Pan-Cancer Analyses of Pyroptosis With Functional Implications in Prognosis and Immunotherapy in Cancer

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Abstract

Background

Programmed cell death is an active and orderly form of cell death regulated by intracellular genes, which plays an important role in the normal occurrence and development of the immune system, and pyroptosis has been found to be involved in the tumorigenesis and development. However, comprehensive analysis and biological regulation about pyroptosis genes are lack in cancers.

Methods

Using the data from the The Cancer Genome Atlas, we established a score level model to quantify the pyroptosis level of cancer. Multi-omics bioinformatical analyses was performed to detect pyroptosis-related molecular features and effect of pyroptosis on immunotherapy in cancer.

Results

In the present study, we performed a comprehensive analysis of pyroptosis and its regulator genes in cancers. Most pyroptosis genes were aberrantly expressed among different cancer types, which is contributed by the CAN frequency and differences of DNA methylation level in cancer. We established the modeling of the pyroptosis level and found that pyroptosis showed dual roles across cancers, while the pyroptosis levels were different in multiple and be significantly associated with clinical prognosis. The dual role of pyroptosis also affect the effects of immunotherapy in several cancers. Multiple pyroptosis genes showed close connections with drug sensitivity across cancers, and may be considered as therapy targets in cancer.

Conclusions

Our comprehensive analyses provide new insight into the functions of pyroptosis in the initiation, development, and progression and treatment across cancers, suggesting corresponding prognostic and therapeutic utility.

Background

Programmed cell death is an active and orderly form of cell death regulated by intracellular genes, which plays an important role in the normal occurrence and development of the immune system[1]. Pyroptosis is a new programmed cell death mediated by caspase-1, characterized by rapid rupture of the plasma membrane, followed by the release of cell contents and pro-inflammatory substances such as IL, triggering an inflammatory cascade that leads to cell damage[2]. Pyroptosis-related signaling pathways include classical pathway mediated by Caspase-1 and non-classical pathway mediated by Caspase-4,

Caspase-5 and Caspase-11. The assembly of inflammatory bodies promotes the activation of Caspase-1 in classical pathways[3]. In the non-classical pathway, lipopolysaccharide enhanced the activation of Caspase-4, 5 and 11, and the activated Caspase-1 or Caspase-4, 5 and 11 reacted by lysing Gasdermin D (GSDMD)[4]. The n-terminal of GSDMD is combined with phospholipid of the cell membrane to form many small holes and water flow in the cell membrane, resulting in cell swelling, cell membrane rupture and release of cell contents, which leads to inflammatory reaction and the occurrence of pyroptosis[5].

With the extensive development of pyroptosis research, its complex biological functions are beginning to emerge. As a new type of programmed inflammatory necrosis, pyroptosis is involved in the occurrence and development of various diseases. In infectious diseases, in addition to salmonella or Shigella discovered in the early stage, candida albicans, Streptococcus pneumoniae, drug-resistant Staphylococcus aureus, hepatitis virus and immunodeficiency virus have been found to induce pyroptosis in recent years[6]. In Alzheimer's disease, Parkinson's disease, Huntington's disease and other neurodegenerative diseases, Caspase-1-mediated classical pyroptosis pathway also exists[7]. Both NLRP3 and Caspase-1 in the pyroptosis signaling pathway have a close relationship with the occurrence and development of atherosclerosis[8]. In addition, the researchers found that pyroptosis is involved in other types of disease, including acute liver injury, rheumatoid arthritis, and acute kidney injury[9–11]. In recent years, pyroptosis has been found to be involved in the tumorigenesis and development[12]. For example, human mesenchymal stem cells can induce pyroptosis by secreting IL-1 β , which can lead to the death of breast cancer cell[13]. The euxanthone can significantly inhibit the proliferation, migration and invasion of hepatoma cell, and this process was mainly involved the mechanism that euxanthone can activate the pyroptosis signaling pathway mediated by Caspase-1[14]. Simvastatin, an anti-hyperlipidemia drug, inhibits proliferation and migration of NSCLC. The mechanism may be related to the activation of NLRP3 inflammatory body, caspase-1, IL-1 β and IL-18 by simvastatin, which induces pyroapoptosis in NSCLC. Caspase-1 inhibitor attenuates the inhibitory effect of simvastatin on non-small cell lung cancer[15]. Conversely, activation of NLRP3 inflammasome promotes proliferation and migration of lung adenocarcinoma A549 cells, which is related to the NLRP3 inflammasome's ability to mediate the release of IL-18 and IL-1 β through caspase-1 dependent or independent pathways[16]. Similarly, NLRP1 inflammasome promotes melanoma growth by increasing Caspase-1 activity and promoting IL-1 β secretion[17]. In addition, pyroptosis was also involved in the immune regulation process in tumor. Elion et al. explored the role of RIG-I mediated innate immune response in breast cancer cells, and found that the activation of RIG-I in breast cancer cells leads to the death of breast cancer cells, partly due to the activation of pyroptosis inducing inflammatory cytokines. The expression of leukocyte recruitment chemokines was associated with the increased expression of major histocompatibility I components[18]. Furthermore, it is suggested that Caspase 3/GSDME-dependent pyroptosis contributes to chemotherapy drug-induced nephrotoxicity. These results showed that pyroptosis is involved in tumor progression and treatment, and a better understanding of the mechanism of pyroptosis will facilitate new approaches to and methods of treating tumors.

In the present study, we firstly performed a comprehensive analysis for molecular patterns, including somatic copy number alterations, mutations, and DNA methylation, pathway enrichment, immune

microenvironment, patients' survival, its effect on immunotherapy and drug resistance in pan-cancer. We also established the model of pyroptosis level based pyroptosis genes across 33 cancer types. Our results highlight the important role of pyroptosis in cancer and new insight into the functions of pyroptosis and therapy in cancer.

Methods

Datasets and source

We downloaded the following data from the University of California SANTA CRUZ (UCSC: <https://xenabrowser.net/datapages/>): marked copy number segment, DNA methylation (illumine human methylation 450), gene expression RNAseq (HTSeq), somatic mutation (SNPx and small INDELs), and survival data. The lists of cancer types were presented in Additional file 1: **Table S1**. The drug response data and genomic markers of sensitivity were downloaded from the Genomics of Drug Sensitivity in Cancer (<https://www.cancerrxgene.org/>) and Cancer Therapeutics Response Portal (<http://portals.broadinstitute.org/ctrp/>) identifying and targeting cancer dependencies with small molecules. Immune-associated data, including immune cells and immunophenoscores were downloaded from ImmuCellAI (Immune Cell Abundance Identifier) (<http://bioinfo.life.hust.edu.cn/ImmuCellAI#!/>). Three immunotherapy datasets (GSE13507: primary bladder cancer; GSE32894: urothelial carcinoma; GSE61676: non-squamous non-small cell lung cancer) were from GEO dataset (<https://www.ncbi.nlm.nih.gov/gds>). Thirty-three pyroptosis-related genes were defined from a previous publication (Additional file 1: **Table S2**) [19].

Differential genes expression analysis

To explore the expression differences of pyroptosis between cancers and normal tissues, we performed differential expression analyses in 32 cancers using the “limma” R package. $|\text{Log2FC}| > 1$ and adjusted P value < 0.05 were defined as significant differential expression levels.

Somatic copy number alteration and mutation analysis

The mutation, fusion, amplification and deletion of homozygously and hetezygous of deletetion and amplification were included to evaluate the copy number alteration and mutation of pyroptosis genes. We defined over five percent were considered as high frequency copy number alteration. We calculated the Pearson's correlation coefficient to evaluate the association between copy number alterations and mRNA expression. The R “maftool” packages were employed to evaluate the overall mutation of pyroptosis genes in cancers.

DNA methylation analysis

DNA methylation (illumine human methylation 450) data of 33 cancers were downloaded from UCSC. Because some cancers do not have normal data of methylation. The differential methylation for 14 cancers between tumor and normal were tested using Wilcoxon rank test. Genes were defined

hypomethylated or hypermethylated according to the adjusted P value ($P < 0.05$). The correlations of methylation and gene expression evaluated using Spearman correlation. $P < 0.05$ was considered as significant level.

Establishing the model of pyroptosis level

To evaluate the pyroptosis level in cancer, we calculated the pyroptosis score using single sample gene set enrichment analysis (ssGSEA) in R 'GSVA' package. The enrichment score was divided into positive or negative components. The normalized differences between positive components minus negative components were defined as pyroptosis level. To evaluate the pathway enrichment of each sample, we also performed gene set variation analysis (GSVA) and estimated the gene set enrichment for pyroptosis genes[20]. The KEGG gene set (c2.cp.kegg. v6.2. symbols) was downloaded from the GSEA database (<http://www.gsea-msigdb.org/gsea/index.jsp>).

Survival analysis

We evaluated the effect of pyroptosis level on survival prognosis in cancers. The following four survival outcomes were included: overall survival (OS), disease specific survival (DSS), progression free interval (PFI), and disease-free interval (DFI). We calculated the hazard ratio of pyroptosis score in each cancer using cox regression. The pyroptosis score were categorized into high and low groups according to the median. The Kaplan-Meier analysis was used to compare the survival curve of high and low pyroptosis groups. $P < 0.05$ was considered as significant level.

Immune features analysis

To investigate the association between pyroptosis and immune microenvironment, we calculated the Pearson correlation coefficient between pyroptosis score and immune parameters including immune score, stromal score, estimated score, and tumor purity.

Immune cells including B cell, T cell, myeloid dendritic cell, endothelia cell, NK cell, macrophage cell, hematopoietic stem cell, and immune cell subsets. Some immune-related pathway, matrix/metastasis-related pathways, and DNA damage repair pathways were also evaluated.

We also investigated the effect of pyroptosis level on survival prognosis in three GEO dataset (GSE13507: primary bladder cancer, survival outcome: OS; GSE32894: urothelial carcinoma, survival outcome: PFS; GSE61676: non-squamous non-small cell lung cancer, survival outcome: OS).

Drug sensitivity analysis and pyroptosis level

To evaluate the association between pyroptosis level and small molecular drugs, we calculated the Pearson correlation coefficients between pyroptosis score and drug sensitivity percent identified by percent viability curve approach. We identified and targeted cancer dependencies with small molecules using Genomics of Drug Sensitivity in Cancer and Cancer Therapeutics Response portal dataset.

Results

Genetic alteration landscape of pyroptosis-related genes in cancer

In this study, we identified thirty-three genes that play critical roles in regulating pyroptosis by reviewing previous studies. The lists of 33 pyroptosis-associated genes were provided in Additional file 1: **Table S2**. To determine the patterns of dysregulation of pyroptosis genes in cancer, we examined the genomic data, including genetic variation, somatic copy number of alternations, mRNA expression, and DNA methylation data of tumor and normal tissues from 32 cancer types. The overall alterations level of pyroptosis genes ranged from 0.7% to 7.7%. Although the DNA alteration level was relatively low, 50% of tumors consisted of at least one type of the alterations (**Fig. 1A**). The mutation, amplification and Homozygously deleted counted for the majority of pyroptosis genes alteration. Among these 33 genes, GSDMC showed the highest alterations frequency (7.7%), which is mainly amplification (5.5%).

The alterations of GSDMD and NLRP3 are similar (6.3% and 6.0%). The alterations of other genes ranged from 0.1% to 3%, and the CASP6 showed the lowest alteration level (0.7%). We further analyzed the alteration pattern among all cancer types, we found difference in alteration among different cancer types. The UCEC showed alteration in all pyroptosis genes, while the alteration levels of KICH and MESO are very low in all genes (**Fig.1A**). Individual pyroptosis genes also showed cancer type different amplification or deletion pattern. High heterozygous amplification frequency almost appeared in all cancers, while the heterozygous amplification frequency is relatively low in THYM, LAML and THCA. The same situation also existed for heterozygous deletion in three types of cancers. However, high homozygous amplification frequency of GSDMC and GSDMD were found in OV, ESCA, UCS, BRCA, STAD, LIHC, and UVM. The high homozygous deletion were scattered in most of cancers (**Additional file 2: Fig. S1A-B**).

The gene mutations mainly consisted of missense mutation, nonstop mutation and multi hit. The mutation levels of these gene range from 0% to 3%. The NLRP3 showed the highest mutation level (3%), NLRP7, NLRP1, CASP8 and NLRC4 showed the same mutations frequencies (2%). The rest genes only had mutation frequencies of 1% (**Additional file 2: Fig. S1C**). For cancer types, the mutation frequencies for pyroptosis genes are relatively low in all cancers relatively high in SKCM. Furthermore, the NLRP3, which encodes a pyrin-like protein containing a pyrin domain and functions as an upstream activator of NF-kappaB signaling, and it plays a role in the regulation of inflammation, the immune response, and apoptosis, showed relatively high mutation frequencies in several cancers including UCEC, SKCM, COAD, LUAD, LUSC (**Additional file 2: Fig. S2**). The NLRP3 mutation were significantly related to PFS, OS and DSS among UCEC. Other genes also showed significant associations with survival prognosis in multiple cancers (**Additional file 1: Table S3**).

Aberrant expression of pyroptosis-related genes among cancers

We performed the differential expression analysis for pyroptosis genes among cancers except MESO and UVM without normal tissues. Our results indicated that all pyroptosis genes were differentially expressed in at least one type of cancer. Some pyroptosis genes presented consistent expression patterns in

multiple cancers. GSDMC, NLRP7, CASP5, PYCARD, IL18, IL1B and GSDMA were significantly upregulated in 22,18,18,18,16 and 17 types of cancers, respectively (**Additional file 2: Fig. S3**). PRKACA, ELANE, MLRP1, PJKV, and CASP9 were significantly downregulated in 25, 24, 22, 25, 23 types of cancers, respectively. Several pyroptosis genes showed cancer type-specific patterns. ELANE were significantly downregulated in almost all cancers but obviously upregulated in GBM (fold change (FC)=2.07) and LAML (FC=12.15). CASP8 mutations are associated with increased risks of cancer, and low expression of CASP8 is closely connected with poor prognosis in patients with cancer but was significantly upregulated in GBM and PAAD[21]. IL6 seems to play a promoting role in the development of cancer[22], showed significantly upregulated in CLBC, GBM, PAAD, TGCT, and THYM, but was downregulated in ACC, BLCA, BRCA, KICH, KIRP, LAML, and LUAD. We also found IL6 showed opposite expression pattern in some subtypes of tumors in the brain and kidney. These results indicated that pyroptosis genes may show different functions in different cancers.

It is known that CNV were involved in the tumorigenesis development, we further evaluated the association between CNV and pyroptosis genes expression. The Pearsons correlation analysis indicated that most of pyroptosis genes were correlated with CNV in most cancers (**Fig. 1B**). For example, TIRAP, which were involved in the TLR4 signaling pathway of the immune system, were significantly correlated with CNV in 29 types of cancers. PRKACA, which participates in cellular processes, including differentiation, proliferation, and apoptosis, were significantly associated with CNV in 27 cancers. These results showed that abnormal copy number of pyroptosis genes in common in most cancers and affect the genes expression levels.

We also assessed the methylation level of pyroptosis genes between tumors and normal tissue. We found that the pyroptosis genes presented complex methylation patterns in the 14 types of cancers (**Fig.1C**), and only ELANE showed hypermethylation in 12 types of cancers. We observed NLRP7 (n=8), AIM2 (n=10), CASP8 (n=6), GSDMA (n=5), GSDMB (n=8), and GSDMC (n=12) mainly showed hypomethylation in most cancers, and PLCG1 (n=11), NLRP6 (n=13), ELANE (n=11), CASP6 (n=5), NLRP4 (n=12), and PYCARD (n=8) showed hypermethylation in most cancers. The methylation level showed significant differences for pyroptosis genes in 14 cancers (**Additional file 1: Table S4**). The Spearman correlation analysis indicated that a negative relationship was found between gene expression and overall methylation levels (**Fig. 1D and Additional file 2: Table S5**). These results showed that DNA methylation might regulate the expressions of pyroptosis genes in cancers.

Estimated modeling of pyroptosis level and its association with prognosis among cancers

To further explore the role of pyroptosis in the development of tumors and understand the pyroptosis-related biological process, we built a estimated modeling of pyroptosis level for all cancer based on enrichment score by single sample GSEA. We observed that LAML showed the highest pyroptosis level, and PCPG showed the lowest pyroptosis level (**Fig.2A**). We further more compared the pyroptosis levels between tumors and normal tissues. We observed that the pyroptosis levels were significantly elevated in

ESCA, HNSC, KIRC, KIRP, and THCA (**Fig. 2B-F**), while pyroptosis levels were significantly decreased in LIHC, LUSC and PRAD (**Fig. 2G-I**).

We further performed the univariate cox regression to evaluate the association between pyroptosis levels and four survival outcomes including OS, DSS, DFI and PFI. The pyroptosis score was significantly associated with OS in eight types of cancers (**Fig. 3A**): KIRC ($P<0.001$), SKCM ($P<0.001$), LGG ($P<0.001$), PAAD ($P=0.002$), UVM ($P=0.006$), BLCA ($P=0.029$), THYM ($P=0.043$), SARC ($P=0.048$), while pyroptosis score was significantly associated with DSS in seven types of cancers (**Fig. 3B**): KIRC ($P<0.001$), SKCM ($P<0.001$), LGG ($P<0.001$), PAAD ($P=0.011$), UVM ($P=0.039$), UCEC ($P=0.044$). However, the pyroptosis score was significantly associated with DFI in only one cancer (**Fig. 3C**): COAD ($P=0.030$). Significant correlation of pyroptosis score with PFI were also found in seven cancers (**Fig. 3D**): KIRC ($P<0.001$), LGG ($P<0.001$), GBM ($P<0.001$), THYM ($P=0.002$), PAAD ($P=0.002$), SKCM ($P=0.017$), and BLCA ($P=0.029$). Among these cancers, the elevated pyroptosis score was associated with poor survival outcomes in twelve types of cancers (**Additional file 2: Fig. S4A**): ESCA, GBM, HNSC, KIRC, LAML, LGG, LUSC, PAAD, THYM, UCEC, UCS, and UVM, while the elevated pyroptosis score favored the survival prognosis in eight types of cancers (**Additional file 2: Fig. S4B**): BRCA, KICH, MESO, SARC, SKCM, STAD, THCA and BLCA. The pyroptosis score showed different clinical effects in some cancer. For example, the pyroptosis is unfavored for KIRC but favor in KICH.

We also evaluated the associations between pyroptosis genes and risk of tumors. For overall cancers, the NLRP3, PJKV, TIRAP, IL18, NLRP1 and NLRP6 usually exist as protective genes (**Additional file 2: Fig. S5A-B**), while the rest of pyroptosis genes seems to risky genes. Some genes showed different risk patterns. For example, IL6 is a risky gene in multiple cancers but a protective in only SARC. Similar result was also observed for PRKACA in several cancers. On the contrary, the TIRAP is a protective gene in KIRC, READ, and STAD but a risky gene in only BRCA. These results indicated that pyroptosis genes may play different roles in tumors.

Pyroptosis-related pathways and immune signature among cancers

To evaluate the association between pyroptosis level and pathways, we calculated the Spearman correlation coefficients between pyroptosis score and other genes and pathways using gene set enrichment analysis (GSEA) in all cancers. As you can see in **Fig. 4**, IL-6/JAK/STAT3 signaling, allograft rejection, inflammatory response, IL2/STAT5 signaling, TNFA signaling via NFkB, apoptosis KRAS signaling up and P53 pathway were enriched in tumors with high pyroptosis level, which means the pyroptosis was positively with these pathways. The spermatogenesis (29 cancers), pancreas beta cells (20 cancers), oxidative phosphorylation (24 cancers), hedgehog signaling (22 cancers), Wnt-beta catenin signaling (22 cancers), peroxisome (20 cancers), and G2M checkpoint (22 cancers) were enriched in most tumors with low pyroptosis level, which indicated that pyroptosis was negatively associated with these pathways. Other common pathways, such as reactive oxygen species pathway, hypoxia, EMT, PI3K/Akt, some metabolism-related pathways were also enriched in multiple cancers. These pathways showed positive association with pyroptosis level. We further performed GSEA in six cancers (significantly

associated with pyroptosis level) from significantly survival analysis (**Additional file 2: Fig. S6A-F**). We observed multiple immune-related pathways were enriched in BRCA, KIRC, LUSC and PAAD, such as innate immune system, cytokine signaling in immune system, and adaptive immune system.

Considering the important function of immune response process in the tumorigenesis, we explored the correlation of pyroptosis with the immune microenvironment in cancers. The results showed that immune score and stromal score were positively associated with pyroptosis score, while pyroptosis score was negatively associated with tumor purity (**Fig. 5A**). Furthermore, we investigated the associations between pyroptosis score and immune-related pathway, matrix/metastasis-related pathways, and DNA damage repair pathways. The results showed that pyroptosis score were positively associated with immune checkpoint, CD8_T effector, and antigen processing machinery in almost all cancers. DNA damage repair were negatively associated with pyroptosis score in most types of cancers, especially in HNSC, TGCT, ESCA, SARC, LAML, CEUS, GBM, and PCPG. Pyroptosis score was positively associated with EMT2 in cancers, pan_F TBRs in 14 cancers, and EMT3 in 16 cancers, while EMT1 showed positive correlation with pyroptosis in 11 cancers (**Fig. 5A**). To better understand the correlations of pyroptosis with immunotherapy, we calculated the Spearman correlation coefficients between pyroptosis score and immune cells infiltration and found pyroptosis score was positively associated with immune cells infiltration score in almost all cancers except THYM and DLBC (**Fig. 5B**). The pyroptosis score was positively with most of T cells such as Tc, Tfh, Tex, Th1, iTreg, CD8_T, CD4_T, Tr1 in most types of cancers. The positive associations between pyroptosis score and macrophage, DC, NK, and T cells in most cancers (**Additional file 2: Fig. S7A-B**). On the contrary, the pyroptosis score showed negative associations with CD8 naïve, neutrophil, and Th17 cells. Some immune cells showed individual pattern. For example, the Th1 and Th2 cells were only negatively associated with pyroptosis level, while Th17 showed a positive association with pyroptosis level in DLBC.

We also investigated the associations between pyroptosis level and MHC genes (**Fig. 5C**), immunosuppressive genes (**Fig. 5D**), and chemokines (**Fig. 5E**) and their receptor (**Fig. 5F**). The results showed that the MHC genes, immunosuppressive genes, and chemokines and their receptor were positively associated with pyroptosis level in most types of cancers. Some negative correlations of pyroptosis with these immune-related genes can be found in some cancers. For example, the HLA-DMA, HLA-DOB, and HLA-DQB1 were negatively with pyroptosis in DLBC, the KDR (immune suppressor genes) was also negatively associated with pyroptosis level in TGCT. Similarly, the chemokines (CXCR4) also show a negative association with pyroptosis in ACC. Some chemokine receptors such as CCL27 (8 cancers), CCL28 (7 cancers), CCL16 (5 cancers), CCL17 (5 cancers), and CCL15 (four cancers) were negatively associated with pyroptosis in several cancers. The CCL11 only show a negative relationship with pyroptosis level in only DLBC. These results indicated a close connection between pyroptosis and immune microenvironment in cancers, and more research is required for illustrating more details.

We further evaluated the correlations of pyroptosis level and microSatellite instability (MSI) and tumor mutation burden (TMB) in cancers that were suggested to be associated with prognosis of multiple cancers after immunotherapy. We observed that pyroptosis level was positively associated with MSI in

COAD, STAD, THCA, PRAD, while negative relationships were observed in LIHC, KIRP, OV, PADD, TGCT and DLBC (**Additional file 2: Fig. S8A**). For TMB, the pyroptosis level showed a positive association in COAD and STAD but negative association in LUAD, PCPG, TGCT and CHOL (**Additional file 2: Fig. S8B**).

To better understand the correlation of pyroptosis with immunotherapy, we investigated the effect of pyroptosis level on prognosis using GEO dataset in three cancers (GSE13507: primary bladder cancer; GSE32894: urothelial carcinoma; GSE61676: non-squamous non-small cell lung cancer). The results showed that high pyroptosis level was associated with poor OS in primary bladder cancer (**Fig. 6A**), DFS in urothelial carcinoma (**Fig. 6B**), and OS in non-squamous non-small cell lung cancer after immunotherapy (**Fig. 6C**). These results showed that pyroptosis might affect the effect of immunotherapy in some cancers.

Identification of potential compounds targeting pyroptosis-related genes

To further understand the association between pyroptosis and drug sensitivity, we calculated the correlation coefficient between pyroptosis genes and drug sensitivity (evaluated by the percent viability curve approach) using CTRP and GDSC datasets. We selected top 30 compounds targeting pyroptosis-related genes ($|r| > 0.3$). The results showed that the expressions of IL-6, IL18 and GSDME were positively associated with these compounds, while CASP4 was positively correlated with sensitivity in 26 cancer drugs (**Fig. 6D**). AIM2, CASP3, NLRC4, TNF, NLRP6 may be associated with tumor drug resistance (**Fig. 6E**). The other results for two datasets were presented in **Additional file 1: Table S6 and Additional file 1: Table S7**. These results indicated that pyroptosis might be associated with sensitivity of multiple drugs in cancers.

Discussion

Pyroptosis is inflammatory programmed cell death, which is characterized by activation of inflammatory Caspases (Caspase 1, 4, 5 and 11) in inflammasomes and secretion of inflammatory cytokines such as interleukin-1 β and interleukin-18[23]. Researchers have found that GSDMA acts as an essential downstream substrate of inflammatory caspases, performing pyroptosis by forming pores in the plasma membrane[24]. Currently, the functions of pyroptosis in the tumorigenesis and tumor treatment have been increasingly concerned. However, comprehensive analysis and biological regulation about pyroptosis genes are lack in cancers. In the present study, we integrated multi-omics data and clinically relevant outcomes across 33 cancers from public dataset and depicted the landscape of alterations, epigenetic and transcriptional levels of pyroptosis genes. We also evaluated the pyroptosis levels across cancers using ssGSEA, and identified the correlations of pyroptosis level with immune features, survival outcomes, immunotherapy and drug sensitivity. The pyroptosis showed different genetic, epigenetic and transcriptional patterns in different cancers, and different functions of pyroptosis on survival and immune treatment were also observed, especially in KIRC, LGG, GBM, PADD, and SKCM. The elevated pyroptosis level has an adverse effect on immunotherapy in primary bladder cancer, urothelial carcinoma

and non-squamous non-small cell lung cancer, which means that pyroptosis level should be considered when immunotherapy is applied in cancers.

The molecular mechanism that pyroptosis is involved in tumorigenesis and development still remains unclear. However, the correlations of pyroptosis with some functions and pathways may provide us some important clues. The results of GSVA indicated that the pyroptosis genes were mainly enriched in two components. The first is inflammation-related pathways such as IL6/JAK/STAT3 signaling, inflammatory response, IL2/STAT5 signaling, TNF-alpha signaling via NFkB, and KRAS signaling up, which have been proved to be associated with tumor. The inflammation response can promote the occurrence and progression of tumor[25]: Sustained oxidative stress in the process of chronic inflammation leads to DNA damage and inhibition on its repair, resulting in inactivation of tumor suppressor genes. Inflammatory cells and inflammatory factors in the micro-environment can induce the expression of a variety of cytokines[26]. These inflammatory cells, cytokines and their downstream products promote the occurrence, development and metastasis of cancer by various ways, such as inhibiting apoptosis, promoting angiogenesis and inducing immune tolerance[27]. The inflammation response is the primary characteristic of pyroptosis. The process of pyroptosis depends on the activation of Caspase-1, and an important function of Caspase-1 is to mediate the cleavage of interleukin-1 β precursor into active IL-1 β , which can recruit and activate other immune cells, induce the synthesis of chemokines, inflammatory factors, adhesion molecules, etc., and eventually form "cascade effect, cascade effect, which amplifies the inflammatory response and leads to a severe inflammatory response[28]. We also found that pyroptosis genes were enriched in immune-related pathways such as allograft rejection, complement, and interferon alpha and gamma response. The results of GSEA analysis indicated that several immune-related pathways such as innate immune system, adaptive immune system, cytokine signaling in immune system appeared in multiple cancers. Pyroptosis, as a programmed cell death, is an important natural immune response of the body. Previous study also reported that inducing pyroptosis in tumors can induce high anti-tumor immune activity and then clear tumors[29,30]. However, our results indicated that pyroptosis showed favorable effect on survival outcomes in BRCA, KICH, MESO, SARC, SKCM, STAD, TCHA and BLCA, while pyroptosis presented adverse effects on prognosis in ESCA, GBM, HNSC, KIRC, LAML, LGG, LUSC, PADD, THYM, UCES, UCS and UVM. Previous studies also reported that pyroptosis inhibits cancer progression in breast, liver, ovarian, stomach, and colon cancers, and promotes cancer progression in melanoma. In lung cancer, NLRP3 inflammasome involved pyroptosis promotes the progression of lung adenocarcinoma but inhibits the progression of non-small cell lung cancer, while GSDMD involved pyroptosis promotes the progression of non-small cell lung cancer[31]. Our results validated the dual roles of pyroptosis in cancers. In addition, the same location tumor also has different prognosis patterns during the same pyroptosis level such as thyroid, kidney, and lung. Furthermore, we analyzed the survival outcomes of patients who received immunotherapy, and found elevated pyroptosis level is an adverse to prognosis in primary bladder cancer; urothelial carcinoma, and non-squamous non-small cell lung cancer. The pyroptosis seems not always exhibit antitumor immune effect.

We further analyzed the correlation between drug sensitivity and pyroptosis genes expression. Previous study reported that GSDMD can be activated by the cleavage of Caspase-3, and induces pyroptosis with the condition of tumor chemotherapy drug[32]. Human neuroblastoma SH-SY5Y cells and human malignant melanoma MeWo cells have a high level of GSDME expression. Under the action of chemotherapy drugs such as Topotecan, Etoposide, Cisplatin and so on, the cells undergo obvious pyroptosis rather than apoptosis[33/34]. Our results indicated that GSDME was positively associated with multiple molecular drugs. GSDME as a tumor suppressor gene is expected to become a new direction of clinical treatment. The NLRP1 expression showed a negative association with drug sensitivity. This result proved the role of promoting tumors. Previous study reported that NLRP1 promotes melanoma growth by enhancing inflammasome activation and suppressing apoptotic pathways[35]. These results highlight the dual roles of pyroptosis in cancers.

Conclusions

In the present study, we performed a comprehensive analysis of pyroptosis and its regulator genes in cancers. Most pyroptosis genes were aberrantly expressed among different cancer types., which is contributed by the CAN frequency and differences of DNA methylation level in cancer. We evaluated the pyroptosis level and found that pyroptosis showed dual roles across cancers, while the pyroptosis levels were different in multiple and be significantly associated with clinical prognosis. The dual role of pyroptosis also affect the effects of immunotherapy in several cancers. Six pyroptosis genes showed close connection with drug sensitivity across cancers, and may be considered as therapy targets in cancer. Thus, our comprehensive analysis highlighted the possibility of pyroptosis-based cancer therapy.

Abbreviations

GSDMD, Gasdermin D; NLRP, Nucleotide-Binding Oligomerization Domain, Leucine Rich Repeat and CARD Domain Containing; RIG, retinoic acid-induced gene; TCGA, The cancer genome atlas; GDSC, Genomics of Drug Sensitivity in Cancer, GSVA, Gene set variation analysis; FC, fold change; DEG, differentially expressed gene; GSEA, Gene set enrichment analysis; TME: Tumor microenvironment; DNA, deoxyribonucleic acid; lncRNA, long non-coding ribose nucleic acid; HR, hazard ratio; CI, confidence interval; OS, overall survival; DSS, disease specific survival; PFI, progression free interval; DFI, disease-free interval; Tem, effective memory T cell; Th1, T helper cell 1; Th17, T helper cell 17; Th2,T helper cell 2; Treg, regulatory T cells

Declarations

Acknowledgements

None.

Authors' contributions

ZZL designed this study and directed the research group in all aspects, including planning, execution, and analysis of the study. LAB drafted the manuscript. NL, LAB collected the data. LZZ provided the statistical software, performed the data analysis, SL arranged the Figures and Tables. SLF revised the manuscript. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

The data underlying this article will be shared on reasonable request to the corresponding author.

Ethics approval and consent to participate

Ethical is not applicable because these data are from public database.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

Figure 1

Genetic alteration landscape of pyroptosis-related genes in cancer. A: gene alterations in cancer. B: correlation of copy number variation and pyroptosis genes expression. C: Methylation level differences in each cancer. D: Correlations between methylation level and pyroptosis genes expression

Figure 2

Establishing modelling of pyroptosis level in cancers. A: pyroptosis level in each cancer. B-F: Elevated pyroptosis levels were found in ESCA, HNSC, KIRC, KIRP, and THCA. G-I: Decreased pyroptosis levels were observed in LIHC, LUSC and PRAD.

Figure 3

Correlations of pyroptosis level with prognosis in each cancer based on cox regression. A: overall survival. B: Disease specific survival. C: progression free interval. D: disease-free interval.

Figure 4

Gene set variation analysis for pyroptosis in cancers

Figure 5

Correlations between pyroptosis level and immune features. A: immune Microenvironment. B: immune cell subsets. C: major histocompatibility complex-related genes. D: immune suppression genes. E-D: Chemokines and their receptors