H), PSA $\leq$ 10(I), PSA>10 (J), Gleason score $\leq$ 7 (K), Gleason score $>$ 7 (L) in GSE70769 set (log-rank test).

Figure 8

Functional analyses based on PS-score model. Gene ontology (GO) enrichment analysis (A) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (B) were then performed based on these DEGs. The results reveal that these DEGs are mainly enriched in muscle system process, contractile fiber, actin binding, and PPAR signaling pathway.

Figure 9

Comparison of ssGSEA scores for immune cells and pathways. Enrichment scores for 16 immunocytes were compared between low- and high-score groups in TCGA cohort (A) and GSE70769 cohort (B). Enrichment score comparisons for 13 immune-related pathways between low- and high-score groups in TCGA cohort (C) and GSE70769 cohort (D). Expression comparisons at common immune checkpoints

between high- and low-score groups in TCGA cohort (E) and GSE70769 cohort (F). (Wilcoxon test, *P < 0.05; **P < 0.01; ***P < 0.001.)

Figure 10

Establishment of nomogram and its performance verification. Nomogram (A) combined with age, T stage, and PRG-score and its calibration diagrams of 3-year (B), 5-year (C), and 7-year (D).

Supplementary Files

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