Identification of the pyroptosis-related prognostic gene signature and the associated regulation axis in lung adenocarcinoma

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INTRODUCTION

Lung cancer remains the most common deadly disease with an estimated 2.09 million new cases and 1.76 million deaths each year [1]. Worse still, the incidence and mortality of lung cancer are rising [1]. Lung adenocarcinoma (LUAD) is the most common histologic subtype of lung cancer, accounting for approximately 40% of all cases [2]. Despite surgery, chemoradiotherapy, targeted therapy, and immunotherapy being used in the treatment of lung cancer, the prognosis remains disheartening [3], and 5-year survival ranges from 4 to 17%, depending on disease and treatment differences [4].

Although many biomarkers or gene signatures have been found to have the potential to predict the prognosis of LUAD, they are still in the molecular research phase and have not yet been applied in clinical practice. Thus, uncovering prognostic gene signatures for the prognosis of LUAD would be of great significance.

Pyroptosis, referred to as cellular inflammatory necrosis, is considered to be gasdermin-mediated programmed necrotic cell death [5]. Triggered by certain inflammasomes, pyroptosis relies on the cleavage of gasdermin D (GSDMD) and activation of inactive cytokines [6]. The correlation between pyroptosis and cancer is extremely complicated. Although pyroptosis can inhibit the oncopogenesis and progression of tumours, it also develops a microenvironment delivering nutrients for cancer and accelerating cancer growth [7]. Increasing studies have demonstrated the effect of pyroptosis on tumour cell proliferation, invasion, and metastasis, thus affecting the prognosis of cancer [8, 9]. For example, a recent study identified a novel pyroptosis-related gene signature for the prognosis of ovarian cancer [10]. In lung cancer, the pyroptosis gene GSDMD can inhibit tumour proliferation by regulating the intrinsic mitochondrial apoptotic pathway and EGFR/Akt signalling [11]. The prognostic value of pyroptosis-related genes (PRGs) in LUAD has not yet been elucidated.

In the current research, bioinformatics analysis was performed to investigate PRG expression profiles and their prognostic significance as well as the associated regulatory axis in LUAD. Our data may provide additional evidence for prognostic biomarkers and therapeutic targets for LUAD.

RESULTS

Defining of the expression of PRGs in LUAD

We first explored the expression of the 33 PRGs in LUAD and normal lung tissues using the TCGA LUAD dataset. A total of 23 PRGs were either upregulated or downregulated in LUAD (Fig. 1A). More
specifically, the expression of PRKACA, NOD1, NLRP1, ELANE, TNF, IL1B, IL18, PYCARD, CASP5, NLRC4, NLRP3, IL6, and CASP1 was increased, while the expression of GSDMB, PIVK, CASP4, NLRP7, CASP3, CASP6, CASP8, GSDM4, GSDMC, and AIM2 was decreased in LUAD compared with normal tissues (Fig. 1A, all <0.001). A protein–protein interaction (PPI) analysis with the minimum required interaction score of 0.9 was constructed to detect the interactions of these PRGs, which revealed that CASP1, CASP5, CASP8, NLRP1, NLRP3, and PYCARD were hub genes (Fig. S1A). Supplementary Fig. S1B shows the correlation network containing all PRGs.

**Landscape of genetic variation of PRGs in LUAD**

We then summarised the incidence of copy number variations and somatic mutations of 33 PRGs in LUAD. As shown in Fig. 1B and Fig. 1C, 116 of 257 (64.59%) LUAD samples demonstrated genetic mutations. Missense mutation was the most common variant classification (Fig. 1B). SNPs were the most common variant type, and C > A ranked as the top SNV class. The results also demonstrated NLRP3 as the gene with the highest mutation frequency, followed by NLRP7 and NLRP2, among the 33 PRGs (Fig. 1C). Figure 1D presents the location of CNV alterations of these 33 PRGs on chromosomes. We also investigated CNV alteration frequency, which revealed that these 33 PRGs showed prevalent CNV alterations. More than half of the 33 PRGs had copy number amplification, while the CNV deletion frequencies of CASP9, GPX4, NLRP7, NLRP2, IL18, ELANE, NLRP6, PLCG1, CASP6, CASP3, NLRP1, and PRKACA were widespread (Fig. 1E).

**Functional enrichment analysis of PRGs**

To clarify the function of PRGs, the pathways were analysed using GO and KEGG databases. We found that these 33 PRGs were mainly...
involved in the positive regulation of cytokine production, interleukin-1 production, regulation of inflammatory response, pyroptosis, inflammasome complex, cysteine-type endopeptidase activity involved in apoptotic process, cysteine-type endopeptidase activity, and cytokine receptor binding in GO analysis (Fig. 2A). Moreover, KEGG pathway analysis suggested that 33 PRGs were mainly involved in the NOD-like receptor signalling pathway, salmonella infection, cytosolic DNA-sensing pathway, TNF signalling pathway, Toll-like receptor signalling pathway, and apoptosis (Fig. 2B).

**Construction of a pyroptosis-related prognostic gene model**
To construct a prognostic gene model, univariate Cox regression analysis was performed to screen those PRG with a prognostic value. As a result, a total of five genes with a prognostic value were identified, and the Kaplan–Meier survival curves are shown in Fig. 3. The results suggested a poor survival rate in LUAD patients with low expression of NLRP7 (Fig. 3A, \( p = 0.021 \)), NLRP1 (Fig. 3B, \( p = 0.008 \)), NLRP2 (Fig. 3C, \( p = 0.014 \)), and NOD1 (Fig. 3D, \( p = 0.047 \)) and high CASP6 expression (Fig. 3E, \( p = 0.048 \)). LASSO

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**Fig. 2**  The functional enrichment analysis of PRG in LUAD. A The enriched item in gene ontology analysis. B The enriched item in Kyoto Encyclopedia of Genes and Genomes analysis. The size of circles represented the number of genes enriched. BP biological process, CC cellular component, MF molecular function, PRG pyroptosis-related gene.

**Fig. 3**  The prognostic value of PRG in LUAD. The overall survival curve of NLRP7 A NLRP1 B NLRP2 C NOD1 D and CASP6 E in LUAD patients in the high-/low-expression group. PRG pyroptosis-related gene, LUAD lung adenocarcinoma.
Cox regression analysis was performed to construct a prognostic gene model based on these five prognostic PRGs (Fig. 4A, B). The risk score = (0.0946) * CASP6 + (0.1573) * NLRP7 + (−0.124) * NOD1 + (−0.1627) * NLRP1 + (−0.0262) * NLRP2. Based on the risk score, LUAD patients were separated into two groups. The risk score distribution, survival status, and the expression of these five genes are presented in Fig. 4C. As the risk score increased, the patients’ risk of death increased, and the survival time decreased (Fig. 4C). The Kaplan–Meier curve revealed that LUAD patients with high-risk scores had a worse overall survival probability than those with low-risk scores (median time = 3.3 years vs. 4.9 years, \( p = 0.00083 \), Fig. 4D), with AUCs of 0.668, 0.591, and 0.612 in the 1-year, 3-year, and 5-year ROC curves, respectively (Fig. 4E).

Building a predictive nomogram

Considering the clinicopathologic features and these five prognostic PRGs, we also built a predictive nomogram to predict the survival probability. Univariate and multivariate analyses revealed that NOD1 expression and pT stage, pN stage, and pM stage were independent factors affecting the prognosis of LUAD patients (Fig. 5A, B). The predictive nomogram suggested that the 3-year and 5-year overall survival rates could be predicted relatively well compared with an ideal model in the entire cohort (Fig. 5C, D).

PRGs were associated with tumour immune infiltration in LUAD

Pyroptosis plays a vital role in the development of the tumour-immune microenvironment. In our study, we also clarified the correlation of the expression of prognostic PRGs (NOD1, CASP6, NLRP1, NLRP2, and NLRP7) and immune infiltration in LUAD using the TIMER database. The data demonstrated a negative correlation between CASP6 expression and the abundance of B cells (Fig. 6A, \( p = 6.6e^{-4} \)) and CD4+ T cells (Fig. 6A, \( p = 0.0157 \)). Moreover, there was a positive association between NLRP7 expression and the immune infiltration level of B cells (\( p = 6.43e^{-5} \)), CD4+ T cells (\( p = 1.21e^{-5} \)), macrophages (\( p = 0.0184 \)), neutrophils (\( p = 4.67e^{-4} \)), and dendritic cells (\( p = 9.9e^{-5} \)) (Fig. 6B). NLRP2 expression showed a positive association with the abundance of CD4+ T cells (\( p = 2.56e^{-4} \)) and dendritic cells (\( p = 0.0426 \)) (Fig. 6C). Figure 6D shows the correlation between NOD1 expression and the abundance of immune cells, which revealed a positive correlation between NOD1 expression and the abundance of B cells (\( p = 5.53e^{-10} \)), CD8+ T cells (\( p = 0.0329 \)), CD4+ T cells (\( p = 1.73e^{-13} \)), neutrophils (\( p = 0.0151 \))

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**Fig. 4** Construction of a prognostic PRG model. A LASSO coefficient profiles of the five PRGs. B Plots of the ten-fold cross-validation error rates. C Distribution of risk score, survival status, and the expression of five prognostic PRGs in LUAD. D, E Overall survival curves for LUAD patients in the high-/low-risk group and the ROC curve of measuring the predictive value. PRG, pyroptosis-related gene; LUAD, lung adenocarcinoma.
and dendritic cells \( (p = 3.74 \times 10^{-11}) \). We also found that NLRP1 expression was positively correlated with the abundance of B cells \( (p = 4.98 \times 10^{-23}) \), CD8\(^+\) T cells \( (p = 0.0158) \), CD4\(^+\) T cells \( (p = 4.28 \times 10^{-48}) \), macrophages \( (p = 2.03 \times 10^{-5}) \), neutrophils \( (p = 2 \times 10^{-14}) \), and dendritic cells \( (p = 1.47 \times 10^{-19}) \) (Fig. 6E). This evidence suggests a significant correlation between PRG and tumour-immune infiltration.

**TMB, MSI, and drug-sensitivity analysis of PRGs**

TMB can be used as a biomarker to predict the efficacy of immunotherapy for lung cancer [12, 13]. Microsatellite instability (MSI) was also suggested as a predictive biomarker for cancer immunotherapy [14]. The above results revealed that the PRG was significantly correlated with tumour immune infiltration. To clarify whether these PRGs could also serve as biomarkers for drug screening, we then analysed the correlation between PRGs and TMB as well as MSI in LUAD. The results revealed a negative correlation between TMB and NOD1 \( (p = 0.0105) \), NLRP2 \( (p = 1.47 \times 10^{-19}) \), and NOD2 \( (p = 0.00211) \) were correlated with clinical stage. However, there was no significant correlation between NOD1 \( (p = 0.339) \), CASP6 \( (p = 0.232) \), and clinical stage. This suggested that NLRP1 and NLRP7 may be involved in tumour progression in LUAD. To clarify the potential molecular mechanism of NLRP1 and NLRP7 in LUAD, we then constructed a network of mRNA–miRNA–lncRNA interactions. The data identified miR-335-5p as the targeting mRNA binding to NLRP1 and NLRP7 according to miRBase and miRBase V.8 (Fig. 8A). Further analysis revealed that miR-335-5p was downregulated in LUAD \( (p = 0.00016) \), and LUAD patients with high miR-335-5p levels experienced better overall survival \( (p = 0.0328) \). According to this result, we also explored its upstream lncRNA targets to construct the miRNA–lncRNA axis. As shown in Fig. 8D, three lncRNAs, lncRNA XIST, lncRNA FTX, and lncRNA KCNQ1OT1, were identified as targets. The ceRNA network is shown in Fig. 8E. The expression of lncRNA targets was also detected, which revealed downregulation of lncRNA FTX (Fig. 8F).


\[ p = 5.9 \times 10^{-5} \]

and upregulation of IncRNA KCNQ1OT1 (Fig. 8G, \( p = 7 \times 10^{-6} \)) in LUAD compared with normal tissues. However, only IncRNA KCNQ1OT1 could reduce the LUAD patients’ survival probability (Fig. 8H, \( p = 0.0361 \)). Thus, the IncRNA KCNQ1OT1/miR-335-5p/NLRP1/NLRP7 regulatory axis may play a vital role in the progression of LUAD.

**DISCUSSION**

Pyroptosis is a newly recognised type of programmed cell death that exerts a dual function in cancer progression and treatment mechanisms. Pyroptosis can release inflammatory factors and stimulate normal cells, resulting in transformation into tumour cells [15]. However, pyroptosis can promote tumour cell death, making pyrolysis a potential prognostic and therapeutic target for cancer [16]. In ovarian cancer, a novel PRG signature has been identified to predict prognosis [10]. However, the role of PRG in LUAD has not yet been elucidated, and our study was performed to clarify this role.

We first clarified the expression and prognostic value of PRGs in LUAD. We found that the expression of PRKACA, NOD1, NLRP1, ELANE, TNF, IL18, PYCARD, CASP5, NLRC4, NLRP3, IL6, and CASP1 was increased, while the expression of GSDMB, PJVK, CASP4, NLRP7, CASP3, CASP6, GSDMA, GSDMC, and AIM2 was decreased in LUAD compared with normal tissues. Prognosis analysis suggested a poor survival rate in LUAD patients with low expression of NLRP7, NLRP1, NLRP2, and NOD1 and high CASP6 expression. These data were consistent with prior results. Edward
et al. suggested that low expression of \textit{NLRP1} was linked to a poor prognosis and immune infiltration in LUAD [17].

We also performed functional enrichment analysis of PRGs, which revealed that these 33 PRGs were mainly involved in the regulation of the inflammatory response, pyroptosis, NOD-like receptor signalling pathway, TNF signalling pathway, Toll-like receptor signalling pathway, and apoptosis. Interestingly, these functions or pathways were correlated with the oncogenesis and progression of LUAD. The induction of Th1-like and cytotoxic immunity by the TLR signalling pathway could result in lung cancer regression or arrest [18]. Moreover, a previous study showed that potentially functional genetic variants in TNF/TNFR signalling pathway genes were associated with prognosis in LUAD [19]. These results suggested that these 33 PRGs may also play a vital role in the oncogenesis and progression of LUAD.

LASSO Cox regression analysis was performed to construct a prognostic gene model based on five prognostic PRGs (\textit{NLRP7}, \textit{NLRP1}, \textit{NLRP2}, \textit{NOD1}, and \textit{CASP6}), which could predict the overall survival of LUAD patients with medium-to-high accuracy. A predictive nomogram suggested that the 3-year and 5-year overall survival rates could be predicted relatively well compared with an ideal model in the entire cohort. A previous study identified several prognostic signatures for LUAD. A study performed by Sijin developed and validated an immune-related prognostic signature in LUAD [20]. Another glycolysis-related gene signature could predict metastasis and survival in LUAD patients [21]. Moreover, an autophagy-related prognostic signature showed good performance in LUAD patient prognosis prediction [22]. In our study, we first identified a pyroptosis-related prognostic gene signature for LUAD, which provides more choices for prognostic prediction in LUAD.

In our study, \textit{CASP6} was found to be one of the gene signatures. Although a previous study revealed that \textit{CASP6} could facilitate the activation of programmed cell death pathways, including pyroptosis, apoptosis, and necroptosis, \textit{CASP6} is typically not associated with pyroptosis [23]. \textit{CASP6} is generally considered to be a vital regulator of innate immunity, inflammasome activation, and host defence [23]. Increasing evidence has revealed that \textit{CASP6} is involved in carcinogenesis and progression by regulating the apoptosis and metastasis of tumours [24]. Moreover, active \textit{CASP6} is thought to be a potential therapeutic target against Alzheimer’s disease [25, 26]. This combined evidence suggested a

![Fig. 7 TMB, MSI, and drug-sensitivity analysis of PRG in LUAD. A–E] The correlation between five prognostic PRG and TMB in LUAD. F–J The correlation between five prognostic PRG and MSI in LUAD. K The correlation between five prognostic PRG and CTRP drug sensitivity in LUAD. TMB tumour mutation burden, MSI microsatellite instability, LUAD lung adenocarcinoma, PRG pyroptosis-related gene, CTRP cancer therapeutics response portal.](https://example.com/fig7.png)

| Drugs | FDR | Correlation |
|-------|-----|-------------|
|       |     |             |
| NLRP2 |     |             |
| NLRP7 |     |             |
| NLRP1 |     |             |
| NOD1  |     |             |
| CASP6 |     |             |

TMB tumour mutation burden, MSI microsatellite instability, LUAD lung adenocarcinoma, PRG pyroptosis-related gene, CTRP cancer therapeutics response portal.
broad role for CASP6. However, studies on the role of CASP6 in pyroptosis are limited. In our study, we found that CASP6 was one of the pyroptosis-related prognostic biomarkers in LUAD. Further in vivo and in vitro studies should be performed to verify whether CASP6 is involved in pyroptosis in LUAD.

Another important finding of our study revealed that the above five pyroptosis-related prognostic genes were significantly correlated with immune infiltration, which further confirmed the fact that pyroptosis plays a vital role in the tumour immune microenvironment. BRAF mutations could regulate the tumour immune microenvironment by regulating the pyroptosis-related signalling pathway [27]. A previous study also found that the pyroptosis gene NLRP1 is correlated with immune infiltration in LUAD [17].

We also constructed a mRNA–miRNA–lncRNA network, which identified a lncRNA KCNQ1OT1/miR-335-5p/NLRP1/NLRP7 regulatory axis. In fact, miR-335-5p could regulate the LUAD cell cycle and metastasis [28]. Moreover, miR-335-5p could suppress TGF-β1-induced EMT in lung cancer [29]. Interestingly, IncRNA KCNQ1OT1 could accelerate LUAD cell proliferation, migration, and invasion [30]. In our study, we also found that miR-335-5p and IncRNA KCNQ1OT1 were linked to the prognosis of LUAD patients. All this evidence suggests that the IncRNA KCNQ1OT1/miR-335-5p/NLRP1/NLRP7 regulatory axis may also play an important role in the progression of LUAD. Further study should be conducted to verify this result.

In conclusion, we performed a comprehensive and systematic bioinformatics analysis and identified the pyroptosis-related prognostic gene signature containing five genes (NLRP7, NLRP1, NLRP2, NOD1, and CASP6) for LUAD patients. Our results also identified a lncRNA KCNQ1OT1/miR-335-5p/NLRP1 regulatory axis, which may also play an important role in the progression of LUAD. Further study should be conducted to verify this result.

**MATERIALS AND METHODS**

**Datasets and preprocessing**
The RNA-sequencing (RNA-seq) data of 486 LUAD patients and the corresponding clinical information were obtained using The Cancer Genome Atlas (TCGA) database on April 1, 2021. The clinical information of the LUAD patients is shown in Table S1. Moreover, somatic datasets and copy number variation (CNV) data for LUAD were also downloaded from TCGA and the University of California, Santa Cruz (UCSC) Xena website, respectively. Data analysis was performed with the R (version 4.0.5) and R Bioconductor packages. The expression data were normalised to transcripts per kilobase million (TPM) values before further analysis.

**Identification of differentially expressed PRGs**
A total of 33 PRGs were obtained from prior reviews [10, 31], which are shown in Table S2. The difference in PRG expression in LUAD and normal tissues was identified using the “limma” and “reshape2” packages. We then constructed a protein–protein interaction (PPI) network for 33 PRGs using the Search Tool for the Retrieval of Interacting Genes (STRING).

**Mutation analysis of PRGs**
The mutation frequency and oncoplot waterfall plot of 33 PRGs in LUAD patients were generated by the “maftools” package. The location of CNV
alteration of 33 PRGs on 23 chromosomes was drawn using the "RCircos" package in R.

**Functional enrichment analysis**

Gene Ontology (GO), including the biological process (BP), cellular component (CC), and molecular function (MF) categories, was conducted with the “goftot2” package in R software. Similarly, this package was also used to perform Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis.

**Development of the pyroptosis-related gene prognostic model**

Cox regression analysis was performed to evaluate the prognostic significance of the PRGs. For Kaplan–Meier curves, p-values and hazard ratios (HRs) with 95% confidence intervals (CIs) were generated by log-rank tests and univariable Cox proportional hazard regression. PRGs with a significant prognostic value were selected for further analysis. Based on these prognostic PRGs, LASSO Cox regression analysis was then used to construct the prognostic model. The TCGA LUAD patients were divided into low- and high-risk subgroups according to the median risk score, and the overall survival (OS) time was compared between the two subgroups via Kaplan–Meier analysis. The predictive accuracy of each gene and the risk score were evaluated by performing time receiver-operating characteristic (ROC) analysis. Considering the clinical characteristics, a predicted nomogram was developed to predict the 1-, 3-, and 5-year overall survival. A forest was used to show the P-value, HR and 95% CI of each variable through the "forestplot" R package.

**Immune infiltration, tumour mutation burden, and microsatellite-instability analysis**

We then analysed the correlation between prognostic PRG and immune infiltration using the Tumour Immune Estimation Resource (TIMER, https://cistrome.shinyapps.io/timer/), a web portal for comprehensive analysis of tumour-infiltrating immune cells. The “Gene” module of TIMER could visualise the correlation of gene expression with the immune infiltration level in LUAD. In tumour mutation burden (TMB) and microsatellite-instability (MSI) analysis, Spearman’s correlation analysis was performed to calculate the correlation between gene expression and TMB and MSI score. A p-value of less than 0.05 was considered statistically significant.

**Competing endogenous RNA network construction**

To clarify the potential function of PRG in LUAD, we then constructed a competing endogenous RNA (ceRNA) network. miRTarBase (http://miRTarBase.cuhk.edu.cn/) and TarBase V.8 (https://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=tarbase&%2Findex) were utilised to predict the mRNA targets binding to the PRGs. Based on the miRNAs identified, StarBase (http://starbase.sysu.edu.cn/) and LncBase Predicted v.2 (https://carolina.imis.athena-innovation.gr/diana_tools/web/index.php?r=LncBase2/index-predicted) were utilised to predict lncRNA targets interacting with miRNAs. We also explored the expression and prognostic value of these miRNA and IncRNA targets using the TCGA LUAD dataset. All analyses were considered statistically significant at P < 0.05.

**DATA AVAILABILITY**

The analysed data sets generated during the study are available from the corresponding author on reasonable request.

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ACKNOWLEDGEMENTS
This study was funded by the Science and Technology Special Fund of Maoming (2020KJZX016), the Science and Technology Project of Guangdong Esophageal Cancer Institute (M201914) and the Science and Technology Special Fund of Guangdong (2020S00061).

COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41420-021-00557-2.

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