Development and verification of pyroptosis scoring system of lower-grade gliomas

Xiao Chen
The First Affiliated Hospital of Xi’an Jiaotong University

Ying Xu
The First Affiliated Hospital of Xi’an Jiaotong University

Maode Wang
The First Affiliated Hospital of Xi’an Jiaotong University

Chunying Ren (✉️ 314610801@qq.com)
The First Affiliated Hospital of Xi’an Jiaotong University

Research Article

Keywords: pyroptosis, lower-grade glioma, tumor microenvironment, immunotherapy, WGCNA

Posted Date: November 22nd, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2259533/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Pyroptosis plays a crucial role in the malignant progression of solid tumors, while the underlying mechanism of pyroptosis has not been systematically investigated in lower-grade glioma (LGG). We retrospectively enrolled TCGA-LGG and CGGA-LGG cohorts, the Genotype-Tissue Expression project, and clinical samples. Pyroptosis-related genes (PRGs) were obtained from the Molecular Signatures Databas. Pyroptosis consensus clustering based on the expression profile of PRGs was used for consistency matrix construction in LGG samples. Subsequently, the “Boruta” algorithm was performed to identify the signature genes among pyroptosis subtypes. Principal component analysis implemented the dimension reduction of the expression profile of signature genes to construct a pyroptosis scoring system. Functional annotation analysis and immune cell infiltrating research depict the immune landscape among different pyroptosis groups. In addition, biomarkers of the pyroptosis scoring system were identified through weight gene co-expression network analysis (WGCNA). 27 PRGs were obtained, 5 of PRGS downregulate while 8 upregulate in LGG. Three pyroptosis patterns identified retain distinct clinical features and tumor microenvironment (TME). Based on the best cut-off pyroptosis value (7.244834), LGG samples were assigned into low and high pyroptosis groups. Patients with higher pyroptosis scores tend to have better prognoses. The pyroptosis score was considered as an indicator predicting the benefits of immunotherapy. In addition, BUB1 and KIF11 were considered the prognostic and pyroptosis-related markers in LGG. The Pyroptosis scoring system constructed in this study could heighten our comprehension of the tumor microenvironment of LGG and assist us in making individualized treatment strategies for patients.

Introduction

Glioma is the most common primary malignant tumor in the central neural system. The median survival of patients depends mainly on the histological grade of the glioma[1]. The lower-grade glioma (LGG) includes World Health Organization (WHO) grades II and III glioma, with a relatively favorable prognosis[2]. The first line of treatment for LGG is still surgical resection[3]. Moreover, although the current radiotherapy and chemotherapy, standard treatments after surgery[4], could improve the clinical outcome of patients, they have limited therapeutic effects on LGG[5, 6]. Immunotherapy has become a rapidly progressing field in the treatment of gliomas[7], especially immune checkpoint blocking (ICB) drugs that have been approved to treat solid cancers[8]. The tumor immune response plays an essential role in glioma, suggesting the promising prospect of immune therapy for glioma therapies[9]. However, the response of glioma patients to the immunotherapy was still far from satisfying[10]. Part of the critical reason is the substantial heterogeneity of the immune microenvironment of LGG, making some patients unable to achieve a satisfactory immunotherapeutic effect through a limited immune response. As a result, it is vital to identify suitable patients benefiting more from immunotherapies by a reliable prediction method.

Pyroptosis, an inflammation-dependent type of programmed cell death, is mediated by inflammasomes[11]. Different from apoptosis, pyroptosis involves cell swelling and lysis[12]. When
activated, caspase-1 cleaves the protein gasdermin D, then pyroptosis occurs, and the gasdermin N subunit is released[13]. It has been observed in several types of solid tumors that this proinflammatory microenvironment is favorable for the initiation and progression of cancer, as increased serum levels of proinflammatory ILs, including IL-1β and IL-18[14]. However, almost no study has systematically studied the underlying role of pyroptosis in LGG. Newly developed strategies have focused on involving pyroptosis to create a more efficacious way to inhibit tumor formation and metastasis[15, 16]. It may provide new ideas and valuable therapeutic targets for tumor treatment by exploring the effect of pyroptosis in the pathogenesis of cancers.

This study identified three pyroptosis patterns in LGG, which were associated with distinct prognoses and tumor immune microenvironment (TIME) features. According to the mRNA expression profiles of pyroptosis pattern-related gene signatures identified through machine learning algorithms, the pyroptosis scoring system was first established to quantify the pyroptosis pathway activity for patients with LGG. Subsequently, we explored the potential correlation between pyroptosis score and clinical traits in LGG and verified these results in the external dataset. The immune landscape analysis investigated the TIME characteristics of the pyroptosis scoring system in LGG. In addition, we also explored the clinical features, mutation landscape, and tumor microenvironment features in different pyroptosis score groups. Through enrichment analysis, we explored the signaling pathways associated with the pyroptosis score in LGG. Finally, we performed weight genes co-expression network analysis (WGCNA) in LGG samples to identify the pyroptosis score-related hub markers. To sum up, we constructed a pyroptosis scoring system to evaluate the pyroptosis activity of LGG samples, quantitatively. Exploring its clinical and prognostic value, investigating the scoring system's potential mechanism through the TME landscape, and finally identifying the hub markers of pyroptosis score aimed to provide a novel and comprehensive perspective for clarifying the possible mechanisms underlying the prognosis of LGG and making individualized treatment strategies for patients.

**Materials And Methods**

The flowchart for this research is shown in Fig. 1.

Public data and samples collection

We collected whole-genome RNA-seq expression data and clinical and molecular information. We also eliminated incomplete clinical information and lacked prognostic information from 529 LGG samples in The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/). 625 LGG glioma samples were screened out from Chinese Glioma Genome Atlas (CGGA, http://www.cgga.org.cn) parts A and B. In this study, cases with ≤ 30 days of survival or those with no survival data were eliminated since they might die of fetal complications rather than glioma. Among the mentioned cases, 447 LGG samples in TCGA and 592 LGG samples in CGGA with complete mRNA expression data and corresponding clinical materials were selected for subsequent analyses. In addition, a control set was used as 940 normal brain samples (including brain tissues in different parts such as cortex, cerebellum, and brainstem) with
complete mRNA_seq data. Considering that batch effects may exist between or within various databases, we used the “normalizeBetweenArrays” function[17] of R package “limma” to remove multiple batch effects from merging the mRNA_seq data of TCGA and GTEx, and CGGA parts A and B.

In addition, Copy number variation (CNV) and somatic mutation data were obtained and analyzed in the cBioPortal dataset (http://www.cbioportal.org/).

Patient samples

The Institutional Ethics Committee approved this study of the Faculty of Medicine at our hospital. All patients using tissue obtained informed consent. Six patients with intracerebral hemorrhage and 24 samples of lower-grade glioma (WHO grade II-III) were collected in May 2019 and April 2021. All patients did not receive chemotherapy or radiotherapy before the operation[18].

Pyroptosis-related gene sets

Gene set, "REACTOME_PYROPTOSIS", was obtained from C2-curated gene sets in the Molecular Signatures Database (http://www.broad.mit.edu/gsea/msigdb/). This gene set contains 27 recognized markers involved in the pyroptosis pathway.

Pyroptosis-based consensus clustering

Performing the ConsesusClusterPlus[19] package in R, unsupervised pyroptosis clusters in the TCGA-LGG dataset were estimated based on the mRNA expression profiles of 27 PRGs, and the construction of pyroptosis clusters was verified by T-distributed stochastic neighbor embedding (t-SNE) algorithm.

Single-sample gene sets enrichment analysis (ssGSEA)

Hallmark gene sets, which summarize and represent specific, well-defined biological states or processes and display coherent expression, were downloaded from the Molecular Signatures Database and chosen for further analysis. The vital biomarkers of 29 immune-related pathways were extracted from the study of Bindea et al. [20]. Based on melanoma mRNA TPM data, single-sample GSEA (ssGSEA)[21] was used to calculate the level of tumor-infiltrating immune cells and activity of hallmark signaling pathway for each sample. (P-value < 0.05 was considered significant). Besides, considering the importance of immune checkpoints (ICPs), including CTLA4, PDCD1, CD274, LAG3, and IDO1 in cancer immunity, we next analyzed their expression levels in both low- and high-score groups.

Dimension reduction and construction of pyroptosis score

In the TCGA-LGG dataset, differentially expressed genes (DEGs) were screened between pyroptosis patterns through the R package “edgeR”. Patients in TCGA-LGG were assigned to different gene clusters through consensus clustering based on the mRNA expression profiles of DEGs among different pyroptosis subtypes. Subsequently, the Boruta algorithm, a wrapper-based feature selection method[22], was performed to handle the identification of the pyroptosis gene signatures (A and B). Principal
component analysis (PCA) was implemented for dimension reduction of the expression profile of gene signatures, which were defined as the signature score A and B (PC1A and PC1B). The pyroptosis score was calculated for each LGG sample, and the formula is as follows:

$$\text{Pyroptosis score (P score)} = \Sigma PC1A - \Sigma PC1B$$

PC1A represents the first component of gene signature A, and PC1B represents the signature B.

Gene sets enrichment analysis

We selected the gene sets "c2.cp.kegg.v7.1.symbols" as the reference. Gene sets enrichment analysis (GSEA) was performed among different pyroptosis score groups. Terms with a false discovery rate (FDR) < 0.05 and nominal P-value < 0.05 were significantly enriched.

Estimation evaluation

According to the ESTIMATE algorithm[24], the relative abundance of stromal and immune cells was quantified as the stromal and immune score based on the whole expression profiles in LGG tissue. The Wilcoxon rank-sum analysis was applied to compare the immune infiltrating level in different pyroptosis score samples.

Tumor mutation analysis in LGG

Somatic mutation data and tumor mutation burden (TMB) among low and high P score groups were visualized using the Maftools package [24]. Then K-M curves were performed to evaluate the correlation between OS and TMB in LGG patients. The association between TMB and pyroptosis was investigated by correlation analysis. (P-value < 0.05 was significant.)

Weight-gene co-expression network analysis (WGCNA)

The weighted gene co-expression network was analyzed by R package "WGCNA"[25]. WGCNA is an algorithm based on the definition of gene co-expressed similarity. This program was performed in the TCGA-LGG dataset based on DEGs among low vs. high P score groups. The Pearson correlation between each pair of differential genes was calculated, and a similarity matrix was obtained. After some alignment, an adjacency matrix was acquired. Then, the topological matrix was created using the topological overlap measure (TOM)[26]. Based on the dynamic hybrid cutting method, we identified the co-expressed gene modules among P score-related genes[27]. Subsequently, we selected the modules with the strongest correlation with P score, and the module genes were chosen for further functional enrichment analysis. Moreover, the genes corresponding to the top ten Maximum Clique Centrality (MCC) according to the algorithm of the “cytoHubba” plug-in[28] were identified as hub genes. In addition, the protein expression levels of these ten hub genes among normal and LGG tissues were investigated in the Human Protein Atlas (HPA, https://www.proteinatlas.org/).

Quantitative real-time PCR
Prognosis-related hub gene RNA was extracted from tissues and cells by Trizol reagent (Invitrogen, Carlsbad, California, USA). cDNA was synthesized using primescript RT Kit (RR047a, Takara, Japan). We used SYBR premix ex Taq II (RR820a, Takara, Kusatsu, Japan) and Bio-Rad CFX Manager 2.1 real-time PCR system to detect mRNA levels according to the specifications provided by the manufacturer. The appropriate Ct method was used to compare the experimental and control groups' data, and GADPH was used as the internal control[29]. We detected the expression of BUB1 and KIF11 samples collected in our hospital, the control and two hub genes' primer sequences are as following: GAPDH 5′-GGAGCGAGATCCCTCCAAAAT-3′(Forward), 5′-GGCTGTTGTCATACTTCTCATGG-3′(Reverse), BUB1 5′-TGGGAAAGATACATACAGTGGGT-3’ (Forward), 5′-AGGGGATGACAGGGTTCCAAT-3’ (Reverse), and KIF11 5′-TCCCTTGGCTGGTATAATTCCA-3′ (Forward), 5′-GTTACGGGGATCATCAACATCT-3’ (Reverse).

Results

Genomics landscape of pyroptosis-related genes (PRGs) in LGG

We investigated the expression of PRGs among non-tumor and LGG tissues. As shown in Fig. 2A, eight genes were upregulated while five were downregulated in LGG tissues. Then, the correlation of PRGs expression was investigated through the PPI network, which showed a strong co-expression correlation among PRGs (Fig. 2B). In addition, Fig. 2C showed the CNV and somatic mutational status of pyroptosis-related genes in TCGA-LGG according to the cBioPortal dataset. Except for BAK1, CHMP2B, and IL1B, most members of pyroptosis genes have a particular frequency of mutation and copy number variation (CNV).

Identification of three pyroptosis patterns in LGG

In the TCGA database, based on the expression profile of 27 PRGs, 529 LGG patients were assigned to the three pyroptosis-related molecular patterns, pyroptosis cluster 1 (C1, n = 284), cluster 2 (C2, n = 203), and cluster 3 (C3, n = 42), respectively (Fig. 3A-C). Principal component analysis (PCA) showed that the three clusters could be well distinguished(Fig. 3D). Moreover, patients in the three pyroptosis patterns had distinct overall survival (OS) (Fig. 3E), which suggested that the pyroptosis pattern might be related to the prognosis of patients. Patients in C1, a group with lower activity of pyroptosis, had a significantly better outcome than other clusters. Figure 3F visualized distinct clinicopathological features among the three patterns. In addition, we found significant differences in the tumor immune microenvironment (TIME) features in the three patterns, including the scores of immune cell hallmarks (Fig. 3G) and immune function pathways (Fig. 3H). The enrichment scores of macrophages and T cells were significantly higher in C3 (a cluster with the most increased activity of pyroptosis). Consistent with the results obtained with the TCGA cohort, the samples were divided into three pyroptosis subtypes with distinct prognoses (figure S1A-D). PCA confirmed the rationality of typing (figure S1E). These results indicated that the pyroptosis pattern could distinguish different characteristics of TME and clinicopathological molecular manifestations.

Development of two pyroptosis-related gene clusters for LGG
There were a total of 3404 DEGs identified among three pyroptosis patterns. Based on the mRNA expression profile of DEGs, LGG patients were divided into two gene clusters (A and B) through unsupervised clustering (Fig. 4A-C). Through the PCA method, dimension reduction was presented based on the pyroptosis gene signatures A and B, discriminated through the “Boruta” algorithm from gene clusters A and B. Heatmap depicted the expression level of these gene signatures and distribution of clinical features among two pyroptosis gene clusters (Fig. 4D). Meanwhile, the survival analysis and three-dimensional PCA also confirmed the rationality of cluster assignments (Fig. 4E, F), the LGG patients in gene cluster B exhibited more inferior OS (p < 0.05). Then, we found different TME features between the two gene clusters. Gene cluster B showed significantly higher infiltrating of hallmark immune cells and activity of immunity-related signaling pathways than gene cluster A (Fig. 4G, H). We confirmed these results in the CGGA cohort (Figure S2A-F). Overall, the consistency between the prognosis and TIME characteristics of the two gene clusters indicated that this classification was of value for further research.

Construction of the pyroptosis scoring system in LGG

According to the formula mentioned above, the pyroptosis score (P score) = \sum PC1A - \sum PC1B, and the pyroptosis scores for LGG samples were calculated. Patients were then assigned to the high (n = 61) and low (n = 386) pyroptosis score groups according to the best cut-off value (7.244834) calculated through survival analysis. We explored the clinical relevance and prognostic value of pyroptosis scores in TCGA and CGGA cohorts. Figure 5A displayed the survival status and gene cluster distribution in two pyroptosis score groups. We found a higher pyroptosis score usually implied an unfavorable prognosis for LGG patients (P < 0.001; Fig. 5B). Meanwhile, multivariate Cox regression analysis further confirmed that pyroptosis score could be an independent factor in predicting the OS of LGG patients (Fig. 5C). The AUC of the ROC curve for predicting 1-, 3- and 5-year OS by pyroptosis scoring system were respectively 0.708, 0.711, and 0.677, which were higher than most clinical risk factors (Fig. 5D-F). In addition, the level of pyroptosis score increased with increasing WHO grade(II-III) of glioma and correlated with the clinical factors, including age, 1p19q co-deletion status, and IDH mutation status (Fig. 5G-K). To investigate the predictive value of pyroptosis score independent of IDH mutation status, 1p19q co-deletion quality, and WHO classification, we divided the samples into four subtypes. As shown in Fig. 5L-P, patients with a higher P score in the different IDH mutation, 1p19q co-deletion status, and WHO III subtypes had worse OS than patients with lower P scores (p < 0.05). While in the WHO II grade, there was no significant prognosis difference among low and high pyroptosis score groups (Fig. 5Q). Generally, patients with low P scores had significantly better prognostic outcomes under the same condition. These results were consistent with our previous conclusions.

Moreover, the P score was associated with the survival and clinicopathological features in the validation cohorts (Figure S3). Similarly, LGG patients with higher P scores had worse OS (Figure S3A). Compared with other clinical factors, the P score was an independent prognosis factor in LGG (Figure S3B). The AUC of the ROC curve for predicting 1-, 3- and 5-year OS by pyroptosis scoring system were 0.856, 0.879, and 0.844, respectively (Figure S3C). Patients older, with wildtype IDH, non-codeletion 1p19q, and higher
grades had higher P scores in LGG (Figure S3D-H). Meanwhile, we demonstrated the prognostic value of P score independent of IDH mutation status, 1p19q co-deletion status, and WHO grade (Figure S3I-K).

The underlying mechanism of pyroptosis score in LGG

The gene sets variation analysis (GSVA) is a non-parametric unsupervised method for assessing gene set enrichment in gene expression microarray and RNA-seq data[30]. GSVA of important hallmarks showed that signaling pathways related to oncogenic transformation and tumorigenesis were enriched in the high pyroptosis score group (Fig. 6A), including PI3k-Akt signaling and Epithelial-mesenchymal transition pathway. Thus, it suggested that these cancer-related signaling pathways activated in the high P score group. Further, Kyoto Encyclopedia of Genes and Genomes (KEGG) results showed that cell cycle, focal adhesion, JAK-STAT signaling, and other critical cancer-related pathways were significantly enriched in the high pyroptosis group (Fig. 6B). Moreover, we explored the activity of immune molecule-related and signaling immune pathways in low and high pyroptosis score groups. Figures 6C and 6D showed that the enrichment scores of immune cell hallmarks and immunity-related signaling pathways were more elevated in high P score groups. In addition, the results of the immune cells infiltrating level analysis (Fig. 6E) showed that CD4 and CD8 T cells, B cells, T regulatory cells (Tregs), and macrophages (M1 and M2) were positively correlated with pyroptosis scores. In addition, LGG patients in the high pyroptosis score group had higher expression levels of five vital immune checkpoints (Fig. 6F). Collectively, pyroptosis score was significantly associated with a higher activity of TIME which may indicate mainly immunosuppressive microenvironment in LGG. As a positive correlation between pyroptosis scores and the expression level of immune checkpoints, we could speculate that LGG patients with higher pyroptosis scores may benefit more from ICB therapies.

The mutation landscape among two pyroptosis score groups

Studies indicated that higher TMB and somatic mutation rates are associated with stronger anti-tumor immunity[31]. Survival analysis revealed that samples with higher TMB had worse prognoses than samples with lower TMB in TCGA-LGG (Fig. 7A). In addition, K-M curves of pyroptosis scores combined with TMB indicated that patients with higher TMB and pyroptosis scores had worse OS than other groups. In comparison, samples with lower risk scores and TMB had the best prognosis (Fig. 7B). These confirmed the relative ability of P score and TMB for predicting the prognosis of patients with LGG. The difference analysis of TMB among low and high-pyroptosis score groups in TCGA-LGG (Fig. 7C) and correlation analysis showed that TMB significantly correlated to pyroptosis score (Fig. 7D).

Furthermore, 20 genes, including IDH1, TP53, and ATRX, were most frequently mutated in each subtype (Fig. 7E, F). These findings indicated that the pyroptosis score was positively correlated to the TMB and somatic mutations in LGG samples. Therefore, we inferred that patients with higher pyroptosis scores might benefit more from anti-cancer immunotherapies.

WGCNA of DEGs among different P score groups
 Crucially, we then need to screen out the relevant biomarkers of P score for further research. A total of 4808 DEGs with adjustable p-value < 0.05 and log|FC| > 1 were identified between the low and high pyroptosis score groups, including 2876 up- and 1,932 down-regulated genes (Fig. 8A). Select 3 as the soft-thresholding power value based on the scale-free fit index and the mean connectivity shown in Fig. 8B, C. Colors of dendrogram branches indicate different gene clusters. In contrast, the upper dendrogram shows sample clustering (Fig. 8D). The blue module (MEblue) positively correlated to the pyroptosis score in LGG samples (MEblue: rho: 0.6, p < 0.001) was selected (Fig. 8E). There was a strong correlation between module membership in the blue module and gene significance for the P score (cor: 0.13, p < 0.001, Fig. 8F). Subsequently, through the protein-protein interaction (PPI) network, we found that blue module genes were strongly co-expressed (Fig. 8G). GO, and KEGG enrichment analysis revealed that MEblue genes were distinctly enriched in cell cycle, P53 signaling pathways, ATPase activity, chromosomal region, and other critical terms in tumors (Fig. 8H).

Verification of hub genes in the blue module

Based on the MCC score calculated by cytoHubba in Cytoscape software (Fig. 9A), the top ten hub genes in the PPI network were selected, including NCAPG, CCNB1, KIF23, KIF11, CCNA2, BUB1B, CCNB2, MELK, TPX2, and BUB1. These hub genes’ mRNA expression levels were upregulated in LGG tissues (Fig. 9B). Further, Cox regression analysis investigated the prognosis value of the ten hub genes. Two prognostic-related hub genes identified, BUB1 and KIF11 could serve as independent risk factors to predict the OS of LGG patients (Fig. 9C). Moreover, we confirmed the protein expression level of KIF11 (Fig. 9D, E) in the HPA dataset, while no valuable data on BUB1 was found.

In addition, we found that the relative mRNA expression level of BUB1 and KIF11 were significantly upregulated in LGG compared to control brain tissues in our hospital samples. (Fig. 9F, G).

Discussion

This work comprehensively analyzed the genomic alterations, clinical implications, and TIME features of pyroptosis-related patterns in TCGA-LGG. Moreover, we constructed a pyroptosis scoring system to assess pyroptosis status for LGG patients. This scoring system could assist researchers in making more efficient and practical immunotherapeutic strategies for patients. These improved the comprehension of pyroptosis for TME in LGG.

Most members participating in the pyroptosis pathway have significant expression levels expressed among normal brain and LGG tissues. Furthermore, some CNVs and single nucleotide polymorphisms (SNP) of PRGs in LGG suggested these genes may involve in the progression of LGG and indicated that relatively low genome stability prevented the occurrence of genomic mutation.

Three molecular pyroptosis patterns developed in LGG had distinct differences in prognosis, immune infiltrations, and functions. Then two gene clusters were identified based on the DEGs correlated with the pyroptosis patterns, which also had distinct clinical outcomes and immune cell infiltrations and roles in
LGG. Moreover, the pyroptosis score was calculated by the Boruta and PCA algorithm for each LGG sample. We found that LGG patients with higher pyroptosis scores always have a poor prognosis and were associated with a higher infiltrating level of immune cells and activation of immune signaling pathways. Pyroptosis is a proinflammatory form of regulated cell death, regarded as a general immune effector in multiple cells[32]. In this study, pyroptosis score was positively correlated to the immune infiltrating level and activity of immune-related signaling pathways.

It was demonstrated that chronic tumor necrosis, induced by pyroptosis of a small population of cancer cells in the central hypoxic region of the tumor, may promote tumor progression[33]. In our study, pyroptosis score was significantly associated with clinicopathological characteristics of LGG patients. Moreover, we proved that pyroptosis score was an independent risk factor for the prognosis of LGG, which had high predictive efficacy for 1-, 3-, and 5-year OS.

Effective immunotherapy requires thorough knowledge of the tumor microenvironment[23], and the suppressive tumor microenvironment is a critical barrier to cancer immunotherapy for gliomas[34]. Immunotherapy designed against human gliomas is fundamentally challenged by the sophisticated interactions between gliomas and their immunological environments[35]. The understanding of the complexity and diversity of TME has been improved with the progress of research, and the impact of TME on immunotherapy has also been studied in depth. By analyzing the special categories and subclasses of tumor immune microenvironment (TIME) in gliomas, the ability to predict and guide immunotherapy will be improved, and new therapeutic targets will be revealed[36]. Therefore, a considerable number of patients with low-grade gliomas may benefit from immunotherapy. It's vital for this study to identify the subtypes that can benefit from immunotherapy through molecular pathological features. Our data indicated that the pyroptosis score could distinguish the immune subtypes among LGG patients. The low score group corresponded to the immune desert type's immune state, while the higher pyroptosis score group represented immunophenotyping of pro-inflammatory. Moreover, the focus of cancer immunotherapy has shifted towards immune checkpoint inhibitors. The effect of immunotherapy in cancer patients depends largely on the expression level of immune checkpoints[37]. In this study, the levels of immune checkpoints were positively correlated to pyroptosis scores. It suggested that LGG patients with a higher pyroptosis score may benefit more from ICB therapies.

The accumulation of genetic mutations may lead to the development of cancers[38], and the higher the mutation load, the higher the malignancy of the tumor[39]. Moreover, it has been reported that the mutation load determines the sensitivity of cancer to ICB[40]. Our data confirmed a distinct difference in genetic and tumor mutations between high and low pyroptosis score groups. Lower TMB was associated with better survival outcomes in patients with LGG, which was consistent with our conclusion[41].

Through WGCNA of DEGs among low and high score groups, we identified two prognosis-related hub genes most associated with pyroptosis score in LGG. BUB1 encodes a serine/threonine-protein kinase that plays a central role in mitosis, and it was reported that this encoded protein might also function in the DNA damage response[42]. In addition, mutations in this gene have been associated with aneuploidy
and several forms of cancer[43, 44]. In this study, BUB1, upregulated in tumor tissues, was considered a novel prognosis-related biomarker in LGG. However, it needs further research. Kinesin family member 11 (KIF11) was identified to encode a motor protein belonging to the kinesin-like protein family involved in various spindle dynamics. Shreds of evidence showed KIF11 is upregulated in colorectal cancer and serves as an oncogene via p53/GSK3β signaling and biomarker for assessing oxaliplatin sensitivity for patients[45]. KIF11 was considered a key driver of malignancy in glioblastoma, and KIF11 could promote the stemness in glioma cells, accompanied by increased proliferation and chemoresistance of glioma[46]. However, the association between KIF11 and pyroptosis in LGG will be the direction of further study.

Conclusion

This study systematically studied the underlying role of pyroptosis hallmarks in LGG. The pyroptosis scoring system constructed in this study is of great clinical significance in evaluating the prognosis and predicting the immunotherapy benefits for LGG patients. In addition, BUB1 and KIF11 were considered critical bio-markers of the pyroptosis score in LGG.

Abbreviations


Declarations

Authors' contributions

XC and MW contributed to conception and design of this study. CR, XC, YX, and MW contributed to the analysis and interpretation of data. All authors read and approved the final manuscript.

Funding

This work was supported in part by the National Natural Science Foundation of China (No. 82173285).

Data availability

Publicly available datasets were analyzed in this study. This data can be found below:

2. GTEx, https://www.genome.gov/Funded-Programs-Projects/Genotype-Tissue-Expression-Project;

**Ethics approval and consent to participate**

This study was conducted in accordance with the Declaration of The First Affiliated Hospital of Xi’an Jiaotong University and was approved by the ethics committee of The First Affiliated Hospital of Xi’an Jiaotong University (ethical batch number: 2021-695). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all subjects/participants and/or legal guardian(s).

**Consent for publication**

Not applicable.

**Competing interest**

The authors declare that they have no conflict of interest.

**Acknowledgments**

We gratefully acknowledge The Cancer Genome Atlas pilot project, Genotype-Tissue Expression Project, and the Human Protein Atlas, which made the genomic data and clinical data of glioma available.

**References**


42. Ricke RM, Jeganathan KB, van Deursen JM. Bub1 overexpression induces aneuploidy and tumor formation through Aurora B kinase hyperactivation. The Journal of cell biology [Internet]. 2011; Available from: http://dx.doi.org/10.1083/jcb.201012035.


Figures
Figure 1

Flowchart of this research.
Figure 2

The genomic characterization of pyroptosis-related genes (PRGs). (A) Heatmap for differentially expressed PRGs among normal brain and lower-grade glioma (LGG) tissues. (B) Protein-protein interaction network of PRGs in STRING database. (C) CNV and SNP of PRGs in LGG according to cBioPortal database. T: tumor, N: normal, CNV: copy number variation, SNP: single-nucleotide polymorphism.
Figure 3

TME characteristics and prognostic outcomes in three pyroptosis patterns. (A) Consensus clustering matrix of TCGA-LGG samples for k = 2 and 3. (B) Consensus clustering CDF for k = 2 to k = 9. (C) Relative changes in the area under the CDF curve by group number. (D) Principal Component Analysis (PCA) in LGG samples when K=3. (E) Survival analysis of 3 clusters in TCGA-LGG datasets. (F) Heatmap for clinicopathological features of the three pyroptosis patterns, terms with red color represented a significant correlation between pyroptosis patterns and clinical factors (p< 0.05). (G) Box plots showed immune cell hallmark scores differenced in the three clusters. (H) Box plots depicted the differences in immunity-related pathways in the three clusters. CDF, cumulative distribution function; Codel, codeletion;
IDH, isocitrate dehydrogenase; Noncodel, non-codeletion; OS, overall survival. ns: not significant; *p<0.05; **p<0.01; ***p<0.001.

**Figure 4**

Pyroptosis-related gene clusters with different prognosis and immunity features in LGG. (A) Consensus clustering matrix of TCGA-LGG samples for k = 2. (B) Consensus clustering CDF for k = 2 to k = 9. (C) Relative changes in the area under the CDF curve by group number. (D) Distinct clinicopathological characteristics in the two pyroptosis gene clusters, terms with red color represented a significant correlation between pyroptosis gene clusters and clinical characteristics (p< 0.05). (E) K-M curves of two gene clusters. (F) PCA about the gene clusters' classification. (G) Box plots depicted immune cell infiltration scores and (H) the differences in immunity pathway scores in the two clusters. Ns: not significant; *p<0.05; **p<0.01; ***p<0.001.
Figure 5

Development and verification of the pyroptosis scoring system for LGG. (A) Alluvial diagram of survival status, pyroptosis score groups, and gene clusters. (B) Survival analysis for two pyroptosis score groups. (C) Forest plot for multivariate Cox regression analysis. (D-F) ROC curve of a predictive test based on pyroptosis score and other clinical factors for 1-, 3- and 5-year OS. (G-K) Box plot depicted the differences in pyroptosis scores among the 1p19q-codeletion status, age groups, survival status, WHO grade, and IDH.
mutation status. Ns: not significant; *p<0.05; **p<0.01; ***p<0.001. (L-Q) Survival analysis of pyroptosis score in different IDH mutation status, 1p19q co-deletion status, and WHO grade in TCGA-LGG.

**Figure 6**

Functional analysis of pyroptosis score in LGG. (A) Heatmap for the contribution of gene set variation analysis (GSVA) scores of Hallmarks in low and high-pyroptosis score groups, terms with red color represent positively correlated with pyroptosis score, and blue terms were negatively correlated with the score. (B) GSEA identified cancer-related pathways enriched in the high pyroptosis score. (C) Box plots depicted immune cell infiltration scores and immune, and stromal scores differed in pyroptosis score groups. (D) Box plots showed the differences in immunity pathway scores in the two groups. (E) The
differences in immune cells’ abundance, calculated by Cibersort, among the two groups. (F) The expression level of five vital immune checkpoints in two groups. Ns: not significant; *p<0.05; **p<0.01; ***p<0.001.

Figure 7

Association between pyroptosis score and tumor mutation burden in LGG. (A) Survival analysis of TMB and OS of the patients with LGG in TCGA. (B) K-M curves of TMB combined with pyroptosis score in TCGA-LGG. (C) Difference analysis of TMB among low and high-risk subtypes in LGG patients. (D) Correlation analysis between TMB and pyroptosis score in different score groups. (E) Top 30 highly mutated genes in high pyroptosis score group. (F) Top 30 highly mutated genes in LGG low-score group. * p < 0.01, ** p < 0.001 and *** p < 0.0001.
Figure 8

WGCNA of DEGs between pyroptosis score groups in TCGA-LGG. (A) Volcano plot diagrams show DEGs. (B) Scale-free fit index and (C) mean connectivity for various soft-thresholding powers (β). (D) DEGs were clustered using hierarchical clustering with a dynamic tree cut and merged based on a dissimilarity measure (1-TOM). (E) Relationship analysis between Traits and modules. (F) Scatterplot of gene
significance (GS. group) versus module membership (KME) for the blue module. (G) PPI network analysis of blue module genes. (H) GO and KEGG analysis of blue module genes.

**Figure 9**

Identify hub genes in the blue module. (A) The top 10 genes in the blue module are ranked by MCC value. (B) The expression analysis of top 10 genes in normal brain and LGG tissues in TCGA-LGG. (C) Multivariate Cox regression analysis of 10 genes. The expression level of KIF11 protein in (D) normal brain and (E) LGG tissue according to the HPA database. Rt-PCR results of (F) BUB1 and (G) KIF11 from patients in our hospital.

**Supplementary Files**
This is a list of supplementary files associated with this preprint. Click to download.

- FigureS1.tif
- FigureS2.tif
- FigureS3.tif