



## Review article

## Role of gasdermin family proteins in the occurrence and progression of hepatocellular carcinoma

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## ABSTRACT

Primary liver cancer is the sixth most common cancer and the third leading cause of cancer mortality worldwide, hepatocellular carcinoma (HCC) is the most common type of liver cancer, accounting for 75%–85% of cases. The occurrence and progression of HCC involve multiple events. Pyroptosis is a gasdermin mediated programmed cell death and is intricately associated with cancerogenesis, including HCC. This review mainly concerns the recent research advances of the gasdermin family members in HCC. The biological roles and specific expression patterns of the family members are discussed, especially those that are involved in the regulatory pathways in the occurrence and progression of HCC. We provide the latest progress into the distinct molecular mechanisms of gasdermin family members involved in the occurrence and development of HCC.

## 1. Introduction

More than 30 years have passed since Saeki et al. [1] first identified Gasdermin (GSDM), a recombinant inducible mutation 3 (Rim3), as a candidate gene associated with mouse skin mutant phenotype. Sato et al. found that Rim3 reveals over-proliferation and differentiation of epidermis and hair follicles in mice [2]. Comprehensive expression analysis demonstrated that GSDM family genes are exclusively expressed in the gastrointestinal and skin epithelium in a highly tissue-specific manner [1, 3, 4]. In 2007, Tamura et al. referred to the previously reported GSDM-associated genes [3, 4, 5] and the three new GSDM genes they identified as the GSDM family [6]. To date, six genes have been categorized as GSDM family members (GSDMs) based on their N-terminal and distinct regions conserved in humans (Table 1), including Gasdermin A (GSDMA) [1, 3], Gasdermin B (GSDMB) [5], Gasdermin C (GSDMC) [7], Gasdermin D (GSDMD), Gasdermin E (GSDME) [8, 9], and PJK [10]. Previous studies have demonstrated that GSDMs are involved in regulating gastric epithelial cell progress and apoptosis [11, 12], pyroptosis [13, 14, 15], gasdermin-like (GSDML) might be a secretory or metabolic product involved in the secretory pathway [16], perform cell death and inflammation [17], the regulation of cancer occurrence and development [18, 19], cancer biology and antitumor immunity [20, 21],

NETosis [22, 23], immune defenses and diseases [24, 25, 26]. Each member of the GSDM family has a different tissue specificity and plays a unique role in physiological or pathological processes [11, 24, 27]. Except for PJK, the N-terminal structural domains released by GSDMA-E exhibit pore-forming activity on cell membranes and are effectors of pyroptosis [13, 26, 28, 29, 30] (Figure 1).

In 2020, primary liver cancer is the sixth most common cancer and the third leading cause of cancer mortality worldwide, with approximately 906,000 new cases and 830,000 deaths [34]. Hepatocellular carcinoma (HCC) is the most common type of liver cancer, accounting for 75%–85% of cases [34, 35]. It is well known that hepatitis virus infection (including hepatitis B and hepatitis C virus) is the most significant risk factor for the development of HCC [36]. Other risk factors include aflatoxin-contaminated foods, heavy alcohol intake, et al. [34]. Non-alcoholic steatohepatitis (NASH), related to metabolic syndrome or diabetes, is becoming the fastest increasing cause of hepatocellular carcinoma, especially in the West country [37]. In addition, reports on mutational characteristics have confirmed aristolochic acid and tobacco as potential causative cofactors of HCC [38]. As research progresses, the role of the GSDM family in tumors is becoming more prominent [20, 39]. Several studies have demonstrated that specific drugs or molecules may trigger GSDMD-mediated pyroptosis in diverse types of cancer,

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**Table 1.** Biological roles of gasdermin family members.

Gene name (Gene symbol)	Alias (Gene symbol)	Related signaling pathways	Biological process or Molecular function	Cancer relations	Pore-forming activity	Related diseases
<i>GSDMA</i>	<i>GSDM</i> [1, 3]; <i>FKSG9</i> ; <i>GSDM1</i> [1,3]	①Upregulated via TGF- $\beta$ ) and LMO1 pathways; ②Active regulation of EMT, apoptosis, cell cycle, DNA damage response, ER pathway;	①Pyroptosis, ②phosphatidylinositol-4,5-bisphosphate binding; ③phosphatidylinositol-bisphosphate binding; ④phosphatidylinositol-4-phosphate binding	Tumor suppressors [11]	Yes [24]	①Asthma [47], ②systemic sclerosis [47, 48], ③Inflammatory Bowel Disease (IBD) [49].
<i>GSDMB</i>	<i>GSDML</i> [5]; <i>PP4052</i> ; <i>GSDMB-1</i> [50]; <i>PRO2521</i> [51]	①Cleaved by caspase-1, -3, -4, -6, -7, -8, and -9 [31, 52]; ②Promotes non-canonical pyroptosis by enhancing caspase-4 activity [29]; ③Active regulation of apoptosis and EMT; ④induces TGF- $\beta$ 1 expression via induction of 5-lipoxygenase [53]; ⑤enhancer-driven HSV thymidine kinase-expressing vector [54];	①Pyroptosis, ②phosphatidylinositol-4,5-bisphosphate binding; ③phosphatidylinositol-bisphosphate binding; ④phosphatidylinositol-4-phosphate binding.	Oncogene [11]	Yes [24]	①Asthma [47], ②gastric cancer [11], ③IBD [49]; ④type 1 diabetes [55]; ⑤ankylosing spondylitis [56]; ⑥inflammatory disorders [57]; ⑦aspirin exacerbated respiratory diseases [58]; ⑧rheumatoid arthritis [59]
<i>GSDMC</i>	<i>MLZE</i> [7]	①cleaved into two fragments by caspase-8 [33]; ②active regulation of apoptosis, DNA damage response, and hormone AR pathway; ③Upregulated by TGFBR2 mutation [60]; ④induced MMP-1 through activation of ERK and JNK pathway [61]; ⑤TRPV1/calcium/calcineurin/NFATc1 signaling [61]	①Pyroptosis, ②phosphatidylinositol-4,5-bisphosphate binding; ③phosphatidylinositol-bisphosphate binding; ④phosphatidylinositol-4-phosphate binding.	①Oncogene [60], ②Tumor suppressor [11].	Yes [24]	lumbar spinal stenosis [62]
<i>GSDMD</i>	<i>DF5L</i> ; <i>DFNA5L</i> [9]; <i>FKSG10</i> ; <i>GSDMDC1</i> [9]	①Cleaved by caspase-1/4/5/8/11 and neutrophil elastase [31, 52]; ②serve as a conduit for the extracellular release of mature IL-1 $\beta$ and IL-18 [63]; ③Involved in the regulation of ERK, STAT3, PI3K/Akt signaling pathways [52]; ④blocking EGFR/Akt signaling pathway [64]; ⑤active regulation of hormone estrogen; ⑥regulation of NOD-like receptor signaling pathway and Salmonella infection; ⑦inflammasome or caspase-1-mediated signaling pathways.	①promote the pyroptosis, ②phosphatidylinositol-4,5-bisphosphate binding; ③phosphatidylinositol-bisphosphate binding; ④phosphatidylinositol-4-phosphate binding. ⑤takes part in host immune defense.	Tumor suppressors [11]	Yes [24]	①Alzheimer's disease [65]; 2 Inflammation-associated diseases
<i>GSDME</i>	<i>DFNA5</i> [8]; <i>ICERE-1</i> [66, 67]	①Cleaved by caspase-3 [68]; ②involve in caspase-3-mediated pyroptosis [69]; ③involved in the antitumor activity of ERK1/2 pathway inhibitors [52]; ④ cell cycle; ⑤ MAPK-related pathways [70]	①trigger pyroptosis by the activation of caspase-3; ②sensory perception of sound; ③sensory perception of mechanical stimuli	Tumor suppressors [71]	Yes [24]	Hearing loss [72, 73]
<i>PJVK</i>	<i>DFNB59</i> [6, 10,74]	①afferent auditory pathway [75];②Involved in Oxidative stress-induced peroxisome proliferation and pexophagy [31, 76]; ③ active regulation of cell cycle pathway and inhibit apoptosis	①sensory perception of sound; ②sensory perception of mechanical stimulus.		Uncertain	Nonsyndromic hearing impairment [8, 74]

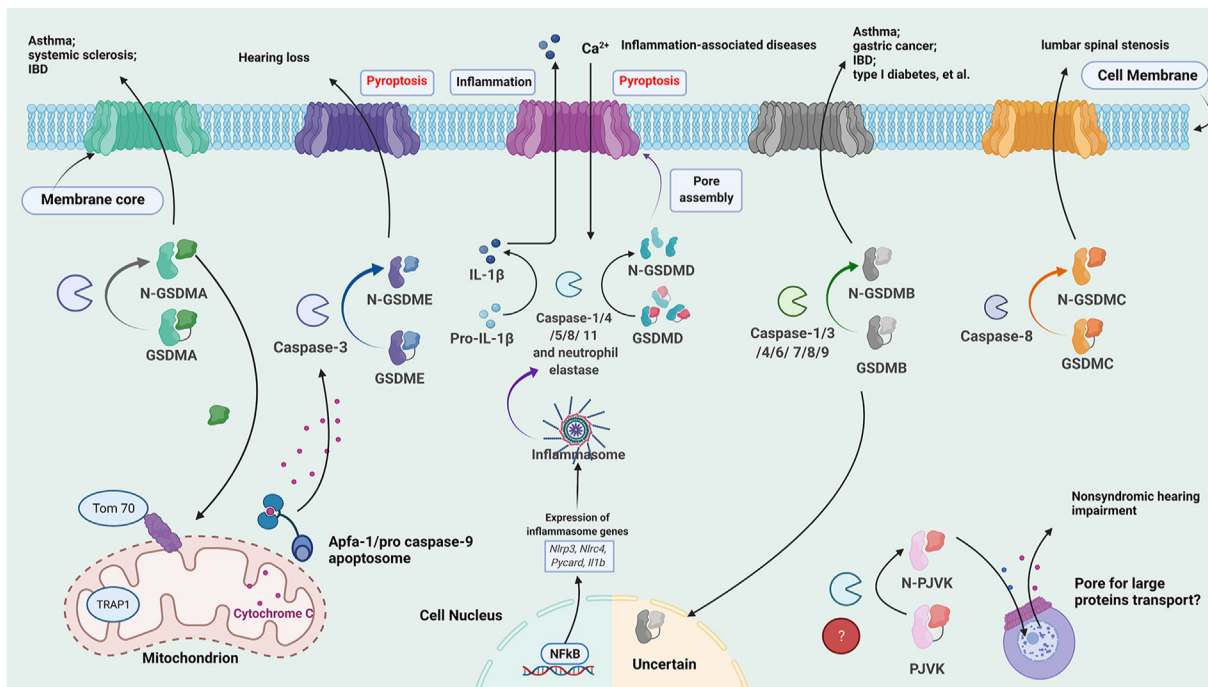
suggesting that this novel programmed cell death is involved in cancer pathogenesis and may be a new target for cancer therapy, such as esophageal adenocarcinoma, gastric cancer, colorectal cancer (CRC), et al. [19, 40]. As we all know, pyroptosis is a gasdermin mediated programmed cell death [14]. Do GSDM family members also play an essential role in the progress of HCC? This review will summarize and discuss the potential impact of GSDM family members on HCC and their role in anticancer therapy. Moreover, we also supplemented the study by obtaining HCC data from The Cancer Genome Atlas (TCGA) database for bioinformatics analysis.

## 2. GSDMA

The GSDMA gene is located on human chromosome 17q21.2 [6] (Figure 2A). Humans have only one copy of the gene encoding GSDMA, while mice have three isomers (GSDMA1-3). GSDMA is predominantly expressed in epithelial cells of the skin, differentiated upper gastrointestinal, and lung but is frequently suppressed in primary gastric cancer and gastric cancer cell lines [11, 41]. Moussette et al. have reported that GSDMA expression is up-regulated in the 5-aza-dC, a DNA methyltransferase inhibitor, treatment adenocarcinoma cell line MCF-7 [42]. Saeki et al. found that GSDMA expression is up-regulated through the

transforming growth factor- $\beta$  (TGF- $\beta$ ) and LIM structural domain 1 (LMO1) pathways, thereby promoting apoptosis in gastric epithelial cells [12]. Lin et al. found that Gasdermin A3 N-terminal functional domain modulates mitochondrial homeostasis through mitochondrial targeting [43].

Hu et al. reported that mRNA expression levels of GSDMA were higher in HCC tissues than in normal tissues by UALCAN databases [44]. We also discovered mRNA expression of GSDMA was accordantly higher in HCC tumor tissues (377 TCGA-LIHC) compared with normal tissues (50 TCGA-LIHC paracancerous tissue and 110 GTEx-normal liver tissues) (Figure 2B). Further analysis of the GSDMA revealed the general existence of copy number variation (CNV) mutations. The genetic alteration rate of GSDMA in HCC is about 0.8% and was mainly focused on the amplification in copy number (Figures 2C, 2D). The correlation analysis between six GSDMs and cancer-related pathways indicated that GSDMA mainly participated in the active regulation of epithelial-mesenchymal transition (EMT), apoptosis, cell cycle, DNA damage response, and hormone estrogen receptor (ER) pathway (Figure 2E). Gene Ontology (GO) functional enrichment analysis indicates GSDMA is associated with the regulation biological process (BP) of pyroptosis, and molecular function (MF) of phosphatidylinositol-4,5-bisphosphate binding, phosphatidylinositol-bisphosphate binding, phosphatidylinositol-4-phosphate binding (Figure 2F, Tables 1 and 2). Receiver operating characteristic (ROC) curve



**Figure 1.** Gasdermin family members and cell cleavage. Except for *PJKV*, the N-terminal structural domains released by *GSDMA-E* exhibit pore-forming activity on cell membranes and are effectors of pyroptosis. 1) The N-terminal end of *GSDMA* can be transmitted to mitochondria via the translocase of outer mitochondrial membrane 70 (Tom70) receptor, which then interacts with the mitochondrial partner TNF receptor-associated protein 1 (TRAP1) to contribute to the production of reactive oxygen species (ROS) [31]. 2) Chemotherapeutic agents or targeted therapeutic agents cause permeabilizes mitochondrial membrane that results in cytochrome c release, and ultimately caspase-3 activation augments. In turn, activated caspase-3 cleaves *GSDME* to produce *GSDMD-cNT*, which forms pores in the plasma membrane and activates pyroptosis. 3) Caspase-1 successively promotes IL-1,  $\beta$ , and IL-18 precursor maturation and cleaves *GSDMD*. The pore form domain of *GSDMD* associates with the plasma membrane to shape the *GSDMD* pore, leading to the release of intracellular contents, including IL-1 $\beta$  and IL-18. Activated caspase-11 (caspase-4/5 in humans) successively cleaves *GSDMD* and induces pyroptosis [32]. 4) *GSDMB* can be cleaved through caspase-1, -3, -4, -6, -7, -8, -9, and the N-terminal structural domain of *GSDMB* (*GSDMB-NT*) was discovered to trigger pyroptosis. 5) *GSDMC* may be particularly cleaved via caspase-8 with TNF $\alpha$  treatment and induces pyroptosis [33]. 6) *PJKV* may be involved in large protein transport. Figure 1 was created with BioRender.com (<https://biorender.com/>).

indicates *GSDMA* had low accuracy in predicting HCC and normal outcomes (area under curve (AUC) = 0.579, confidence interval (CI) = 0.501–0.658, Figure 3A). Besides, *GSDMA* was positively associated with infiltration of CD8+ T cell, B cell, neutrophil, and dendritic cell [44]. Spearman correlation analysis between expression of *GSDMA* and infiltration levels of 24 immune cells [45] was performed using single-sample gene-set enrichment analysis (ssGSEA), which is gene set variation analysis (GSVA) package built-in algorithm [46]. We found that *GSDMA* expression is a significant positive correlation with the infiltration level of activated dendritic cell (aDC), B cells, CD8 T cells, Cytotoxic cells, dendritic cell (DC), Eosinophils, immature dendritic cell (iDC), Macrophages, Mast cells, Neutrophils, NK CD56 bright cells, NK CD56 dim cells, NK cells, plasmacytoid dendritic cell (pDC), T cells, T helper cells, T effector memory cell (Tem), T follicular helper cell (TFH), T helper 1 cells (Th1 cells), and Th2 cells in HCC. In contrast, *GSDMA* expression is a significant negative correlation with the infiltration level of Th17 cells ( $P < 0.001$ , Figure 3H, Table 3).

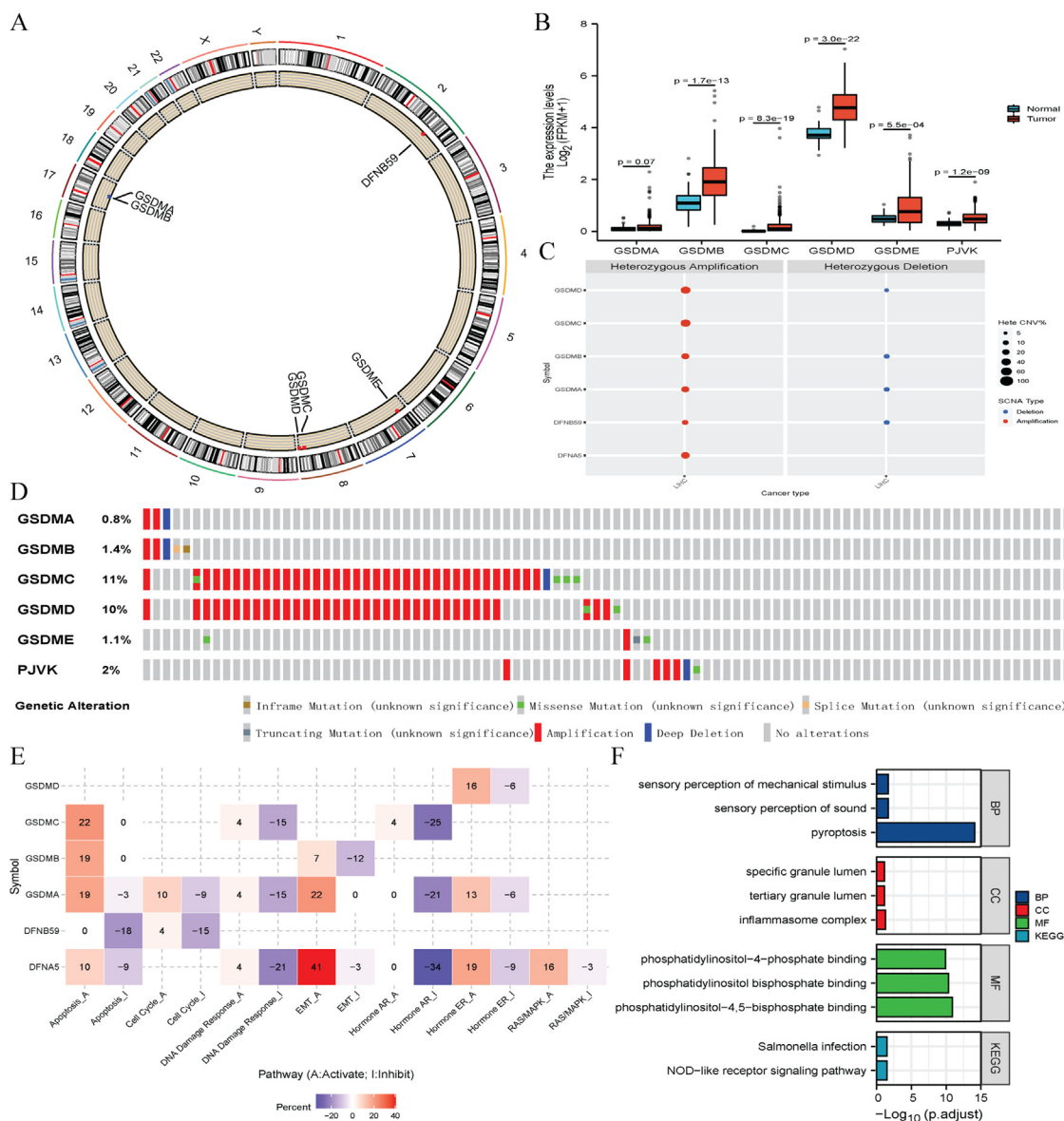
### 3. GSDMB

The *GSDMB* gene is chromosomally mapped at 17q12 [11] (Figure 2A). The N-terminal structural domain of *GSDMB* (*GSDMB-NT*) was discovered to trigger pyroptosis [79]. *GSDMB* can be cleaved through caspase-1, -3, -4, -6, -7, -8, and -9 [31, 52]. *GSDMB* can be explored in the esophageal, gastrointestinal tract, liver, colon, lung, and lymphocyte epithelium [6, 11, 53]. Previous studies have indicated that *GSDMB* is highly expressed in some cancer cells, such as gastric cancer, HCC, CRC, and cervical cancer, et al. therefore, it is recognized to be an oncogene [11, 54, 80, 81]. *GSDMB* upregulation was associated with poor effect and promoted invasion and metastasis in breast cancer cells

[50]. Hu et al. found that expression levels of *GSDMB* were higher in HCC samples than in normal samples [44]. We also discovered the *GSDMB* expression is accordingly higher in HCC tumor samples compared with normal samples (Figure 2B). Researchers have indicated that *GSDMB* is located in amplicons, a region of the genome that is frequently amplified during cancer development [82]. We found the genetic alteration rate of *GSDMB* in HCC is about 1.4 % and also was mainly focused on the amplification in copy number (Figures 2C, 2D). The correlation analysis between six *GSDMs* and cancer-related pathways indicated that *GSDMB* has mainly participated in the active regulation of apoptosis and EMT (Figure 2E). GO functional enrichment analysis indicates *GSDMB* is participating in the regulation BP of pyroptosis and MF of phosphatidylinositol-4,5-bisphosphate binding, phosphatidylinositol-bisphosphate binding, phosphatidylinositol-4-phosphate binding (Figure 2F, Tables 1 and 2). ROC curve indicates *GSDMB* had good accuracy in predicting HCC and normal outcomes (AUC = 0.821, CI = 0.764–0.878, Figure 3B). Besides, *GSDMB* expression is significant positive associated with the infiltration level of NK CD56 bright cells; and negative related to the infiltration level of CD8 T cells, iDC, Macrophages, Mast cells, Neutrophils, NK CD56 dim cells, NK cells, and Tgd ( $P < 0.05$ , Figure 3I, Table 3).

### 4. GSDMC

*GSDMC* gene is mapped at 8q24 on the human chromosome [6] (Figure 2A). *GSDMC* was originally found to be highly expressed in metastatic melanoma cells and is known as a melanoma-derived leucine zipper-containing extranuclear factor (MLZE) [7]. Human *GSDMC* is expressed in epithelial cells of the upper gastrointestinal tract, trachea, and spleen [11]. In addition, *GSDMC* exhibited cell-growth inhibition



**Figure 2.** Multi-omics analysis of GSDMs in HCC (A) The copy number variation (CNV) alteration site of six GSDMs on 23 human chromosomes. We obtained the CNV frequencies of the six GSDMs in each HCC patient from an online exploration tool- UCSC Xena (<http://xena.ucsc.edu/>) [77]. The R package “Rcircos” was used to visualize the location of six GSDMs in human chromosomes (B) Differential expression of six GSDMs in normal and tumor samples of HCC (C, D) CNV variation frequency of six GSDMs in the TCGA-HCC cohort. The CNV was divided into two subtypes, homozygous CNV and heterozygous CNV, which represent the occurrence of CNV on both two chromosomes or only one. Percentage statistics based on subtypes of CNV used GISTIC processed CNV data, and calculation of correlation used mRNA RSEM data and raw CNV data (C) A red dot indicates amplification, and a blue dot indicates the deletion of CNV type (D) Horizontal coordinates represent the genetic alteration type of GSDMs, and the vertical coordinates represent the alteration frequency of CNV in each GSDM. Different rectangular blocks represent different mutation types. Brown, inframe mutation; green, missense mutation; orange, splice mutation; dark blue, truncating mutation; red, amplification; blue, deep deletion; grey, no alterations (E) The cancer-related signaling pathways are regulated by six GSDMs. Red indicates pathway activation; blue indicates pathway inhibition. GSCALite (<http://bioinfo.life.hust.edu.cn/web/GSCALite/>), a web-based analysis platform for gene-pooled cancer analysis [78], was used to explore the GSDMs involved in the regulation of cancer-associated pathways (activation and inhibition). The pathway we included: Apoptosis pathways, Cell Cycle, DNA Damage Response, EMT, Hormone AR, Hormone ER, PI3K/AKT, RAS/MAPK, RTK, and TSC/mTOR. They are all famous cancer-related pathways. The Gene expression was divided into two groups (High and Low) through the median expression, student T-test was used to define the difference of pathway activity score (PAS) between two groups, p-value was adjusted by FDR, FDR≤0.05 is considered as significant. When PAS (Gene A in groupHigh) > PAS (Gene A in groupLow), we consider gene A might have an activating effect on a pathway, otherwise have an inhibiting effect on a pathway [78] (F) Functional annotation analysis for six GSDMs through GO enrichment analysis and KEGG pathway analysis. We use the clusterProfiler package [version 3.14.3] to analyze the data, and the ggplot2 package [version 3.3.3] to visualize the results. The length of the bars from longest to shortest indicates the number of enriched genes from most to least. GO, Gene Ontology; blue indicates biological processes (BP); red presents cellular components (CC); green demonstrated molecular functions (MF), and sky blue presents Kyoto Encyclopedia of Genes and Genomes (KEGG).

activity in some esophageal squamous cell carcinomas cells and gastric cancer cells, which implies its role as a tumor suppressor [11]. However, GSDMC may have a contrary effect in specific cancers, such as GSDMC was up-regulated in CRC and over-expression of GSDMC facilitated the

proliferation and tumorigenesis in CRC cells [60]. These conflicting results suggest that the role of GSDMC in tumor development is ambiguous and needs to be confirmed by further studies. Similar to other GSDMs, GSDMC is also involved in regulating the process of pyroptosis. Hou et al.



**Table 2.** GO functional enrichment and KEGG pathway analysis of GSDMs.

Ontology	ID	Description	GeneRatio	BgRatio	P value	p.adjust	Gene ID	Count
BP	GO:0070269	pyroptosis	5,6	11,18670	1.47e – 16	8.07e – 15	GSDME, GSDMB, GSDMC, GSDMD, GSDMA	5
BP	GO:0007605	sensory perception of sound	2,6	145,18670	8.80e – 04	0.021	GSDME, PJVK	2
BP	GO:0050954	sensory perception of mechanical stimulus	2,6	165,18670	0.001	0.021	GSDME, PJVK	2
CC	GO:0061702	inflammasome complex	1,6	14,19717	0.004	0.051	GSDMD	1
CC	GO:1904724	tertiary granule lumen	1,6	55,19717	0.017	0.075	GSDMD	1
CC	GO:0035580	specific granule lumen	1,6	62,19717	0.019	0.075	GSDMD	1
MF	GO:0005546	phosphatidylinositol-4,5-bisphosphate binding	5,5	75,17697	1.19e – 12	1.31e – 11	GSDME, GSDMB, GSDMC, GSDMD, GSDMA	5
MF	GO:1902936	phosphatidylinositol bisphosphate binding	5,5	110,17697	8.47e – 12	4.66e – 11	GSDME, GSDMB, GSDMC, GSDMD, GSDMA	5
MF	GO:0070273	phosphatidylinositol-4-phosphate binding	4,5	30,17697	3.35e – 11	1.23e – 10	GSDMB, GSDMC, GSDMD, GSDMA	4
KEGG	hsa04621	NOD-like receptor signaling pathway	1,1	181,8076	0.022	0.031	GSDMD	1
KEGG	hsa05132	Salmonella infection	1,1	249,8076	0.031	0.031	GSDMD	1

Note: BP, biological processes; CC, cellular components; MF, molecular functions; KEGG, Kyoto Encyclopedia of Genes and Genomes.

[33] disclosed that GSDMC may be particularly cleaved via caspase-8 with TNF $\alpha$  treatment and induces pyroptosis in breast cancer cells. Moreover, high expression of GSDMC correlates with poor survival. Similarly, GSDMC overexpression was related to poor prognosis in lung adenocarcinoma [83]. However, unfortunately, the role of GSDMC in HCC has little been reported until now.

Hu et al. found that the GSDMC expression is higher in HCC samples than in normal samples by UALCAN databases [44]. We also discovered that the mRNA expression level of GSDMC was higher in HCC tumor samples than in corresponding normal samples (Figure 2B). GSDMC has the highest rate of genetic alterations among GSDMs in HCC, about 11%, and was mainly focused on amplification (Figures 2C, 2D). The correlation analysis between six GSDMs and cancer-related pathways indicated that GSDMC was mainly involved in the active regulation of apoptosis, DNA damage response, and the hormone AR pathway (Figure 2E). GO functional enrichment analysis demonstrates GSDMC is associated with the regulation BP of pyroptosis and MF of phosphatidylinositol-4, 5-bisphosphate binding, phosphatidylinositol-bisphosphate binding, phosphatidylinositol-4-phosphate binding (Figure 2F, Tables 1 and 2). ROC curve indicates GSDMC had good accuracy in predicting HCC and normal outcomes (AUC = 0.885, CI = 0.846–0.924, Figure 3C). Besides, we found that GSDMC expression has a significant positive correlation with the infiltration level of aDC, iDC, Macrophages, Mast cells, Neutrophils, NK CD56 bright cells, NK cells, T helper Tem, TFH, Th1 cells, and Th2 cells. On the contrary, GSDMC expression is a significant negative correlation with the infiltration level of CD8 T cells and Th17 cells ( $P < 0.05$ , Figure 3J, Table 3).

## 5. GSDMD

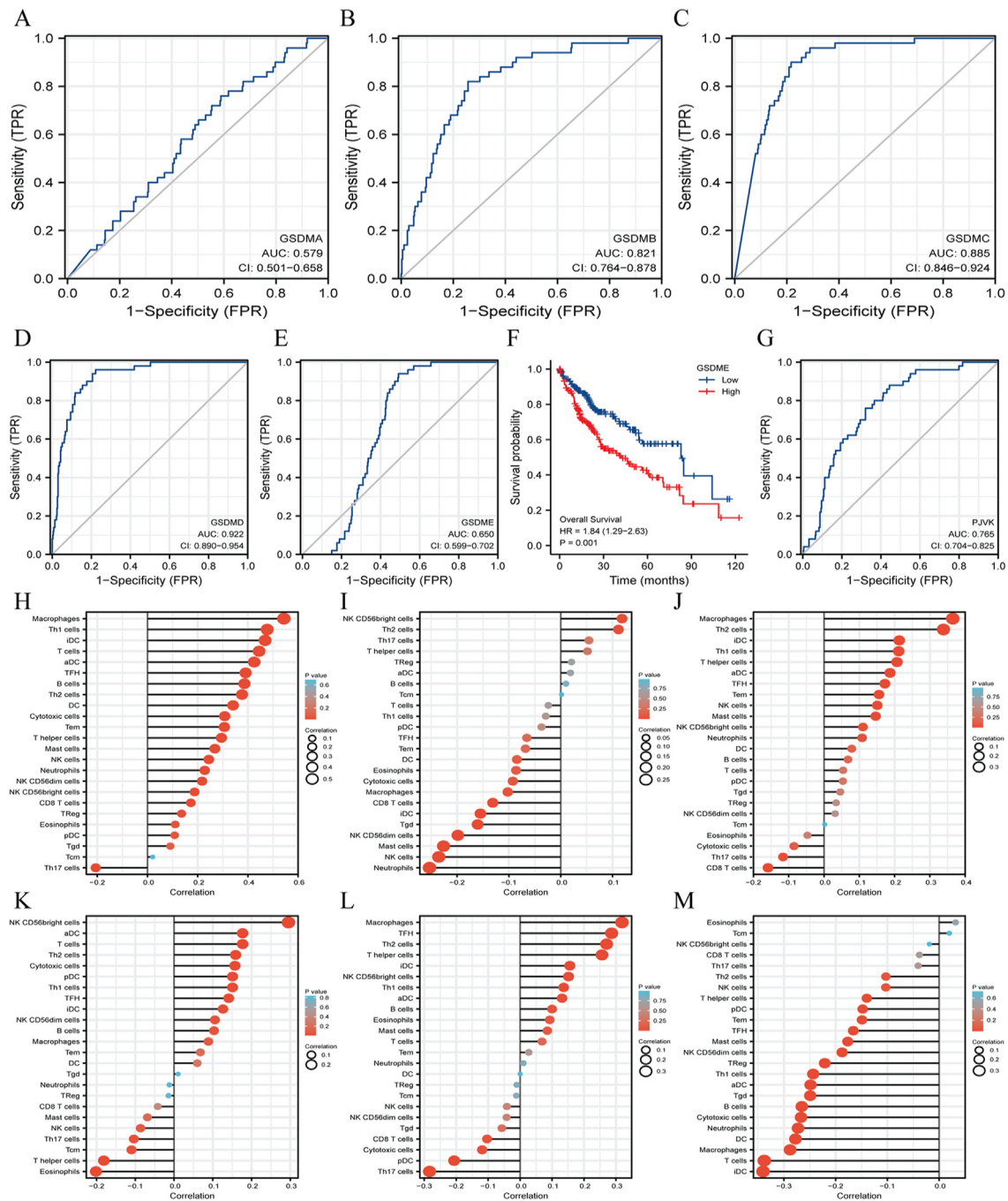
GSDMD is the most characteristic member of the GSDM family and has been recognized as an end-effector of pyroptotic [52]. GSDMD gene locates at 8q24.3 in the human chromosome (Figure 2A) and appears to play a role in the regulation of epithelial proliferation, such as skin, esophagus, and stomach epithelium [5, 11]. Among all GSDMs, GSDMD has the most potent inhibitory effect on cell growth and is considered to be a tumor suppressor gene [11]. The GSDMD molecule maintains auto-inhibitory accordance relying mainly on the function of its C-terminal structural domain (GSDMD-CT) [28]. Since the identification of GSDMD, pyroptosis has been reconsidered as GSDM-mediated programmed necrosis [14, 15, 63]. Several studies have recently discovered that pyroptosis can influence tumor proliferation, invasion, metastasis, and anticancer immunity and is regulated by several non-coding RNAs and other molecules [40, 84, 85]. Of course, GSDMD is also involved in regulating the process of cancer development. Wang et al. reported that

GSDMD expression is down-regulated in GC cells and tissues compared to normal tissues [86]. Reduced GSDMD expression significantly facilitated GC cell proliferation by activating some signaling pathways, such as ERK, STAT3, PI3K/Akt, et al. [52]. Gao et al. found that down-regulation of GSDMD inhibits tumor proliferation by inhibiting EGFR/Akt signaling and endogenous mitochondrial apoptotic pathway and predicts good prognosis in non-small cell lung cancer [64].

Hu et al. found that GSDMD expression was higher in HCC tissues than in normal tissues [44]. We also discovered that the GSDMD expression is significantly higher in HCC tissues than in corresponding normal tissues (Figure 2B). We found the genetic alteration rate of GSDMB in HCC is about 10 % and was mainly focused on the amplification in copy number (Figures 2C, 2D). The correlation analysis between six GSDMs and cancer-related pathways indicated that GSDMD was mainly involved in the active regulation of the hormone estrogen (Figure 2E). GO functional enrichment analysis indicates GSDMD participates in the regulation BP of pyroptosis, CC of inflammasome complex, tertiary granule lumen, specific granule lumen, MF of phosphatidylinositol-4,5-bisphosphate binding, phosphatidylinositol-bisphosphate binding, phosphatidylinositol-4-phosphate binding. KEGG pathway analysis indicated that GSDMD is involved in regulating the NOD-like receptor signaling pathway and Salmonella infection (Figure 2F, Tables 1 and 2). ROC curve indicates GSDMD had excellent accuracy in predicting HCC and normal outcomes (AUC = 0.922, CI = 0.890–0.954, Figure 3D). Besides, GSDMD expression is a significant positive correlation with the infiltration level of aDC, B cells, Cytotoxic cells, iDC, NK CD56 bright cells, NK CD56 dim cells, T cells, TFH, Th1 cells, Th2 cells; and a negative correlation with the infiltration level of Eosinophils and T helper cells ( $P < 0.05$ , Figure 3K, Table 3).

## 6. GSDME

GSDME, initially identified as deafness autosomal dominant 5 (DFNA5), is positioned at 7p15 on the human chromosome [72, 73] (Figure 2A). GSDME can be explored in the heart, kidney, brain, and placenta [87]. Wang et al. discovered that GSDME expression was different in cancer cells and normal tissues [69]. Since the discovery of GSDME, numerous studies have revealed its underlying role in gastric cancer [88], CRC [89, 90], and breast cancer [91]. Several previous studies have reported GSDME as a tumor suppressor. Kim et al. found that GSDME can significantly inhibit CRC cell colony-forming and growth ability [89]. Rogers et al. discovered that GSDME-deficient melanoma cells grew larger than wild-type melanoma cells [71]. Reversal of low GSDME expression in gastric cancer tissues by decitabine enhances the efficacy of chemotherapeutic agents [68]. Wang et al.



**Figure 3.** Prognostic analysis of GSDMs and correlation analysis of immune cell infiltration. ROC curves of GSDMs in HCC. R (version 3.6.3) software is used for statistical analysis and visualization, in which R package “pROC” [version 1.17.0.1] is used to analyze data, and R package “ggplot2” [version 3.3.3] is used to visualize the results. The AUC is between 0.5 and 1. The closer the AUC is to 1, the better the diagnostic effect. AUC has lower accuracy when 0.5–0.7, AUC has certain accuracy when 0.7–0.9, and AUC has higher accuracy when it is above 0.9 (A) GSDMA; (B) GSDMB; (C) GSDMC; (D) GSDMD; (E) GSDME; (G) PJKV (F) Survival curves (OS) of GSDME in the high and low expression groups. R package “Survival” [version 3.2–10] is used for statistical analysis of survival data, and R package “survminer” [version 0.4.9] is used to visualize the results (H–M) Immune cell correlation analysis between different GSDMs and 24 immune cells. The data were collected from RNAseq data in level 3 HTSeq-FPKM format of TCGA (<https://portal.gdc.cancer.gov/>) LIHC (hepatocellular carcinoma) project, which is converted to TPM (transcripts per million reads) format and transformed into log<sub>2</sub>, and then filtered (excluding control/normal (not all items have control/normal)). The markers of 24 kinds of immune cells came from Bindea’s study [45]. We use the built-in algorithm ssGSEA of the “GSVA” package [version 1.34.0] to calculate immune cell infiltration, and then use the Spearman method to analyze the correlation. The results were shown by lollipop charts (H) GSDMA; (I) GSDMB; (J) GSDMC; (K) GSDMD; (L) GSDME; (M) PJKV. We performed the ROC curves and immune cell correlation analysis of the six GSDMs in HCC patients by an online exploration tool- Xiantao Academic (<https://www.xiantao love/>).

found that GSDME inhibits HCC cell proliferation through inducing cell cycle arrest [92]. Chemotherapeutic agents or targeted therapeutic agents usually cause GSDME-mediated pyroptosis, unlike GSDMD-mediated pyroptosis [19]. Zhang et al. reported that multitone induces

cell death in HCC cells by GSDME-mediated pyroptosis [93]. Shang-guan et al. found that Cannabidiol suppresses HCC cell growth in vivo and in vitro by inducing GSDME-mediated pyroptosis [94]. Curcumin enhanced the expression of the GSDME N-terminus and related proteins

**Table 3.** Spearman correlation analysis between GSDMs expression and immune cell infiltration in HCC.

Immune cells	Spearman Correlation coefficient/ <i>P</i> -value					
	GSDMA	GSDMB	GSDMC	GSDMD	GSDME	PJKV
aDC	<b>0.425***</b>	0.019	<b>0.187***</b>	<b>0.177***</b>	<b>0.131*</b>	<b>-0.249***</b>
B cells	<b>0.386***</b>	0.010	0.068	<b>0.103*</b>	0.100	<b>-0.266***</b>
CD8 T cells	<b>0.173***</b>	<b>-0.131*</b>	<b>-0.159**</b>	-0.042	-0.103*	-0.038
Cytotoxic cells	<b>0.308***</b>	-0.092	-0.085	<b>0.157**</b>	<b>-0.119*</b>	<b>-0.267***</b>
DC	<b>0.341***</b>	-0.085	0.078	0.060	0.000	<b>-0.278***</b>
Eosinophils	<b>0.110*</b>	-0.086	-0.047	<b>-0.202***</b>	0.093	0.032
iDC	<b>0.469***</b>	<b>-0.155**</b>	<b>0.213***</b>	<b>0.126*</b>	<b>0.156**</b>	<b>-0.340***</b>
Macrophages	<b>0.543***</b>	<b>-0.102*</b>	<b>0.365***</b>	0.089	<b>0.319***</b>	<b>-0.288***</b>
Mast cells	<b>0.268***</b>	<b>-0.226***</b>	<b>0.146**</b>	-0.069	0.085	<b>-0.177***</b>
Neutrophils	<b>0.228***</b>	<b>-0.253***</b>	<b>0.108*</b>	-0.012	0.010	<b>-0.273***</b>
NK CD56 bright cells	<b>0.188**</b>	<b>0.118*</b>	<b>0.110*</b>	<b>0.295***</b>	<b>0.152**</b>	-0.019
NK CD56 dim cells	<b>0.218***</b>	<b>-0.199***</b>	0.032	<b>0.106*</b>	-0.042	<b>-0.188***</b>
NK cells	<b>0.244***</b>	<b>-0.235***</b>	<b>0.151**</b>	-0.087	-0.041	<b>-0.103*</b>
pDC	<b>0.108*</b>	-0.037	0.053	0.151	<b>-0.206***</b>	<b>-0.148**</b>
T cells	<b>0.445***</b>	-0.024	0.054	<b>0.177***</b>	0.069	<b>-0.338***</b>
T helper cells	<b>0.295***</b>	0.052	<b>0.207***</b>	<b>-0.181***</b>	<b>0.256***</b>	<b>-0.140**</b>
Tcm	0.021	0.002	0.002	-0.110	-0.012	0.020
Tem	<b>0.306***</b>	-0.068	<b>0.156**</b>	0.068	0.027	<b>-0.149**</b>
TFH	<b>0.391***</b>	-0.065	<b>0.172***</b>	<b>0.141**</b>	<b>0.287***</b>	<b>-0.166**</b>
Tgd	0.091	<b>-0.160**</b>	0.046	0.010	-0.057	<b>-0.249***</b>
Th1 cells	<b>0.477***</b>	-0.029	<b>0.212***</b>	<b>0.151**</b>	<b>0.136**</b>	<b>-0.244***</b>
Th17 cells	<b>-0.206***</b>	0.054	<b>-0.116*</b>	-0.104	<b>-0.284***</b>	-0.040
Th2 cells	<b>0.377***</b>	0.111	<b>0.338***</b>	<b>0.159**</b>	<b>0.271***</b>	<b>-0.103*</b>
TReg	0.136	0.021	0.034	-0.014	-0.011	<b>-0.221***</b>

Note: aDC, activated dendritic cell; DC, dendritic cell; iDC, immature dendritic cell; pDC, plasmacytoid dendritic cell; Tcm, T central memory cell; Tem, T effector memory cell; Tfh, T follicular helper cell; Tgd, T gamma delta cell. TH, T Helper.

during the pyroptosis, promoted HCC HspG2 cell pyroptosis, and increased intracellular ROS levels [95]. GSDME expression increased the sensitivity of drug-resistant melanoma cells to therapeutic agents via triggering caspase-3-mediated apoptosis [66]. Peng et al. reported that GSDME enhances cisplatin susceptibility for reversing non-small cell lung carcinoma by inducing pyroptosis to induce antitumor immunocyte infiltration [96].

The GSDME gene is expressed in some tumors but is episodically silenced in most tumor cells due to promoter hypermethylation [97]. Hu et al. found that the GSDME expression was higher in HCC samples than in normal samples by UALCAN databases. Besides, high GSDME expression was significantly related to shorter OS in HCC patients [44]. We also discovered that the mRNA expression level of GSDME was higher in HCC tumor tissues than in corresponding normal tissues (Figure 2B). GSDME has the lower rate of genetic alterations among GSDMs in HCC, about 1.1%, and was mainly focused on amplification, Figures 2C, 2D. The correlation analysis between six GSDMs and cancer-related pathways indicated that GSDME was mainly involved in the cell cycle pathway's active regulation and inhibited apoptosis (Figure 2E). GO functional enrichment analysis indicates GSDME is involved in the regulation BP of pyroptosis, sensory perception of sound, sensory perception of the mechanical stimulus, and MF of phosphatidylinositol-4,5-bisphosphate binding, phosphatidylinositol-bisphosphate binding, phosphatidylinositol-4-phosphate binding (Figure 2F, Tables 1 and 2). ROC curve indicates GSDME had moderate accuracy in predicting HCC and normal outcomes (AUC = 0.650, CI = 0.599–0.702, Figure 3E). High GSDME gene expression in HCC has a worse prognosis (HR = 1.84, P = 0.001, Figure 3F). Besides, we found that GSDME expression is a significant positive correlation with the infiltration level of aDC, iDC, Macrophages, NK CD56 bright cells, T helper cells, TFH, Th1 cells, and Th2 cells. On the contrary, GSDME expression is significantly negatively associated with the infiltration level of Cytotoxic cells, pDC, and Th17 cells ( $P < 0.05$ , Figure 3L, Table 3). Based

on the given evidence, GSDME could become crucial biomarkers to improve the diagnosis and prognosis of HCC better.

## 7. PJKV

Pejvakin (PJKV), also known as autosomal recessive deafness type 59 (DFNB59) [8,74], is mapped at 2q31.1-2q31.3 on the human chromosome [72, 74] (Figure 2A). Unlike other GSDMs, the N-terminal structural domains of PJKV cannot be perforated by its C-terminal fragment blocking [14, 24]. The PJKV protein has a shorter C-terminal split, unlike other GSDMs, which share nearly 45% sequence homology, and it is less conserved than other GSDMs [6, 24, 98]. PJKV encoded protein is necessary for the appropriate function of auditory pathway neurons. Defects in this gene are a reason for non-syndromic sensorineural DFNB59 [74]. GSDMA-E has membrane perforation activity and induces pyroptosis, which may be associated with cancer. Research on PJKV has mainly focused on the field of hearing loss [74, 99, 100, 101, 102], while there is little research on the relationship between PJKV and the development of cancer. Hu et al. discovered that the PJKV expression was higher in HCC tissues than in normal tissues in UALCAN databases [44]. We also discovered that PJKV expression was higher in HCC tumor tissues than in corresponding normal tissues (Figure 2B). PJKV has a lower rate of genetic alterations among GSDMs in HCC, about 2%, and was mainly focused on amplification (Figures 2C, 2D). The correlation analysis between six GSDMs and cancer-related pathways indicated that PJKV was mainly involved in the cell cycle pathway's active regulation and inhibited apoptosis (Figure 2E). GO functional enrichment analysis indicates PJKV is involved in the regulation BP of sensory perception of sound and mechanical stimulus (Figure 2F, Tables 1 and 2). ROC curve indicates GSDME had good accuracy in predicting HCC and normal outcomes (AUC = 0.765, CI = 0.704–0.825, Figure 3G). Interestingly, in the immune cell infiltration correlation analysis, PJKV exhibited

different results from other GSDMs, with its expression significantly and negatively correlated with the level of most immune cell infiltration. Such as aDC, B cells, Cytotoxic cells, DC, iDC, Macrophages, Mast cells, Neutrophils, NK CD56 dim cells, NK cells, pDC, T cells, T helper cells, Tem, TFH, Tgd, Th1 cells, Th2 cells, Treg ( $P < 0.05$ , Figure 3M, Table 3).

## 8. Conclusion

GSDMs play modulatory functions in various essential cellular processes, such as cell proliferation, inflammation, and especially pyroptosis. Each member of the GSDM family demonstrates tissue- and stage-specific expression profiles. GSDMs are related to different human diseases, including cancer and inflammation-associated disorders. In addition to GSDMB, which promotes the proliferation of some cancer cells, GSDMA, GSDMD, and GSDME all exhibit growth-inhibiting properties of some cancer cells. In addition, the role of GSDMC in cancer cells remains contradictory, with some cancer cells exhibiting oncogenes and some cancer cells exhibiting tumor suppressor genes. The role of PJVK in cancers is still uncertain. All six GSDMs are highly expressed in HCC and have relatively good predictive prognostic power. High GSDME gene expression in HCC has a worse prognosis and can be an essential biomarker to improve the diagnosis and prognosis of HCC better. Besides, the expression of six GSDMs was associated with cancer-related pathways and immune cell infiltration in HCC, but the underlying mechanism is still poorly understood.

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