**Gasdermin Family as an Underlying Key Factor in Glioma**

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**Abstract**

Pyroptosis is a programmed cell death process mediated by gasdermins (GSDMs). The prognostic value of pyroptosis-related genes in different tumor types has been gradually demonstrated recently. However, the prognostic impact of GSDMs expression in glioma remains unclear. Here, we present a comprehensive bioinformatic analysis of gasdermin family member gene expression, producing a prognostic model for glioma and creating a competing endogenous RNA (ceRNA) network. We identify a significant association between expression of GSDMD and GSDME and glioma stage, and demonstrate that high expression of both GSDMD and GSDME is associated with shorter survival. Conversely, low expression of GSDMC was associated with shorter survival. Least absolute shrinkage and selection operator (LASSO) cox regression analysis was used to construct a prognostic gene model based on the four prognostic gasdermin family genes (GSDMC, GSDMD, GSDME and PJVK). This model was able to predict overall survival of glioma patients with high accuracy. We show that gasdermin family genes are expressed primary by immune cells, endothelial cells and neuronal cells in the tumor microenvironment, rather than by malignant tumor cells. T cells were significantly activated in high-risk patients; however, the activation induced cell death (AICD) pathway was also significantly activated, suggesting widespread expiration of cytotoxic T lymphocytes (CTLs), facilitating tumor progression. We also identify the lncRNA/miR-296-5p/GSDMD regulatory axis as an important player in glioma progression. In conclusion, we have conducted a comprehensive bioinformatic analysis identifying the importance of gasdermin family members in glioma; a prognostic algorithm containing 4 genes (GSDMC, GSDMD, GSDME, and PJVK) was constructed.

**Keywords**: Gasdermin，Pyroptosis，Glioma，Prognostic model, AICD

**Abbreviations**

ceRNA competing endogenous RNA

LASSO Least absolute shrinkage and selection operator

AICD activation induced cell death AICD

CTLs cytotoxic T lymphocytes CTLs

PFD pore-forming domain

RD repressor domain

TCGA The Cancer Genome Atlas

CGGA Chinese Glioma Genome Atlas

GSVA Gene set variation analysis

scRNA-seq Single-cell RNA-seq

DAC dexitabine

TMZ temozolomide

TMB tumor mutation burden

GBM glioblastoma multiforme

LGG lower grade glioma

**Introduction**

Glioma is the most common and fatal tumor in adult central nervous system. (Louis, Perry et al. 2021). Molecular research in glioma has advanced substantially; modern molecular classification is now combined with traditional histological classification to optimize glioma diagnosis, patient prognostication and prediction of treatment response(Mischel and Cloughesy 2003, Schonberg, Lubelski et al. 2014, Molinaro, Taylor et al. 2019). However, these classification systems do not fully resolve individual patient variation and are therefore suboptimal in providing risk stratification for glioma patients. Integration of further data is therefore required, with gene expression profiling representing a leading candidate method for further improving glioma classification(Tirosh and Suva 2018).

In 2015, pyroptosis was defined as a programmed death process mediated by gasdermins (Kayagaki, Stowe et al. 2015, Shi, Zhao et al. 2015). The gasdermin superfamily (GSDMs) consists of human gasdermin A/B/C/D (GSDMA/B/C/D), gasdermin E (also known as DFNA5), and DFNB59 (also known as PJVK) (in mice by GSDMA1/2/3 , GSDMC1/2/3/4, GSDMD, DFNA5, and DFNB59)(Broz, Pelegrin et al. 2020). Except PJVK, all gasdermin proteins are composed of C-terminal restriction fragments(RD) and N-terminal pore-forming fragments(PFD) (Shi, Zhao et al. 2015). When stimulated, gasdermins are cleaved by granzyme or caspase, C-terminal RD solved the limitation of N-terminal PFD, and then the N-terminal shifted to the cell membrane for punching. Pores are formed on the cell surface leading to the release of inflammatory molecules and cell pyroptosis. Early studies identified that pyroptosis mainly occurs in macrophages; in a study of mouse macrophage treatment with anthrax lethal toxin, Friedlander demonstrated rapid release of cell contents and cellular death(Friedlander 1986). Gasdermin proteins have been linked to human diseases in the scientific literature; however, the specific mechanisms by which gasdermin proteins play a role in disease processes is poorly understood.

Pyroptosis has recently been identified to play a dual role in tumor development and therapeutic response. Pyroptosis plays a dual role in tumor, promoting tumor death on the one hand and leading to immunosuppressive microenvironment on the other hand(Tan, Huang et al. 2020). Recent studies have determined the relationship between pyroptosis-related genes and prognosis in lung and ovarian cancer(Lin, Chen et al. 2021, Ye, Dai et al. 2021). However, these gene sets primarily comprise inflammasome-related genes. GSDMs are the effectors of cell pyroptosis. In vitro investigations have demonstrated that gasdermin activation induces cell death in glioma cells(Kay, Wang et al. 2020). However, Gliomas are composed of malignant and non-tumor cells. These non-malignant populations dilute the purity of glioma cells and play an important role in tumor biology(Zhang, Cheng et al. 2017). When studying the effects of gasdermin family members on glioma in tumor specimens, attention should be paid to its expression distribution among different cell types within the tumor microenvironment. At present, no research has systematically characterised the relationship between the gasdermin family on the prognosis and clinical characteristics of glioma.

In the present study, we perform bioinformatic analysis to characterize the expression of gasdermin family members in patients with gliomas. We utilize the Cancer Genome Atlas (TCGA) and the Chinese Glioma Genome Atlas (CGGA) RNA sequencing data sets to investigate the relationship between gasdermin gene expression profiles, comparing these to patient survival. We use the Least Absolute Shrinkage and Selection Operator (LASSO) to develop 4 genetic risk characteristics. We investigated the relationship between risk score and clinical characteristics in patients with glioma. The Single-cell RNA-seq (scRNA-seq) results demonstrate that expression of gasdermins is mainly confined to monocytes/macrophages and CD8+ T cells. Gene set variation analysis(GSVA) showed that gasdermins are significantly related to the AICD pathway of T cells. Western blot results showed that antigen treatment could significantly activate caspase-1/GSDMD pathway and induce T cells to pyroptosis. We also construct an mRNA-miRNA-lncRNA interaction network to elucidate the underlying molecular mechanism of GSDMD in glioma. This study is the first to investigate the expression of GSDMs and its effect on clinical and molecular characteristics.

**Result**

**Relationships of GSDMs expression with clinical and molecular characteristics, mutation landscape, and tissue localization in glioma**

Six genes in the GSDM family were analyzed using the glioma transcriptome data from TCGA and CGGA. This showed that GSDMD and GSDME were expressed highly in gliomas relative to GSDMA, GSDMB, GSDMC and PJVK and there was a significant correlation between them. For GSDMD, expression was significantly higher in WHO IV than in WHO II/III(Fig 1A). Protein-Protein Interaction(PPI) network demonstrated that genes related to pyroptosis interacted with one another, and the GSDMD is a hub gene with local clustering coefficient was 0.625(Fig 1B) . Figure 1C shows the position of CNV changes within these 6 genes on the respective chromosome. GSDM family genes were common targets of copy number amplification and deletion (Fig 1C). The frequency of mutation in GSDMA, GSDMB, GSDMC, GSDMD, GSDME and PJVK were 0.4%, 0.1%, 3%, 3%, 0.6%, 0.5%, respectively (Fig 1D), and the main genomic aberrations type is amplification. Both amplification and deep deletion events were observed in GSDMD，while both amplification and missense mutation events were identified in GSDMC (Fig 1D). In order to determine the source cell type of GSDM family gene expression within glioma tissue, we analyzed two single-cell RNA-seq (scRNA-seq) datasets ((GSE89567, GSE102130, GSE103224 and GSE135437)). This identified monocytes/macrophages, T cells and neurons as the major GSDM-expressing cell types, with low expression in malignant cells (Fig 1E).

**TMB, drug sensitivity analysis and prognostic impact of GSDMs**

Temozolomide (TMZ) is an oral alkylating agent that can cross the blood-brain barrier to reach the foci. It is one of the first-line commonly used drugs in clinical chemotherapy for gliomas(Zhao and Ma 2021). Next, we discussed the relationship between gasdermin gene expression level and treatment sensitivity of temozolomide.There was a significant positive correlation between GSDMD and TMZ sensitivity (Fig 2A). It has been found that the GSDMD promoter region of tumor cells is hypermethylated, which inhibits its expression level. Next, we explore the expression of GSDM family genes in brain lower grade glioma(LGG). Therefore, we used dexitabine (DAC) combined with temozolomide（TMZ）to treat glioma cells U251.The cells were pretreated with DAC for 24 hours and then incubated with temozolomide. CCK8 showed that dexitabine could evidently enhance the sensitivity of U251 to temozolomide. At the same time, western blot results showed that GSDMD expression was up-regulated by dexitabine treatment. Combined treatment could significantly activate caspase-1/GSDMD pathway(Fig 2B, C).The correlation between GSDM family genes and tumor mutation burden (TMB) was investigated; TMB was positively correlated with GSDMA and GSDMD, and negatively correlated with GSDMC and PJVK. GSDMB or GSDME has no correlation with TMB (Fig 2D).

Then we analyzed the prognostic value of gasdermin family by univariate Cox regression analysis, and analyzed prediction accuracy for one-year, three-year and five-year survival by ROC analysis. The survival of glioma patients with high GSDMA expression was poor (Fig. 2E, p = 0.0042); similarly, survival time was low in patients with high expression of GSDME (Fig. 2I, p = 0.00027) and GSDMD (Fig. 2H, p < 0.0001). Conversely, low GSDMC expression was associated with poor survival (Fig. 2G, p = 0.00051). ROC curve analysis demonstrated our model had good predictive efficacy (GSDMA: AUC = 0.64 for 1 year, 0.56 for 2 year, and 0.52 for 3 year survival; GSDMC: AUC = 0.63 for 1 year, 0.61 for 2 year, and 0.55 for 3 year survival; GSDMD: AUC = 0.78 for 1 year, 0.75 for 2 year, and 0.68 for 3 year survival; GSDME: AUC = 0.61 for 1 year, 0.61 for 2 year, and 0.54 for 3 year survival) (Fig 2E-J).

**Construction of a prognostic model**

The prognostic models by 6 genes in GSDM family were constructed by LASSO Cox regression analysis. Figure 3A shows the general situation of LASSO coefficients of 6 genes. Figure 3B shows the ten times the cross-validation error rate. Riskscore=(0.2483)\*GSDMC+(0.7607)\*GSDM-D+(0.1264)\*GSDME+(-0.2989)\*PJVK. Patients with glioma were divided into two groups according to risk score. Survival status , risk score distribution, and expression of the four genes are shown in Figure 3C. Increased risk score was associated with shorter survival time and increased risk of death(Fig 3C). Besides Kaplan–Meier analysis showed poorer survival in patients with higher risk scores compared to lower risk scores (Fig. 3D), with the ROC curve, the AUC of 1, 3 and 5 years were 0.819, 0.789, and 0.672, respectively (Fig 1D).

Finally, we harnessed two glioma patient datasets of CGGA to demonstrate the association between high risk score and shorter survival. CGGA data for 301 and 325 patients also showed that patients with high risk glioma score had lower overall survival probability than patients with low risk glioma score (Fig 3E-F). At the same time, we found that riskscore can predict the prognosis and recurrence of radiotherapy in patients with LGG well(Figure S2-3). But in GBM, the prediction effect of the model is was not satisfying. (Figure S1).

**Riskscore in relation to clinical and molecular characteristics, mutations, and methylation in glioma**

Next, we explored differences in risk score according to molecular and pathological characteristics of glioma. Higher risk was parallels with higher histopathological glioma grade (Fig 4A-C). The classical and mesenchymal subtypes of glioma demonstrated higher risk scores (Fig 4D-E). ROC analysis was used to assess the ability of risk score to distinguish between glioma mesenchymal cell subtypes and classical cell subtypes. The ROC AUC was 86.6% and 89.2% in the TCGA and CGGA cohorts, respectively (Fig 4D-E). These data suggest a potentially important role for the calculated risk score in glioma progression. To some extent, the risk score can represent the classical subtype. The risk score for WHO IV was higher than that of WHO II and III (Fig 4F). Moreover, the risk score of IDH mutation cases was lower than those with IDH wild type(Fig 4G); cases with chromosome 1p/19qnon-co-deletion also demonstrated a higher risk score. The unmethylated MGMT cases also demonstrated a higher risk score (Fig 4H-I).

Together, these data show that glioma patients in the wild-type IDH,1p/19q non-co-deletion, unmethylated MGMT and WHO IV groups have higher risk scores. This is consistent with the poor prognosis reported in IDH wild type cases and relatively favorable outcome of patients with 1p/19q co-deletion or MGMT methylation(Barthel, Johnson et al. 2019, Brandner, McAleenan et al. 2021, Pirozzi and Yan 2021).

**The relationship between risk score and immune infiltration**

We further explored the relationship between risk score and immune infiltration within the tumor. We first calculated the ssGSVA scores of all kinds of immune cells in glioma tissues. Burden of the major immune cell types DCs, B cells, T cells in tumor microenvironment were associated with high risk scores (Fig 5A). The immune score, stromal score, and ESTIMATE score were determined using on the TCGA cases. There were apparent correlations between the risk score and stromal, immune and ESTIMATE scores (Fig 5B). Spearman's correlation analysis demonstrated significantly correlation between the risk score and immune contexture (r = 0.6, p < 2.2e-16), stromal contexture (r = 0.59, p < 2.2e-16) and Estimate scroe (r = 0.62, p < 2.2e-16) (Fig 5B).

Next, we analyzed the correlation between risk score and immune checkpoint molecules such as PD1, PD-L1, LAG3， CTLA4, and IDO1. We identified significant positive correlations between the risk score and immune checkpoint molecules (Fig 5C). We then confirmed the relationship between GSDMs and T cell immune response in gliomas using GSVA analysis. An important result that emerged from the data was that the GSDMs gene is associated with activation-induced cell death (AICD) of T cells(Fig 5D), which is very important to mediate T cell tolerance. (Huang, Chen et al. 2018). Flow cytometry showed that Jurkat treated with cell fragments could significantly induce the increase of Annexin V**-**PI**+** cells. This suggests that antigen stimulation may induce pyroptosis of tumor cells. Furthermore, using western blot detection, we found that antigen stimulation could significantly induce the activation of caspase-1/GSDMD pathway in tumor cells (Fig 5E, F).Next, we will selected the genes with risk score correlation coefficient R > 0.5 for gene enrichment analysis; 1163 genes were identified in TCGA and 755 genes were identified in the CGGA set for gene ontology analysis (Fig 5G). The results showed that high risk score was closely related with immune response, neutrophil activation and T cell activation, alongside other pathways(Fig 5H).

**Risk score is associated with different patterns of genomic changes**

The TCGA dataset investigates genomic change patterns based on risk scores: cases are divided into high risk scores and low risk scores. Mutation frequency comparison revealed higher numbers of somatic mutations in the high risk score cases. Mutations in CIC IDH1, NOTCH1and FUBP1 were significantly enriched in the low risk score cases. High risk score cases demonstrated more frequent mutation of PTEN and NF1. Significant differences in mutations were also detected with high / low risk scores(Fig 6A-B). Next, we studied the changes of somatic cell copy number between patients with low risk score and high. As shown in figure 6C, gliomas with higher risk score demonstrated frequent Chr7 amplification and Chr10 deletion(Fig 6C). However, the incidence of 1p/19q co-deletion decreased with the increase of risk score expression in gliomas. We also examined the correlation between riskscore and genome variation. As the results show, riskscore is positively correlated with TMB, fraction genome altered and aneuploidy Score(Fig 6D).

**Building a predictive nomogram**

We established nomogram to predict survival probability. The result showed that GSDMA expression and GSDMD expression were independent factors affecting the prognosis of glioma patients (Fig 7A). Compared with the ideal model in the whole queue, the 1-year, 3-year and 5-year overall survival rate can be predicted with good efficacy compared using the prognostic nomogram (Fig 7B-C).

**Construction of a network of mRNA–miRNA–lncRNA**

In order to clarify the potential molecular mechanism of GSDMD in glioma, we constructed an mRNA-miRNA-lncRNA interaction network. We identified 4 miRNAs as target mRNAs bound to GSDMD according to miRDB and starBase (Fig 8A). Among them, hsa-miR-296-5p demonstrated the highest target score; we therefore explored its upstream lncRNA target to construct the miRNA-lncRNA axis. Four lncRNAs (KCNQ1OT1, LINC01278, MIRLET7BHG, NEAT1) were identified as target lncRNAs (Fig 8B and 8E). A ceRNA network was visualized; KCNQ1OT1 and LINC01278 were positively correlated with glioma staging, while MIRLET7BHG and NEAT1 were negatively correlated with staging(Fig 8C). Survival analysis demonstrated that high LINC01278 and MIRLET7BHG were associated with better prognosis, while NEAT1 was associated with poorer survival (Fig 8D). There was no significant association between KCNQ1OT1 and survival.

**Discussion**

Pyroptosis can promote tumor development by secreting inflammatory factors leading to immunosuppressive microenvironment(Xia, Wang et al. 2019). Conversely, pyroptosis can promote the immunogenic death of tumor cells and activate anti-tumor immunity, making it a potential tumor prognostic and therapeutic target (Xia, Wang et al. 2019). In ovarian cancer and lung cancer, PRG signature has been identified to predict prognosis(Lin, Chen et al. 2021, Ye, Dai et al. 2021). However, the role of PRG in glioma is not yet clear; we aim to dissect the role of PRG in this disease context.

Glioma is composed of both immune cells and stromal cells, alongside malignant tumor cells. Microglia are non-malignant myeloid-derived cells found in the brain parenchyma; these cells can produce macrophage-like reactions under pathological conditions(Keane, Cheray et al. 2021). Large numbers of microglia have been found within glioma masses, and the degree of microglial infiltration has been positively associated with the degree of glioma malignancy(Gibson and Monje 2021, Xuan, Lesniak et al. 2021). Previous studies have focused on the pyroptosis of malignant cells themselves without considering the impact of pyroptosis in non-malignant cells in the tumor microenvironment.

Immune cell pyroptosis was first discovered in monocytes/macrophages and is an important form of immune cell death6. Therefore, when exploring the impact of PRG in glioma, its "tissue specificity" must be considered. We demonstrate that gasdermin family genes are mainly expressed in immune cells rather than tumor cells. We also identify a large number of aggregates in endothelial and neuronal cells. We found that due to the methylation of GSDME gene promoter, GSDME expression was reduced in multiple tumor cell lines, while GSDME was widely expressed in normal tissues. In Gsdme-/- mice, intraperitoneal injection of 5-FU resulted in severe gastrointestinal injury due to infiltration of immune cells(Wang, Yin et al. 2018). In addition, flagellin AN/C can inhibit radiation-induced ROS production, reduce NLRP3 activity, and ultimately inhibit caspase-1-dependent burning, which may be an important factor in protecting intestinal epithelial cells from radiation injury. (Wu, Han et al. 2018).

In this study, we first studied the expression of gasdermin family genes in gliomas and their prognostic value. We found that the stage of glioma was positively correlated with the expression of GSDMD and GSDME. Prognostic analysis showed that gliomas with low expression of GSDMC and high expression of GSDMD or GSDME had a low survival rate. By LassoCox regression analysis, a prognostic gene model was established based on 4 Gasdermin family prognostic genes (GSDMC, GSDMD, GSDME and PJVK), which could predict the overall survival rate of glioma patients with moderate and high accuracy. Predictive normograms showed that overall 3 - and 5-year survival could be predicted relatively well compared to ideal models across the entire cohort. However, this model is not suitable for glioblastoma multiforme(GBM). Previous studies have demonstrated that prognostic characteristics associated with autophagy and ferroptosis can well predict the prognosis of PATIENTS with GBM(Zhuo, Chen et al. 2020, Chen, Wu et al. 2021). In our research, we first determined the prognostic gene characteristics of the gasdermin family of glioma, which provides more options for the prognosis prediction of LGG.

We further explored the relationship between this model and the clinical features of glioma. A high risk score is related to the degree of malignancy, such as a high WHO level. In addition, risk score was higher in mesenchymal glioma subtype. This subtype is characterized by NF1 mutations and mesenchymal differentiation, showing poor immunoparticipatory and aggressive clinical behavior. This is the first study on expression patterns of Gasdermin family members in gliomas based on WHO classification system, TCGA subtype or WHO molecular classification in 2016(Louis, Perry et al. 2016). In this study, we explored different genomic changes based on risk scores.We identified somatic mutations and CNA events corresponding to the differential risk score.Carcinogenic drivers such as CDK4, EGFR, PIK3C2B and PDGFRA were detected in the high risk.

In the gene ontology analysis of biological functions, we found that risk score is significantly correlated with the infiltration of various immune cell types. Moreover, numerous immune checkpoint molecules were highly expressed with increasing risk score. We also calculated the GSVA score of T cell related pathways to explore the relationship between risk and T cell function. This analysis identified that while T cells are significantly activated in the high-risk group, the AICD pathway was also activated. This may represent one mechanism by which CTLs are inactivated within this patient group in order to facilitate tumor progression. We demonstrate for the first time that antigenic stimulation can significantly induce activation of the caspase1/GSDMD pathway in tumor cells. This provides a new idea to explore the mechanism of AICD.

Cancer immunotherapy has demonstrated marked efficacy in some clinical contexts. Clinical trials of immune checkpoint blocking for gliomas are ongoing(Guan, Zhang et al. 2018). Many investigators have posited that TMB-based detection methods can help identify patient groups that respond to immunotherapy(Bortolomeazzi, Keddar et al. 2021, Hodi, Wolchok et al. 2021, Valero, Lee et al. 2021). However, this detection method has not achieved optimal predictive power in glioma(Samstein, Lee et al. 2019). Our findings suggest that this may be due to immune cells with gliomas of the high TMB group, which are rich in GSDMs member expression, which are prone to AICD, which in turn modules sensitivity to immunotherapy.

We also constructed an mRNA–miRNA–lncRNA network. We found that KCNQ1OT1/LINC01278/MIRLET7BHG/NEAT1 can be used as the upstream target of miR-296-5p to regulate the progression of glioma. Studies have found that silencing KCNQ1OT1 reduces pyroptosis by targeting miR-214-3p and caspase-1(Yang, Qin et al. 2018). Neat1 is associated with AIM2, NLRC4 and NLRP3 inflammasome in mouse macrophages and is involved in assembly of these inflammasome and activation of caspase1 (Zhang, Zheng et al. 2019, Zhang, Cao et al. 2019). Our research suggests for the first time that NEAT1 may directly regulate the expression of GSDMD through has-miRNA-296-5p and thus affect cell pyroptosis. Further research is required to independently confirm these findings.

In summary, our study investigated the biological significance of gasdermin family genes using large cohorts of glioma cases. Our findings indicate that gasdermin family members may serve as important biomarkers within glioma, and their expression is associated with differences in tumor mutation spectrum, copy number events, histology and clinical features.

**Method**

**Data collection**

The 698 glioma samples within the TCGA RNA-seq database were accessed (https://portal.gdc.cancer.gov/) and used as a training cohort. The CGGA RNA sequencing (RNA-seq) data set (mRNAmicroarray\_301, mRNAseq\_325) and corresponding clinical and molecular information were retrieved for use as a validation set. Cases with mismatching identifier between the transcriptome data and clinical annotation were removed prior to analysis. Drug sensitivity related data were obtained from the Cancer Cell Line Encyclopedia (https://sites.broadinstitute.org/ccle)

**Mutation analysis of Gasdermin family gene**

The mutation frequencies of gasdermin family genes and corresponding oncoplot/waterfall plot in LGG patients were generated using the "maftools" package. CNA positions on chromosome 23 were mapped using the "RCircos" package.

**Development of the prognostic model**

Raw RNA-sequence data (level 3) and corresponding clinical annotation for the LGG and GBM tumors were obtained from the Cancer Genome Atlas (TCGA) database (https://xenabrowser.net/datapages/). Survival differences were analyzed using the log rank test. The above analysis were implanted using the glmnet package in R.

For Kaplan-Meier survival curves, the hazard ratio (HR) and 95% confidence interval (CI) were obtained by univariate Cox proportional hazard regression models. All of the above analysis methods and R software packages are implemented using v4.0.3 version R software (R Foundation for Statistical Computing, 2020). A threshold of p<0.05 was considered statistically significant.

**Competing endogenous RNA network construction**

To illustrate the potential capabilities of GSDMD in LGG, StarBase (http://starbase.sysu.edu.cn/) and MiRDB (http://mirdb.org/) were used to predict miRNA targets. We also used the TCGA dataset to explore the expression and prognostic value of these miRNA and lncRNA targets. Results were considered statistically significant when P <0.05.

**Cell lines and antibodies**

The Jurkat and U251 were cultured with RPMI-1640 with 10% Fetal Bovine Serum (Gibco).The antibodies were used:anti-caspase-1 (abclonal), anti-GSDMD (Sigma), anti-GAPDH (abclonal).

**Western blot analyzes**

The protein was extracted according to the protocol and stored in -20℃. Proteins were separated by electrophoresis, and the target protein samples were prepared by lytic cells or tissues. After SDS-PAGE electrophoresis, proteins with different molecular weights were separated. The electrophoretic bands were transferred from the gel to the NC/ PVDF membrane. The commonly used method is electroelution or electrophoretic transfer to form a "negative electrode-sponge-three-layer filter paper-glue-membrane-three-layer filter paper-sponge-positive" membrane structure. After applying the electric field, the protein is removed from the polyacrylamide gel and adsorbed on the membrane surface. After antibody incubation, the membrane was treated with the antibody (first antibody) of the target protein, and the unbound antibody was rinsed to remove the unbound antibody. The membrane contained only the first antibody bound by the target protein. Then the labeled secondary antibody was used for enzyme immunolocalization. Development analysis, exposure with x-ray film, according to the strength of the signal to adjust the exposure time or multiple pressing at different times to achieve the best results. After the exposure is completed, take out the x-ray film and quickly immerse it in the developer to develop, stop developing after the strip is noticeable, and analyze the results.

**Statistical analysis**

R language (v. 3.6.3), SPSS software (v. 22.0), and GraphPad Prism (v. 8.0) for Windows were used for statistical analyses and generating figures. All error bars in graphical data represent the mean ± SD. Spearman’s rank correlation was used to analyze the association. Student’s two-tailed t test and Wilcoxon test were used to determine the statistical relevance between groups, and p < 0.05 was considered significant.

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**Data availability**

The data could be download at (https://portal.gdc.cancer.gov/, https://xenabrowser.net/, and http://www.cgga.org.cn/) and the code used during the current study are available from the corresponding author on reasonable request.

**Conflict of interest**

The authors declare no competing interests.

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