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Pyroptosis: Turning Up the Heat on Cancer

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Abstract

The major effector cells of antitumor immunity are killer lymphocytes that recognize and eliminate tumor cells. The fact that tumor cells look a lot like normal cells poses a challenge to antitumor immune control. A danger signal from the tumor or from antigen-presenting cells that have taken up dying tumor cells is needed to distinguish tumor cells from normal cells to fully activate killer cell effector functionality and memory and thereby control the tumor. How a tumor cell dies strongly affects whether the immune system sees it as dangerous. Activation of innate immunity in the tumor, including interferon signaling and necrotic cell death (e.g., necroptosis and pyroptosis), sounds a potent immune alarm. Pyroptosis plays an important role in tumor immunity by generating an inflamed tumor microenvironment. However, it is a double-edged sword that can both promote tumorigenesis and increase the effectiveness and cytotoxicity of cancer therapy. In this article, we review what is known about the role of tumor cell pyroptosis, which is arguably the most inflammatory type of cell death, in antitumor immunity and discuss whether it could be safely harnessed to broaden the range of tumors that respond to immunotherapy.

1. INTRODUCTION

Approximately one million cells die every second in each of us. How they die has significant implications for health and disease. Under basal conditions, most cell deaths maintain homeostasis, and most dying cells undergo apoptosis, which, as the least immunological form of cell death, is mostly invisible to the immune system. However, some cells that die in response to stress, infection, or therapy undergo more immunogenic forms of cell death that can trigger inflammation and both protective and pathological outcomes.

Pyroptosis ("fiery death") is an especially rapid and inflammatory form of immunogenic programmed cell death mediated by cleavage and activation of pore-forming proteins, called gasdermins (GSDMs), whose N-terminal cleavage fragments assemble to form membrane pores (1, 2). Under basal conditions, the GSDMs, as their name suggests, are mostly expressed in the skin and other mucosal tissues, including the gut, as well as in myeloid cells in blood, lymph, and tissues, which serve as immune sentinels of pathogens and sterile danger. Pyroptosis is characterized by cell lysis (also called necrosis, meaning cell membrane integrity is disrupted) and the release of danger signals, including inflammatory cytokines that lack secretion signals, which are released through GSDM pores to alert the immune system to invasive infection and tissue damage (1, 2). The wide distribution of the sensors and mediators of pyroptosis and the accompanying processing and release of the inflammatory IL-1 family cytokines (such as IL-1β and IL-18) contribute to making pyroptosis one of the most, if not the most, inflammatory forms of cell death in the body.

The human GSDM family contains five active GSDMs (GSDMA–E) (**Figure 1**; **Table 1**). A sixth GSDM, GSDMF (also known as DFNB59 or PJVK), is less homologous, has a truncated C terminus, shows limited expression in most tissues, and has not been shown to cause cell death (3). The most well-studied GSDM is GSDMD, which is expressed in myeloid cells and in

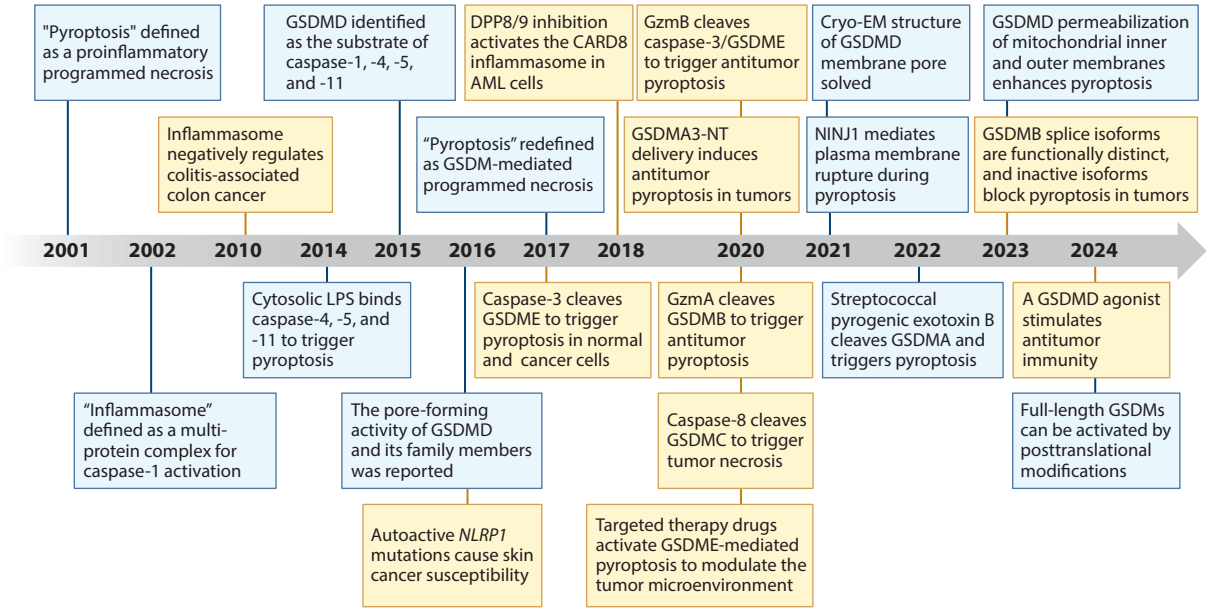


Figure 1

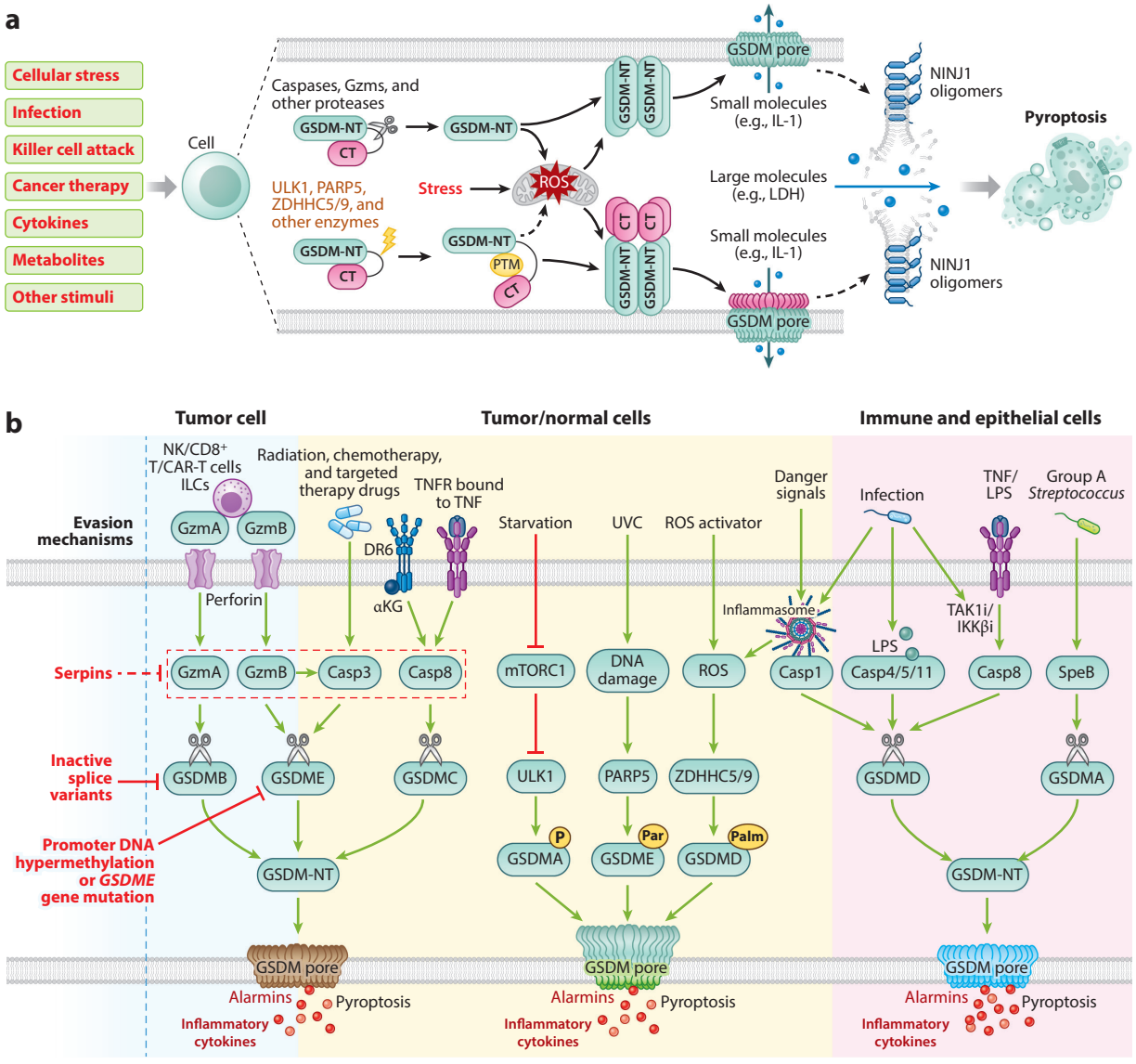
A timeline of key pyroptosis research findings in cancer. The yellow boxes indicate studies directly related to cancer. Abbreviations: AML, acute myeloid leukemia; CARD8, caspase recruitment domain–containing protein 8; cryo-EM, cryogenic electron microscopy; DPP8/9, dipeptidyl peptidase 8/9; GSDM, gasdermin; Gzm, granzyme; LPS, lipopolysaccharide; NINJ1, ninjurin-1; NT, N-terminal domain. Figure adapted from images created in BioRender; Zhang Z. 2025. **https://BioRender.com/l4avj0a**.

Table 1 Human gasdermin genes, their expression, and their functions in cancer[a]

Gene	Pore formation	Expression in tissues	Expression in tumors	Activation	Function	Disease link(s)	References
GSDMA (*Gsdma1–3*)	Yes	Gastrointestinal tract and skin	**Upregulated**: glioma **Downregulated**: gastric cancer	SpeB Phosphorylation	Defense against infection	Alopecia Asthma IBD Cancer	5, 6, 76, 80, 131, 193, 194
GSDMB (NA)	Yes	Gastrointestinal tract, airway, liver, bladder, and breast	**Upregulated**: oral, cervical, gastric, bladder, and breast cancers **Downregulated:** esophageal and gastric cancers **Splicing isoforms:** differentially expressed in many cancers	GzmA	Defense against infection and tumor Tumorigenesis Epithelial repair Transcription	Cancer Asthma IBD Systemic sclerosis	12, 84–88, 129–136
GSDMC (*Gsdmc1–4*)	Yes	Esophagus, skin, spleen, cervix, and tonsil	**Upregulated**: melanoma and colorectal, breast, and lung cancers **Downregulated**: gastric cancer	Casp8 Casp6 Mcpt1	Defense against tumor and worm infection Tumor necrosis IL-33 secretion	Cancer	51, 89–91, 131, 195–198
GSDMD (*Gsdmd*)	Yes	Most tissues, including spleen, liver, lung, and gastrointestinal tract	**Upregulated:** adenoid cystic carcinoma and lung, breast, and liver cancers **Downregulated**: gastric and colon cancers	Casp1 Casp4/5/11 Casp8 Elastase Ctsg Palmitoylation	Defense against infection and tumor Tumorigenesis IL-1β secretion IL-33 secretion Mucin secretion Transcription	Sepsis CRS Autoimmune diseases Cancer	7–9, 14–21, 70–74, 96, 103, 104, 131, 147–151, 199–201
GSDME/DFNA5 (*Gsdme/Dfna5*)	Yes	Most tissues, including cochlea, placenta, brain, and heart	**Upregulated**: pancreatic and lung cancers **Downregulated**: gastric, colorectal, and breast cancers	GzmB Casp3 PARylation	Defense against tumor Mucin secretion Transcription	Cancer Hearing loss CRS Tissue damage ALS	10, 11, 53, 54, 77, 119, 124, 126, 138–144
GSDMF/DFNB59 (*Gsdmf/Dfnb59*)	No	Many tissues, including cochlea, testis, breast, and skin	Not known	NA	Peroxisome proliferation	Hearing loss	3, 202, 203

[a]The expression of human *GSDM* genes in tissues is mostly based on The Human Protein Atlas (**https://www.proteinatlas.org**) and indicated references. The expression pattern of gasdermins in tumors are from published studies, which are based on The Cancer Genome Atlas database and experimental validation. The mouse *Gsdm* homologs are listed in parentheses.
Abbreviations: ALS, amyotrophic lateral sclerosis; Casp8, caspase-8; CRS, cytokine release syndrome; Ctsg, cathepsin G; Gzm, granzyme; IBD, inflammatory bowel disease; Mcpt1, mast cell protease-1; NA, not applicable; PARylation, poly(ADP-ribosyl)ation.

mucosal epithelial and endothelial cells and which is activated by inflammatory caspases (caspase-1 in mice and humans, caspase-11 in mice, and caspase-4 and -5 in humans) after signs of invasive infection or sterile danger are sensed by cytosolic sensors called inflammasomes. GSDMC and GSDME can also be activated by apoptotic caspase-8 and -3, respectively, in cells that express these GSDMs under certain conditions to convert noninflammatory apoptosis to inflammatory pyroptosis (4) (**Figure 2**). The GSDMs can also be activated independently of the caspases by other proteolytically active proteases in the cytosol, where the GSDMs are mostly localized (4). These proteases include pathogen proteases, such as a virulence factor of a bacterial skin pathogen, which cleaves GSDMA, mostly expressed in the skin (5, 6); neutrophil granule proteases [e.g., neutrophil elastase and cathepsin G (Ctsg), which cleave GSDMD] (7–9); and cytotoxic lymphocyte



(*Caption for* ***Figure 2*** *appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Molecular mechanisms that activate and inactivate pyroptosis. (*a*) Molecular steps in pyroptosis are shown. GSDM-NTs generated by protease cleavage (1) permeabilize mitochondrial membranes and generate mitochondrial ROS (68), which drive *S*-palmitoylation of GSDM cysteines required for GSDM-NT membrane binding (70–74). GSDM-NTs then form pores that perforate the plasma membrane (16–21). These pores trigger the oligomerization of the integral membrane protein NINJ1, enabling plasma membrane rupture and release of larger molecules, such as tetrameric LDH (75). In parallel, PTMs in the full-length GSDM can disrupt the CT inhibition of the pore-forming NT to trigger pyroptosis (70, 76, 77). (*b*) Molecular signaling pathways that activate pyroptosis and known mechanisms that tumors can employ to avoid pyroptosis. Killer lymphocytes, including NK cells, $CD8^+$ T cells, ILCs, and CAR-T cells, can deliver GzmA and GzmB into tumor cells, where they cleave and activate GSDMB and GSDME, respectively, to induce pyroptosis (10, 12). GzmB can also activate Casp3, which, in turn, cleaves GSDME to amplify pyroptosis (10, 11). Radiation, chemotherapy, and targeted therapy drugs can activate Casp3 to cleave GSDME and activate pyroptosis (50, 54, 79, 106–111). GSDMC can be activated by active Casp8 downstream of death receptors TNFR and DR6 (51, 89). However, tumors evolve various strategies to evade antitumor pyroptosis. Gzms and caspases can be suppressed by the endogenous Serpin protease inhibitors. Tumors also overexpress inactive GSDMB splice isoforms to inhibit GSDMB-mediated pyroptosis (85). In addition, tumors can evade pyroptosis via epigenetic repression or mutation of *GSDME* (10, 119). Upon sensing infection or tissue danger, inflammasomes activate Casp1 to cleave GSDMD and induce pyroptosis (14, 15). Inflammatory Casp4, Casp5, and Casp11 can be activated to cleave GSDMD and trigger pyroptosis by binding to cytosolic LPS or oxidized host lipids (96, 97). GSDMD can also be activated by Casp8 in response to infection or TNF when TAK1 or IKKβ is inhibited (103, 104). During infection, the Group A *Streptococcus* protease and virulence factor SpeB can cleave and activate GSDMA to induce pyroptosis in keratinocytes (5, 6). Starvation-induced phosphorylation of GSDMA (76), ROS- and inflammasome-induced *S*-palmitoylation of GSDMD (70), and UVC-induced PARylation of GSDME (77) (indicated by *orange circles*) all activate full-length GSDMs to cause pyroptosis. Abbreviations: αKG, α-ketoglutarate; CAR, chimeric antigen receptor; Casp3, caspase-3; CT, C-terminal domain; GSDM, gasdermin; Gzm, granzyme; IKKβ, inhibitor of κB kinase β; IKKβi, IKKβ inhibition; ILC, innate lymphoid cell; LDH, lactate dehydrogenase; LPS, lipopolysaccharide; mTORC1, mammalian target of rapamycin complex 1; NINJ1, ninjurin-1; NK, natural killer; NT, N-terminal domain; PARP, poly (ADP-ribose) polymerase; PARylation, poly(ADP-ribosyl)ation; PTM, posttranslational modification; ROS, reactive oxygen species; TAK1, TGF-β-activated kinase 1; TAK1i, TAK1 inhibition; TNFR, TNF receptor; ULK1, unc-51-like autophagy-activating kinase 1; ZDHHC, zinc finger and DHHC domain–containing protein. Panel *a* adapted from images created in BioRender; Zhang Z. 2025. **https://BioRender.com/6kdfhjy**. Panel *b* adapted from images created in BioRender; Zhang Z. 2025. **https://BioRender.com/i6rj3e7**.

granule proteases [called granzymes (Gzms)] delivered into target cells. GzmA activates GSDMB, while GzmB activates GSDME (10–13). Additional host and pathogen proteases that cleave the GSDMs and trigger pyroptosis likely will be identified in the future.

The study of pyroptosis took off after the genetic identification of GSDMD in 2015 as the executioner of pyroptosis in macrophages (14, 15) and of membrane pore formation as its mechanism of action one year later (16–21) (**Figure 1**). Before that, pyroptosis was identified as a programmed form of cell death triggered by infections and sterile danger signals (22) and mediated by activation of inflammasomes that destroy the host cell to suppress bacterial intracellular replication, leading to processing and secretion of IL-1 family cytokines; the cytokines recruit immune cells to sites of infection and danger and help activate them. Pre-2015 studies examined the role of pyroptosis in cancer by investigating the roles of inflammasome components and of IL-1 family cytokines in tumor promotion and protection. These studies suggested that pyroptosis could either promote tumorigenesis or protect against tumors, depending on the context (23). For example, germline mutations in the inflammasome gene *NLRP1* that constitutively activate NLRP1 [NOD (nucleotide-binding oligomerization domain)-like receptor family pyrin domain–containing protein 1] increase skin inflammation and susceptibility to skin cancer (24), melanoma (25), and asbestos-associated mesothelioma (26). Similarly, a gain-of-function variant of *NLRP3* increases the risk of melanoma and colorectal cancer (CRC) (25, 27). The detrimental role of these inflammasome gene variants depends, at least in part, on elevated IL-1β, which promotes tumor growth by increasing tumorigenesis, angiogenesis, proinflammatory transcription factor expression, tumor cell malignancy, and recruitment of immunosuppressor cells (28–32), although the tumor-protective role of IL-1β has also been reported in other circumstances (33–35).

Because of the well-documented role of inflammation in tumorigenesis (36), most cancer immunologists have steered away from studying potential protective roles of pyroptosis in stimulating antitumor immunity or considering the triggering of pyroptosis as a component of immunotherapy. However, pyroptosis protects against azoxymethane (AOM)- or dextran sulfate sodium (DSS)–induced CRC tumors, and mice genetically deficient in key pyroptosis pathway genes (i.e., *Nlrp3*, *Asc*, *Casp1*, *Nlrp1*, *Nlrc4*, *Nlrp6*, *Pyrin*, or *Card9*) have increased tumor burden (37–46). Some of this antitumor protection may be mediated by the ability of IL-18 to promote gut epithelial barrier repair (47). *Il18*$^{-/-}$ mice have increased tumor burden, and IL-18 treatment of mice deficient in inflammasome genes reduces tumor burden (37–40). However, IL-18 protection depends on tumor type. A polymorphism that upregulates *IL18* expression is associated with increased lymphoma risk (48), and *Il18*$^{-/-}$ mice are protected from multiple myeloma progression (49).

After 2016, pyroptosis research expanded rapidly to consider the role of pyroptosis in cancer and of other GSDM family members that are activated independently of inflammasomes (**Figure 1**) (10, 12, 50–52). The apoptotic caspase caspase-3 was soon found to activate inflammasome-independent pyroptosis by cleaving GSDME (53, 54), and GSDME-mediated pyroptosis was shown to contribute to the toxicity of cancer therapy in normal tissues (11, 54) and to play essential roles in mediating antitumor immunity (10, 50). GSDME expression and activity are often suppressed in human cancers, and this finding suggests that GSDME might be a tumor suppressor gene and that figuring out ways to induce pyroptosis in tumor cells could improve antitumor immunity or tumor response to immunotherapy. Recent studies have directly examined pyroptosis in cancer cells by measuring GSDM cleavage and membrane permeabilization in cells expressing wild-type, inactive, or cleavage-resistant GSDMs. In most cancer cells, inflammasome-independent pyroptosis does not directly or indirectly activate the inflammatory caspases required for IL-1 maturation, as inflammasome components are often silenced or mutated in those cells (55). Moreover, IL-1 family cytokines are frequently not expressed in tumor cells. However, IL-1 production has been reported in tumors undergoing inflammasome-independent pyroptosis. In tumors, IL-1 processing and secretion could result from secondary activation of inflammasome-mediated pyroptosis in tumor-infiltrating or bystander immune cells, such as macrophages (11).

Antitumor immunity relies on killer lymphocyte recognition and elimination of tumor cells, especially by CD8^{+} cytotoxic T lymphocytes. Reflecting the thinking of cancer immunologists developing cancer immunotherapy at the time, the original model of the cancer-immunity cycle, proposed over a decade ago by Chen and Mellman (56), focused on antigen-specific, T cell–mediated antitumor immunity. The original cancer-immunity cycle model begins with antigen-presenting cells (APCs) phagocytosing dying cancer cells in the tumor, processing and presenting tumor antigens, and migrating to draining lymph nodes, where they activate tumor-specific T cells. These T cells return to infiltrate the tumor and kill tumor cells, and the cycle restarts. Since cancer cell death is both the beginning and end point of this cycle, how cancer cells initially die and the state of the tumor microenvironment (TME) determine how effectively antitumor immunity is stimulated and whether tumor-specific cytolytic T cells control the tumor.

To avoid autoimmunity, killer cells need to distinguish transformed tumor cells from the normal cells from which they arise—a challenging task since many tumors do not express many tumor antigens, and tumors also suppress antigen processing, antigen presentation, and MHC expression as part of immunoediting to avoid immune elimination (57, 58). Moreover, the development of T cell antitumor functionality, including killing, cytokine secretion, and memory, which are critical for effective antitumor immunity, depends not only on T cell or natural killer (NK) cell receptor recognition of tumor antigens or cellular stress (signal 1) and costimulation (signal 2) but

also on a third danger signal (signal 3) (59–61). The danger signal is generally an innate immune alarm. It can be provided by cytokines, such as interferons, which are well-recognized to promote T cell activation, and also by inflammatory cytokines, which have not been as well-studied. Necrotic forms of programmed cell death—pyroptosis, necroptosis, and probably ferroptosis—are all immunogenic and promote tumor immunity (10, 62, 63). The relative potency of interferons versus inflammatory forms of necrotic cell death in promoting antitumor immunity has not been directly compared, but inflammatory cell death may sound a stronger immune alarm by releasing multiple danger molecules. A recent study in a genetically engineered mouse model (GEMM) of $Her2^+$ breast cancer found that cancer immunoediting suppresses all the innate immune pathways in tumor cells that generate danger signals, and this finding suggests that all these pathways contribute to immunosurveillance (64). In contrast, apoptosis, except for some chemotherapy- and radiotherapy-induced forms of cell death that cause ER stress (65), is generally immunologically silent and can also induce immunotolerance, suppressing antitumor immunity (56, 66).

Importantly, although killer lymphocyte–mediated programmed cell death was previously thought to be noninflammatory like apoptosis, tumor-specific killer lymphocytes [e.g., NK cells, T cells, and tissue-resident innate lymphoid cells (ILCs)] can trigger caspase- and inflammasome-independent pyroptosis in some GSDM-expressing tumor cells via the action of cytotoxic granule proteases, GzmA and GzmB (10–13). Pyroptosis and other immunogenic forms of cell death make the TME inflamed, and this inflammation stimulates antitumor immunity (10, 12, 50, 52, 62, 63). Restoring GSDM expression in tumor cells, where it is often epigenetically repressed, can increase solid tumor immune surveillance or responsiveness to immunotherapy (12, 52).

This article is based on the idea that providing a pyroptotic danger signal can help reestablish tumor immunosurveillance and potentially expand the range of solid tumors that respond to immune checkpoint blockade (ICB) and other immunotherapies. We review what is known about the role of pyroptosis in cancer. Our understanding of the molecular basis of pyroptosis, the complex interaction of cancer cells with the TME, and the role of pyroptosis in immune control of cancer in different cell types is still limited and evolving. Figuring out the tumor types and situations in which pyroptosis promotes antitumor immunity versus those in which pyroptosis promotes tumorigenesis or metastasis will be critical for evaluating new ways to effectively manipulate the cancer-immunity cycle.

2. THE MOLECULAR BASIS OF PYROPTOSIS

Pyroptosis is now defined as programmed necrotic cell death mediated by GSDMs (2, 67). GSDMA–E contain an active N-terminal domain (NT) and an autoinhibitory C-terminal domain (CT), connected by a flexible linker (1). Proteolytic cleavage in the interdomain linker releases active GSDM-NT, which binds to cardiolipin in mitochondrial inner and outer membranes to form pores that permeabilize both membranes and cause profound mitochondrial damage (68). Mitochondrial damage occurs before plasma membrane damage and is necessary for pyroptosis (68, 69). Mitochondrial ROS enhances *S*-palmitoylation of cysteines (i.e., Cys-191 of human GSDMD, Cys-192 of mouse GSDMD), which is required for the active N-terminal fragment of GSDMs to bind to membranes (70–74). GSDM-NTs then bind to acidic phospholipids on the inner leaflet of the plasma membrane, oligomerize, and form pores that perforate the plasma membrane (16–21), which trigger (through an unknown mechanism) the oligomerization of the integral membrane protein ninjurin-1 (NINJ1) to mediate plasma membrane rupture and release of larger molecules, such as tetrameric lactate dehydrogenase (LDH) (75) (**Figure 2*a***). The GSDMs are cleaved and activated by a variety of proteases that include not only inflammatory caspases (i.e., caspase-1, -4, -5, and -11) but also apoptotic caspases (i.e., caspase-3 and -8), hematopoietic cell granule

proteases, and bacterial protease virulence factors (**Figure 2*b***; **Table 1**) (4–13). In fact, the flexible interdomain linker of the GSDMs is highly susceptible to proteolytic cleavage. Thus, the GSDMs can be viewed as sensors of active cytosolic proteases, which are a sign of cellular danger. In addition to proteolytic cleavage, genetic mutations and posttranslational modifications (PTMs), including palmitoylation, phosphorylation, and PARylation [poly(ADP-ribosyl)ation], can also disinhibit and activate full-length GSDMs under some circumstances, likely by disrupting the interactions of GSDM-NT with the inhibitory CT (**Figure 2**) (17, 70, 76, 77).

GSDM pores are large, with an inner diameter of approximately 200 Å, which should allow the release of small soluble molecules, including smaller proteins and other molecules, such as metabolites and ATP (21, 78). However, protein release is not solely determined by size. The GSDMD-NT pore (and presumably that of other GSDMs) has an acidic cytosolic rim that repels molecules with an acidic surface charge, such as the unprocessed pro-IL-1 family cytokines. These cytokines can be secreted after processing to their mature forms, which have a basic surface (21). This "intelligent design" of the pore serves to optimize inflammation by releasing only the biologically active cytokines. The release of larger molecules that are too big to pass through the GSDMD pore, such as LDH, depends on NINJ1-mediated plasma membrane rupture (75).

GSDM-triggered pyroptosis not only plays essential roles in host defense against both infection and cancer but also can cause inflammatory pathology, including cytokine storm, vascular leak, sepsis, and neurodegeneration (1). GSDME-mediated pyroptosis has been shown to exacerbate normal cell toxicity caused by cancer chemotherapy and radiation and the dangerous cytokine storm complication of CAR (chimeric antigen receptor)-T cell therapy (11, 54, 79). Pyroptosis is also dysregulated to cause autoinflammatory disease when upstream activators, such as inflammasomes, are mutated to become constitutively active (1). More work is needed to understand better where GSDMs are expressed, the stimuli and pathways that activate them, how their expression and activity are regulated, and their normal physiological functions and pathological roles in disease (**Table 1**).

A brief description of what is known about each GSDM follows.

GSDMA is selectively expressed in the gastrointestinal tract and dermis (80). SpeB, which is the group A *Streptococcus* cysteine protease virulence factor, activates GSDMA by cleaving it after Gln-246 to induce pyroptosis in keratinocytes and enhances defense against *Streptococcus* infection (5, 6). GSDMA in amphibians, reptiles, and birds is activated by caspase-1 (81), but mammalian activators of GSDMA are not known. GSDMA can also be activated independently of proteolytic cleavage by Ser-353 phosphorylation (76) or by mutations that disrupt the interaction between the NT and CT (17, 82).

GSDMB, most highly expressed in the gastrointestinal tract and lung epithelia, is activated by the killer lymphocyte cytotoxic granule protease, GzmA, when it is delivered into the cytosol of target cells during lymphocyte attack (12). GzmA cleaves GSDMB after Lys-244 (major cleavage site) and Lys-229 (minor site). The pore-forming activity of GSDMB-NT in mammalian cells was verified in some studies but not others (83, 84). These discrepancies were later clarified by studies showing that only two of the five GSDMB splicing variants (isoforms 3 and 4) contain an intact exon 6 and can form pores (85–88), while the other isoforms are dominant-negative proteins that inhibit pore formation (85). Mice do not encode for a GSDMB ortholog. Killer cell–triggered GSDMB pyroptosis enhances host defense against cancer and infection (12, 84).

GSDMC, most highly expressed in the spleen, skin, and salivary glands, causes pyroptosis triggered by cytokines and metabolites. *GSDMC* expression is induced by hypoxia (51), a common feature of poorly vascularized tumor regions. TNF-α activates the death receptor–associated caspase-8 to cut GSDMC after Asp-365 and thereby to convert apoptosis to pyroptosis in tumor cells. The tricarboxylic acid cycle metabolite α-ketoglutarate also induces GSDMC-mediated

pyroptosis in tumor cells by activating the death receptor DR6 and caspase-8, which cuts GSDMC after Asp-240 (89). Why caspase-8 cuts GSDMC at different sites under different conditions is unclear. Caspase-6, activated by killer cell GzmB, was also reported to cleave GSDMC after Asp-365 to trigger pyroptosis and enhance responses to PARP [poly (ADP-ribose) polymerase] inhibitors (90). GSDMC can also be cleaved and activated in epithelial cells by mast cell protease 1; GSDMC-NT pores secrete IL-33 without causing cell death during helminth infection (91).

GSDMD is the most well-studied GSDM. GSDMD-mediated pyroptosis in macrophages, epithelial cells, and endothelial cells is activated by inflammasomes and inflammatory caspases in response to infection and danger signals, as reviewed elsewhere (92–94). GSDMD is also expressed weakly in most solid cancers, as shown in an analysis of The Cancer Genome Atlas (TCGA) database (95). When immune cytosolic sensors (i.e., NOD-like receptors, AIM2-like receptors, pyrin) encounter pathogen-associated molecular patterns, they organize into micron-sized complexes (canonical inflammasomes) that recruit and activate the inflammatory caspase-1 in humans and mice to process and release the pro-IL-1 family cytokines and cause pyroptosis. In addition, in the noncanonical inflammasome pathway, human caspase-4 and -5 and mouse caspase-11 are activated by binding to cytosolic lipopolysaccharide (LPS) (96) or oxidized host lipids (97) to process GSDMD and trigger pyroptosis (98–100). Noncanonical inflammasome activation by LPS causes sepsis and vascular leak. Activation of the noncanonical inflammasome in humans is much more inflammatory than in mice, in part because human caspase-4, but not mouse caspase-11, is constitutively expressed and also processes the inflammatory cytokine pro-IL-18 (101, 102). GSDMD can also be cleaved after Asp-276 by caspase-8 when TAK1 (TGF-β-activated kinase 1) is inhibited by the *Yersinia* effector YopJ (103, 104). In addition, GSDMD is cleaved and activated by neutrophil elastase and Ctsg and is implicated in the generation of neutrophil extracellular traps (NETs) and neutrophil cell death (NETosis) (7–9). GSDMD can also be weakly activated independently of proteolytic cleavage by its palmitoylation (70). On the other hand, the apoptotic effector caspases can inactivate GSDMB and GSDMD by cleaving their NTs (86, 105).

GSDME is the GSDM most strongly linked to cancer. GSDME is activated by caspase-3, which cleaves it after Asp-270 to convert noninflammatory apoptosis to inflammatory pyroptosis in GSDME-expressing cells (53, 54). GSDME can also be activated independently of proteolytic cleavage by its PARylation and oxidation in UVC-treated cells (77). Unlike the other GSDMs, GSDME is normally expressed in tissues where inflammation would seem undesirable—the brain, placenta, heart, and other muscles—and its function in those tissues is not well-understood. GSDME is not expressed or is mutated to cause loss-of-function in many tumors. GSDME-mediated pyroptosis causes immunogenic cell death (ICD) that stimulates antitumor immunity in mouse immunocompetent tumor models (10). GSDME-mediated pyroptosis increases the efficiency of chemotherapy, targeted therapy, and radiotherapy by modulating the TME (50, 79, 106–111) but also aggravates cancer therapy–induced toxicity in normal tissues (11, 54, 79). GSDME can also be cleaved and activated by the cytotoxic lymphocyte serine protease GzmB, which cleaves after aspartic acid at the same site as caspase-3 during killer lymphocyte attack (10). GzmB also cleaves and activates caspase-3 in target cells; caspase-3 activation amplifies killer cell pyroptosis of target cells and tumor immunogenicity (10, 11).

3. ROLES OF GASDERMIN-MEDIATED PYROPTOSIS IN CANCER

The roles of GSDM activation and pyroptosis in cancer are complex, and their study has just barely begun. This section will review the evidence concerning the pro- and antitumorigenic functions of pyroptosis (**Figure 3**; **Table 1**). Pyroptosis plays different and sometimes conflicting

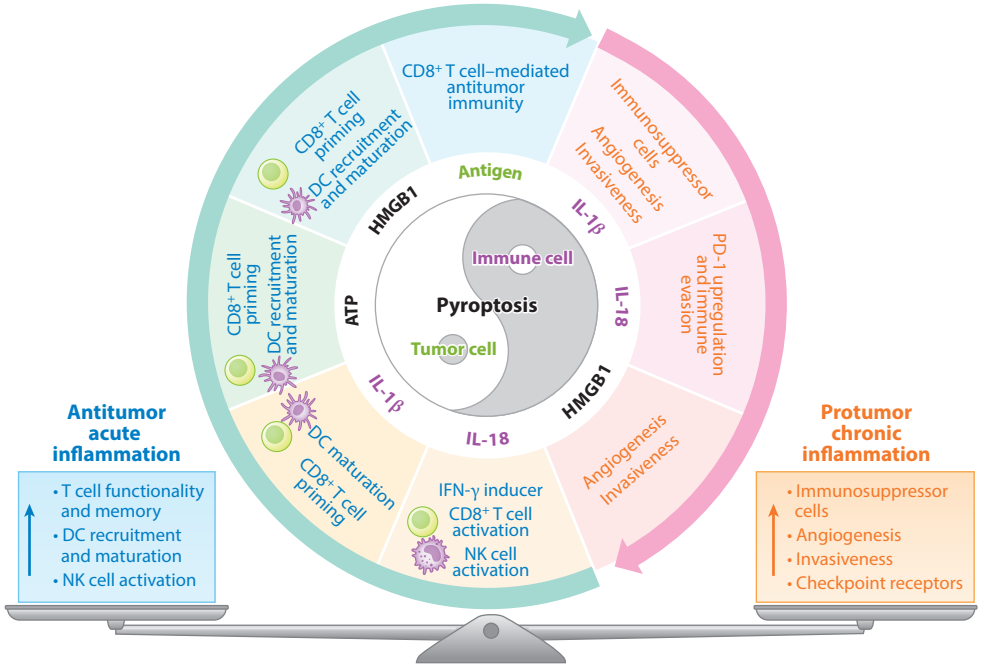


Figure 3

Pyroptosis plays dual roles in cancer. In acute inflammation, antitumor effects may dominate, while in chronic inflammation, tumorigenesis tends to increase (36). The antitumor effects of pyroptosis include, but are not limited to, increased T cell functionality and memory, DC recruitment and maturation, and NK cell activation. Protumor effects include, but are not limited to, increased expression and signaling by inflammatory transcription factors, which promote tumorigenesis, as well as dysfunction and suppression of antitumor immunity by increasing tumor-infiltrating immunosuppressor cells, tumor angiogenesis and invasiveness, and expression of immune checkpoint receptors. Consistent with these effects, some representative danger signal molecules released by pyroptotic cells (*inner circle*) have been shown to have both anti- and protumor effects (*outer circle*). The molecules whose names are shown in black could be released by both pyroptotic tumor and immune cells, while those whose names are shown in purple are mainly released from immune cells, and that whose name is shown in green is released from tumor cells. Abbreviations: DC, dendritic cell; HMGB1, high-mobility group box 1; NK, natural killer; PD-1, programmed death 1. Figure adapted from images created in BioRender; Zhang Z. 2025. **https://BioRender.com/t95mu5s**.

roles in cancer, depending on the tumor, its subtype, host tissue, stage of tumor development, and which cells are undergoing GSDM activation or pyroptosis. Epidemiological evidence suggests that chronic inflammation, caused by infection, autoimmunity, and environmental or dietary exposure, often promotes tumorigenesis. The CANTOS trial evaluated the activity of an IL-1β inhibitory antibody (canakinumab) in cardiovascular disease and unexpectedly showed significantly reduced overall cancer mortality, lung cancer incidence, and lung cancer death (112). Since IL-1β secretion generally depends on GSDMD activation, the study strongly suggests that chronic GSDMD activation promotes tumor formation or malignancy. However, acute inflammation can also promote antitumor immunity by providing alarm signals that recruit and activate APCs and tumor-infiltrating lymphocytes (TILs) and suppress tumor growth (**Figure 3**) (36). The complex effects of the IL-1 family cytokines on tumorigenesis and antitumor immunity are well illustrated by IL-33. IL-33 not only can increase KRAS-driven pancreatic tumorigenesis and recruit and activate immunosuppressive T regulatory cells (Tregs) in hepatocellular carcinoma (113) but

also can amplify antitumor immunity by activating ILC2s to promote lymphotoxin-dependent formation of intratumoral tertiary lymphoid structures (114–117). Therefore, the overall effect of pyroptosis may depend on whether the tumor is developing or is already formed, on the tumor type, and on features of the TME (36).

The basal expression of GSDMs is highly tissue specific. The online mRNA and protein expression data for the GSDMs are not a reliable indication of their expression during infection and other diseases because GSDM expression can be induced by a variety of still poorly defined inflammatory stimuli, including cytokines, such as IFN-γ and TNF-α (12), and the transcription factor IRF-2 (interferon regulatory factor 2) (118). All the pore-forming GSDMs have been shown to be upregulated in some tumors and downregulated in others and to have conflicting correlations with patient outcome (**Table 1**). The expression of some GSDMs (e.g., GSDME, GSDMB, and GSDMA) is epigenetically suppressed by promoter hypermethylation in some tumors, and this low expression correlates with worse outcome (119–121). A comprehensive pan-cancer analysis using TCGA database compared *GSDM* expression in tumors with that in normal tissue, analyzed the frequency of mutations and copy number variations (CNVs) in different cancer types, and linked *GSDM* expression in tumors to prognosis and ICB responsiveness (122). *GSDM* CNVs were much more common than genetic mutations and were especially common in ovarian, esophageal, uterine, and breast carcinomas (observed in 20–40% of cases). In general, higher *GSDM* expression was linked to a higher immune score and more stromal cells infiltrating the tumor and to higher tumor mutational burden, microsatellite instability, and expression of immune checkpoint genes, which are predictors of ICB responsiveness. These results are consistent with GSDM promotion of antitumor immunity.

The expression of different GSDMs correlates both positively and negatively with survival in a variety of human cancers; this relationship likely reflects the complex interaction between inflammation and cancer. Because mRNA expression is easy to measure and because clinically linked data are available for many human cancers in TCGA and other web databases, many papers have looked at correlations of *GSDM* expression with survival and clinical responses to cancer therapy and ICB. *GSDM* expression is not an indicator of pyroptosis because the GSDMs need to be cleaved or posttranslationally modified to be activated. Moreover, induction of pyroptosis is also controlled by other factors, such as the sensing of danger signals, the presence of ROS, protease and cytotoxic lymphocyte activation, protein expression, the stability of upstream sensors and mediators of inflammatory death, and posttranslational GSDM modifications (including ubiquitylation, phosphorylation, and palmitoylation), which are all more challenging to measure but are more relevant to biological function (1, 94, 123).

In addition, GSDMs can exert nonpyroptotic functions by pore-dependent release of cytokines, chemokines, and other inflammatory host proteins, called alarmins. In one example, *GSDME* promotes, independently of pyroptosis, mucus barrier formation, which protects pancreatic ductal adenocarcinoma (PDAC) cells from digestion by pancreatic enzymes and which promotes their survival in immunodeficient mice (124). Under some circumstances, the cell membrane pores formed by activated GSDMs are repaired by the cell membrane repair machinery present in all cells, and the cells with activated GSDMs do not die (97, 125). These surviving cells, described as hyperactivated, nonetheless continue to secrete copious amounts of inflammatory cytokines and alarmins. Hyperactivated cells can be more inflammatory and immunogenic than pyroptotic cells because pyroptotic cells die within minutes and stop releasing inflammatory mediators, but hyperactivated cells keep secreting for as long as a day. Hyperactivation has been described mostly in myeloid cells, especially macrophages and dendritic cells (DCs), and more recently in neurons (126). What determines whether a cell with activated GSDMs dies or survives as hyperactivated is not known—is death or survival determined by the relative amounts of sensors, GSDM-activating

proteases, and GSDMs in the cell relative to the membrane repair capacity of the cell, the levels of posttranslational modifiers or known or unknown pathways that cause GSDM inactivation or degradation, or the localization and extent of membrane damage? One study suggests that the mitochondria of hyperactivated cells are less metabolically damaged than those of cells that go on to die (127). In neurons wherein the membrane damage may be localized to the neurites, the cell can survive by pinching off damaged neurites. An intriguing cancer vaccine study in mice showed that hyperactivated DCs are more potent APCs than are pyroptotic, apoptotic, or untreated DCs, and vaccination with them can induce memory T cells to turn "cold" mouse tumors "hot" and control their growth (128). Therefore, the roles of GSDMs in cancer could be shaped by both their pyroptotic and nonpyroptotic functions, which could have opposing effects on tumorigenesis.

Below we summarize some of the studies that have correlated *GSDM* expression with cancer outcome. These studies together suggest that the role of individual GSDMs in cancer is context and cancer-type specific.

GSDMB is upregulated in oral squamous cell carcinoma and in cervical, bladder, and $HER2^+$ breast cancer, and it may promote tumorigenesis in those cancers (12, 129–134). *GSDMB* expression correlates with poor outcome in $HER2^+$ breast cancer (135) and renal cell carcinoma (12). In contrast, *GSDMB* is suppressed in primary esophageal and gastric tumors, and its expression correlates with better outcome in bladder carcinoma and melanoma (12). Differential splicing of *GSDMB* that generates GSDMB isoforms with distinct pore-forming ability might explain some of the disparate prognostic correlations with *GSDMB* expression (and possibly with expression of other *GSDM*s, although splicing of other *GSDM*s has not been studied). Although the expression of individual GSDMB isoforms in HeLa cells does not affect in vitro cell proliferation and migration, the isoforms that lack exon 6 can act as dominant-negative suppressors of pore formation by isoforms that include exon 6 (85). A pan-cancer analysis showed that noncytotoxic GSDMB1 and GSDMB2 are frequently overexpressed in multiple primary tumors, while cytotoxic GSDMB3 and GSDMB4 are often downregulated in tumors (85). Changes in *GSDMB* splicing were observed in gastrointestinal and hepatic cancers (136). Noncytotoxic GSDMB2, but not other isoforms, are upregulated in $HER2^+$ breast cancer, and overexpression of *GSDMB2* in the breast cancer cell line MCF7 promotes cancer cell migration and invasion independently of pyroptosis (133). Although *GSDMB2* knock-in does not alter the frequency of spontaneous neoplasia in mice, the incidence of breast cancer significantly increases when *GSDMB2* is coexpressed with *Her2/Neu* in mice (137). These data suggest that expression of cytotoxic isoforms of GSDMB may reduce tumor risk, while nonfunctional isoforms may promote tumor development.

GSDME is epigenetically silenced by promoter DNA hypermethylation in gastric, colorectal, and breast cancers (138–142), and hypermethylation correlates with worse 5-year survival and increased lymph node metastases in breast cancer (141). *GSDME* expression, restored by the methyltransferase inhibitor 5-aza-2′-deoxycytidine (decitabine) in vitro, suppresses colony formation and tumor cell proliferation in gastric cancer, melanoma, and colorectal cancer and reduces the invasiveness of breast cancer (139–141, 143). *GSDME* expression suppresses the growth of melanoma, breast cancer, and colorectal cancer cell line implants in immunocompetent, but not immunodeficient, mice (10). GSDME deficiency does not alter tumor cell growth or malignant properties or act as a cell-intrinsic tumor suppressor—its suppression of tumor growth disappears in NSG (NOD-*scid* $IL2Rg^{null}$) mice lacking lymphocytes and in mice deficient in perforin, which is required for lymphocyte killing of tumor cells; this loss of tumor control suggests that the antitumor effect of GSDME is immune mediated. These data together suggest that GSDME is a cell-extrinsic tumor suppressor in some tumors. However, *GSDME* overexpression in PDAC promotes mucus barrier formation, which enables PDAC cells to avoid digestion by pancreatic

enzymes (124). GSDME was also proposed to support the stability and activation of EGFR (epidermal growth factor receptor) to promote non–small cell lung cancer tumorigenesis (144).

Genetic alterations in *GSDM* genes shed light on the roles of GSDM-mediated pyroptosis in cancer. *Gsdma* deficiency in HGC27 and N87 human gastric carcinoma cell lines promotes tumor growth under starvation conditions in athymic nude mice, presumably through blocking starvation-induced GSDMA activation and pyroptosis (76). Homozygotes for the minor allele of *GSDMB* single nucleotide polymorphism rs11078928 barely express pore-forming GSDMB3 and GSDMB4 but express inactive GSDMB1 and GSDMB2 (145), and loss of cytotoxic GSDMB isoforms in rs11078928 is associated with higher cervical cancer risk (85); these observations suggest a protective role of pore-forming GSDMBs in cervical cancer. *GSDMC* somatic mutations correlate with poor prognosis and low antitumor immune response, although these mutations have not been characterized (146). *Gsdmd*$^{-/-}$ mice exhibit increased AOM and DSS-induced CRC tumor burden (147) but show reduced sporadic colorectal tumorigenesis (148), chemically induced hepatocarcinogenesis (149), and lung cancer metastasis (150), which were attributed to decreased IL-1β secretion by myeloid cells. However, the growth of EO771 breast and Hepa1-6 liver tumors and their immune cell infiltration are unchanged in *Gsdmd*$^{-/-}$ mice (151). These data suggest that the role of GSDMD in cancer is context and cancer type dependent. Although *GSDME* is epigenetically repressed in many human tumors and tumor cell lines, some tumors that express it have mutated *GSDME*. Most tumor GSDME-NT mutations are loss-of-function mutations that reduce pyroptosis, and this finding suggests that GSDME is in fact a tumor suppressor gene (10). Interestingly, *GSDME* I217N, a lung cancer mutation, can block pyroptosis by wild-type GSDME (152).

How cancer cells die strongly affects antitumor immune activation and the effectiveness of therapeutic interventions. APCs that phagocytose cancer cells that died by noninflammatory apoptosis are less effective in stimulating T cell effector functions and memory than are APCs that phagocytose cells that died by programmed necrosis (e.g., necroptosis, pyroptosis, ferroptosis) (1, 2, 153). Moreover, apoptosis can induce immune tolerance (e.g., by inducing TGF-β, a major immunosuppressive cytokine) and can thereby inhibit the establishment of T cell–mediated antitumor responses and memory (66). Cell death that induces an immune response that protects mice vaccinated with dying tumor cells from subsequent tumor challenge is defined as ICD (154, 155). Necrotic programmed cell death, but not usually apoptosis, causes ICD. However, some chemotherapy drugs and radiation that trigger apoptosis and cause ER stress can induce ICD (65, 156). GSDME-mediated pyroptosis in the tumor increases the number and function of tumor-infiltrating DCs, CD4$^+$ T cells, CD8$^+$ T cells, and NK cells but reduces Tregs and myeloid-derived suppressor cells (MDSCs) (10, 50, 157). In models that used doxycycline-inducible GSDME-NTs to trigger pyroptosis in colon and lung cancer cells, CD103$^+$Xcr1$^+$ type I conventional DCs (cDC1s) in tumors and draining lymph nodes play an important role in modulating the TME and enhancing antitumor immunity (157). Similarly, when GSDMA3-NT was delivered into 4T1 triple-negative breast cancer (TNBC) tumor implants in mice, tumor-infiltrating CD4$^+$ T, CD8$^+$ T, and NK cells and the M1:M2 macrophage ratio increased, but the numbers of monocytes, neutrophils, and MDSCs decreased (52). In this study, although GSDMA3-NT delivery alone only induced pyroptosis in approximately 10–15% of tumor cells, orthotopic 4T1 tumors were substantially suppressed. Low levels of pyroptosis induced by GSDMA3-NT nanoparticle treatment also enhanced tumor control when combined with the administration of anti–programmed death 1 (anti-PD-1) antibody (52).

In immunocompetent mice, pyroptosis in cancer cells increases the efficacy of not only anti-PD-1 immunotherapy (12, 52) but also targeted therapy [i.e., BRAF and MEK inhibitors for *BRAF* V600E/K mutant melanoma (50) and PARP inhibitors for *BRCA*-mutated cancers (90)],

chemotherapy [e.g., cisplatin and doxorubicin (106–109)], and radiotherapy (79, 110, 111). Activated tumor-infiltrating killer lymphocytes, the major executors of antitumor immunity, can also further amplify immune control of GSDMB- or GSDME-expressing tumors by inducing tumor target cell pyroptosis (10, 12). On the other hand, TNF-α activation of caspase-8 to trigger GSDMC-triggered pyroptosis in hypoxic tumor cells leads to tumor necrosis and chronic inflammation, which promotes tumor progression and causes drug resistance (51). However, this result was found in a human breast cancer implant model in immunodeficient mice, and the tumor-protective effects of pyroptosis in other models depend completely on an immune response and killer lymphocytes. Although these experiments suggest that GSDMs might have both protective immune effects and tumor-promoting nonimmune effects, further experiments to compare the effects of GSDMC in immunocompetent and immunodeficient mice are warranted.

4. PYROPTOSIS, DANGER SIGNALS, AND CANCER

Antitumor immune control relies on killer lymphocyte recognition and elimination of tumor cells. The development of T cell memory and antitumor functionality, which are critical for effective antitumor immunity, depend not only on T cell receptor recognition of tumor antigens and costimulation but also on a danger signal (59–61). The danger signal is generally an innate immune alarm. In cancer cells, mislocalized DNA in the cytosol may be the most important danger-associated molecular pattern; cytosolic DNA activates interferons, inflammatory pyroptosis, and necroptosis through distinct cytosolic sensors. A recent study of cancer cell immunoediting, which enables cancer cells to avoid immunosurveillance, in a $Her2^{+}$ breast cancer GEMM found that immunoediting represses not only tumor antigen processing and presentation genes to suppress T cell receptor activation but also the expression of key genes in all innate immune pathways known to generate danger signals (64). Moreover, treating these GEMM mice, as well as immunocompetent mice bearing a variety of tumor cell line implants, with a DNA methyltransferase inhibitor derepressed these innate and adaptive immune-stimulating genes and restored immunosurveillance, which depended on these danger-signaling pathways. These results suggest that various innate immune danger signals, including not only pyroptosis but also interferons, interferon-stimulated genes, necroptosis, and ferroptosis, can contribute to the development of protective immunity. While the stimulation of type I interferons has been well-studied and has been shown to promote antitumor immunity, the roles of other innate immune alarm systems in antitumor immunity are less well-understood and warrant further investigation.

Pyroptosis causes ICD (10). T cell memory and enhanced functionality are induced by vaccination with pyroptotic tumor cells but not usually with apoptotic tumor cells. As a result, immunocompetent, but not immunodeficient, mice are protected from tumor rechallenge (10, 154). Danger signals released from pyroptotic tumor cells play essential roles in priming and activating antitumor immunity (**Figure 3**). Many proteins and other molecules that potentially act as danger signals are released during pyroptosis. These molecules include the inflammatory cytokines, especially members of the IL-1 family, which lack a secretion signal and are released via GSDM pores; chemokines; activated pyroptosis mediators, including processed inflammatory caspases and the N-terminal pore-forming fragments of GSDMs; and HMGB1 (high-mobility group box 1) and ATP, which might further increase inflammation. In fact, extracellular vesicles that contain GSDMD-NT and that are released from pyroptotic cells can be taken up by phagocytic cells in the TME to trigger pyroptosis (158). ATP can be sensed by seven-transmembrane purinergic P2RY2 receptors (159, 160), and HMGB1 can be sensed by TLR4 (Toll-like receptor 4), RAGE (receptor for advanced glycation end products), and other cell surface receptors (161) in tumor-infiltrating immune cells and bystander cells to reshape the TME. Although GSDM-NT

neither recognizes lipids on the outer membrane of healthy cells nor kills bystander cells from the outside, it binds to phosphatidyl serine (PS), which is flipped to the outer membrane during both apoptotic and necrotic cell death (69, 162). Externalized PS could in principle trigger myeloid cell phagocytosis to remove dying tumor cells and also serve as a binding site for secreted GSDM-NT to trigger pyroptosis in any dying cells in the TME. Phagocytosis of pyroptotic tumor cells would be expected to increase tumor antigen presentation, but it is hard to predict whether it would reduce or increase inflammation in the TME.

5. TUMOR EVASION OF PYROPTOSIS

Because excessive pyroptosis leads to or exacerbates many diseases (1), pyroptosis needs to be tightly regulated. Although much about GSDM regulation remains unknown, GSDMs are known to be regulated by both transcription (118) and PTMs (123), including phosphorylation, ubiquitylation, palmitoylation, succination, and PARylation (53, 70–74, 76, 77, 163–167). For example, phosphorylation of a conserved Thr-6 GSDME residue by AMP-activated protein kinase (AMPK) blocks pyroptosis (53, 166). Similarly, phosphorylation of GSDMD at Ser-46 by AMPK and at Thr-213 by an unidentified kinase blocks pyroptosis (164, 165). Succination of GSDMD-critical cysteines, such as Cys-191, also blocks pyroptosis (163). Pyroptosis is also enhanced by ROS and mitochondrial damage (68, 143), which is required for GSDM palmitoylation and membrane binding to form pores (70, 71), and suppressed by ESCRT (endosomal sorting complex required for transport)-III-dependent membrane repair (168). In addition, many upstream regulatory mechanisms of inflammasomes have been identified and reviewed (93, 94). The effect of these PTMs on tumor growth and antitumor immunity has not been well-studied.

Evasion of cell death is a hallmark of cancer (169). Not surprisingly, tumors employ various strategies, such as mutation, alteration of splicing, or suppression of *GSDM* expression, as discussed above, to evade pyroptosis (**Figure 2*b***). Some of the more salient recent papers that describe how tumor cells evade pyroptosis are described below. These papers support the concept that activating pyroptosis may induce more effective antitumor immunity in some settings, but which settings are most effective for therapeutic targeting needs to be better defined.

5.1. Epigenetic Silencing

GSDM expression is suppressed epigenetically in many tumors arising from tissues and cell types that normally express GSDM. In multiple human cancer cell types (e.g., gastric, breast, and colorectal cancers), the *GSDME* promoter was found to be hypermethylated (80, 119, 138–142). *GSDMA* and *GSDMB* are similarly repressed by DNA methylation in some cancer cell lines (120, 121). In addition, inflammasome components, including the genes encoding the adapter ASC, caspase-1, and IL-1β, are epigenetically silenced by DNA methylation in many tumors (55). However, how the *GSDM* and inflammasome genes and their associated histones are epigenetically modified and how these modifications are regulated have not been well-studied. A recent study suggests that DNA hypermethylation in tumors could be driven by an elevated methionine flux, which can cause resistance to pyroptosis (170).

A recent study using an aggressive Her2$^+$ breast cancer GEMM and breast cancer, lung cancer, and melanoma cell line implants found that tumor cells broadly suppress the expression of key genes in all the major innate immune pathways in addition to suppressing tumor antigen processing and presentation by TILs (64). Consistently, treatment of late tumors with low, noncytotoxic doses of decitabine restored expression of genes in all these innate (and T cell) immune pathways, which included pyroptosis, necroptosis, interferon signaling, and inflammatory cytokines. Moreover, decitabine treatment of tumor-bearing mice increased the number, function, and memory

phenotype of TILs and cDC1 cells; reduced infiltration of suppressor cells; and induced tumor cell pyroptosis and necroptosis in the tumor. Tumor growth was dramatically suppressed, and tumor suppression depended on lymphocyte killing, since it disappeared in perforin-deficient mice. Knockout experiments in the tumor suggested that interferon induction, pyroptosis, and necroptosis all contributed to decitabine-induced tumor control (64). This study suggests that, at least in some cancers, tumor evasion of immune surveillance requires suppression of both innate and adaptive immune responses to the tumor, and immune evasion can be reversed by epigenetic manipulation. Future studies in other types of tumors and with other epigenetic modifiers are needed to evaluate the importance of pyroptosis and other innate immune pathways in immune tumor control and to determine whether and how they contribute to responsiveness to immunotherapy.

5.2. Alternative Splicing

Four recent studies similarly demonstrated that GSDMB isoforms exhibit distinct pore-forming activity (85–88). Three studies suggested that the conserved region encoded by exon 6 of GSDMB is required for oligomerization and/or lipid binding. However, these studies were based on multiple mutations, which may have disrupted the proteins, and mostly relied on liposome leakage and cell death assays, which only showed the overall activity of GSDMB. Our study (85), which directly compared isoforms without relying on mutagenesis, used assays that dissected each step of pore formation (lipid binding, oligomerization, and insertion). We found that all the *GSDMB* isoforms similarly bound to lipids and oligomerized, while the GSDMB-NTs lacking exon 6 could not insert into the membrane. In this scenario, hetero-oligomers containing a mixture of cytotoxic and noncytotoxic isoforms exhibited reduced pore-forming activity. Consistently, expression of noncytotoxic GSDMB-NT isoforms blocked pyroptosis caused by cytotoxic GSDMB-NTs in a dose-dependent and dominant-negative manner. The expression of cytotoxic *GSDMB3* or *GSDMB4* isoforms, but not of *GSDMB1* or *GSDMB2* isoforms, which are frequently upregulated in tumors, was associated with better outcome in human bladder and cervical cancers; these findings suggest that GSDMB3- and GSDMB4-mediated pyroptosis was protective in those tumors (85). This study shows that nonfunctional GSDMB isoforms inhibit pyroptosis and that tumors may block and evade killer GSDMB-mediated pyroptosis by alternative splicing. *GSDME* also has two other isoforms generated by alternative splicing (according to UniProt), both of which lack part or all of the NT and may function as negative regulators of GSDME-mediated pyroptosis; however, this intriguing possibility has not been experimentally examined.

5.3. Serpins

Because pyroptosis is mediated by proteases, including caspases and hematopoietic cell granule proteases [e.g., Ctsg (9), neutrophil elastase (7, 8), and granzymes (10, 12)], the Serpin proteins, a family of endogenous protease inhibitors, regulate pyroptosis. Both mouse Serpinb1 and Serpinb6 inhibit Ctsg to block neutrophil death mediated by Ctsg cleavage of GSDMD (9). Similarly, human SERPINB1 can suppress pyroptosis in immune cells by blocking the activity of inflammatory caspases (i.e., caspase-1/-4/-5/-11) (171). Some tumors overexpress Serpins to block pyroptosis. SERPINB9, an inhibitor of GzmB, is commonly amplified in human tumors, and high *SERPINB9* expression correlates with poor tumor cell killing by CAR-T cells (172). Serpinb6b is an inhibitor of Ctsg (9) and GzmA (173), which blocks GSDMD cleavage [and likely GSDMB activation by GzmA (12), although this has not been shown] and suppresses T cell–induced tumor cell pyroptosis. The long noncoding RNA *Malat1*, which upregulates expression of *Serpinb6b* and other *Serpin* genes, was recently shown to promote dormant tumor cell reactivation at metastatic sites by suppressing TIL $CD8^+$ T cells from killing tumor cells (174, 175). This result suggests that

induction of tumor cell pyroptosis may play an important role in preventing the development of macroscopic metastases in some settings.

6. HARNESSING PYROPTOSIS TO TREAT CANCER

Pyroptosis can both directly eliminate tumor cells and elicit robust tumor-specific immune responses that can control or reject implanted tumors in mouse models (10, 52). But the low expression of GSDMs in many tumors and pyroptosis-triggered adverse effects on normal tissues need to be carefully considered when designing strategies to harness pyroptosis for its immune-stimulating effects. Because excessive activation of pyroptosis can cause tissue damage and autoimmunity (11, 54, 79, 176), activation of pyroptosis must be finely controlled. Assays to evaluate in vivo pyroptosis, such as the measurement of tumor and normal cell uptake of intravenously injected propidium iodide in animal models and serum measurement of secreted GSDMs and IL-1 family cytokines released specifically during pyroptosis, can be useful to assess how therapeutically induced pyroptosis contributes to not only tumor elimination but also toxicity.

Several strategies for enhancing antitumor immunity have been proposed to activate pyroptosis in tumors (**Figure 4**). However, the efficiency, specificity, safety, and effects on tumor growth and immunity of those strategies have not yet been clinically evaluated. This section focuses on strategies to activate pyroptosis in already formed tumors.

6.1. Gasdermin Agonist

A recent high-throughput liposome leakage screen to identify compounds that activate GSDMD, which is widely expressed at low levels in human cancers, identified quinoxaline 6,7-dichloro-2-methylsulfonyl-3-*N*-tert-butylaminoquinoxaline (DMB) as a direct and selective human and mouse GSDMD agonist (95). DMB covalently binds to the same reactive cysteine in GSDMD-NT (Cys-191 in humans and Cys-192 in mice), whose palmitoylation is required for GSDMD-NT to bind to membranes and form pores. Unexpectedly, DMB activates GSDMD pores and pyroptosis without cleaving GSDMD. In a few mouse tumor models, DMB treatment, given in a pulsed dosing regimen, induced pyroptosis in a small proportion of tumor cells but did not increase pyroptosis of tumor-infiltrating macrophages, which highly express GSDMD, or of other immune cells in the TME. The reason for the selective pyroptosis in tumor cells is uncertain but might be due to secondary amplification of tumor cell killing by tumor-specific cytotoxic T cells or NK cells. In fact, protection by DMB was abrogated in NSG mice lacking lymphocytes. There was no apparent toxicity or sign of systemic inflammation. Vaccination with DMB-treated cancer cells protected mice from secondary tumor challenge, and this finding indicates that DMB triggers ICD, as GSDME-mediated pyroptosis previously was shown to do (10). DMB treatment also synergized with anti-PD-1 in an ICB-resistant tumor (95). This study suggests that the development of GSDM agonist drugs to potentiate antitumor immunity with acceptable toxicity might be possible. Because cysteine-reactive drugs have off-target effects, further work to identify noncovalent GSDM activator compounds may be needed to identify a useful drug candidate for clinical testing.

6.2. Pyroptosis Activators

Pyroptosis-inducing drugs have been reviewed in detail elsewhere (177–180), but their tumor specificity, toxicity, and effectiveness in vivo require further investigation. A few examples of pyroptosis activators tested in mouse cancer models or human cancer cell lines in vitro are described below.

Val-boroPro is a nonselective inhibitor of postproline-cleaving serine proteases. Its inhibition of DPP8 (dipeptidyl peptidase 8) and DPP9 proteases activates NLRP1 and CARD8 (caspase

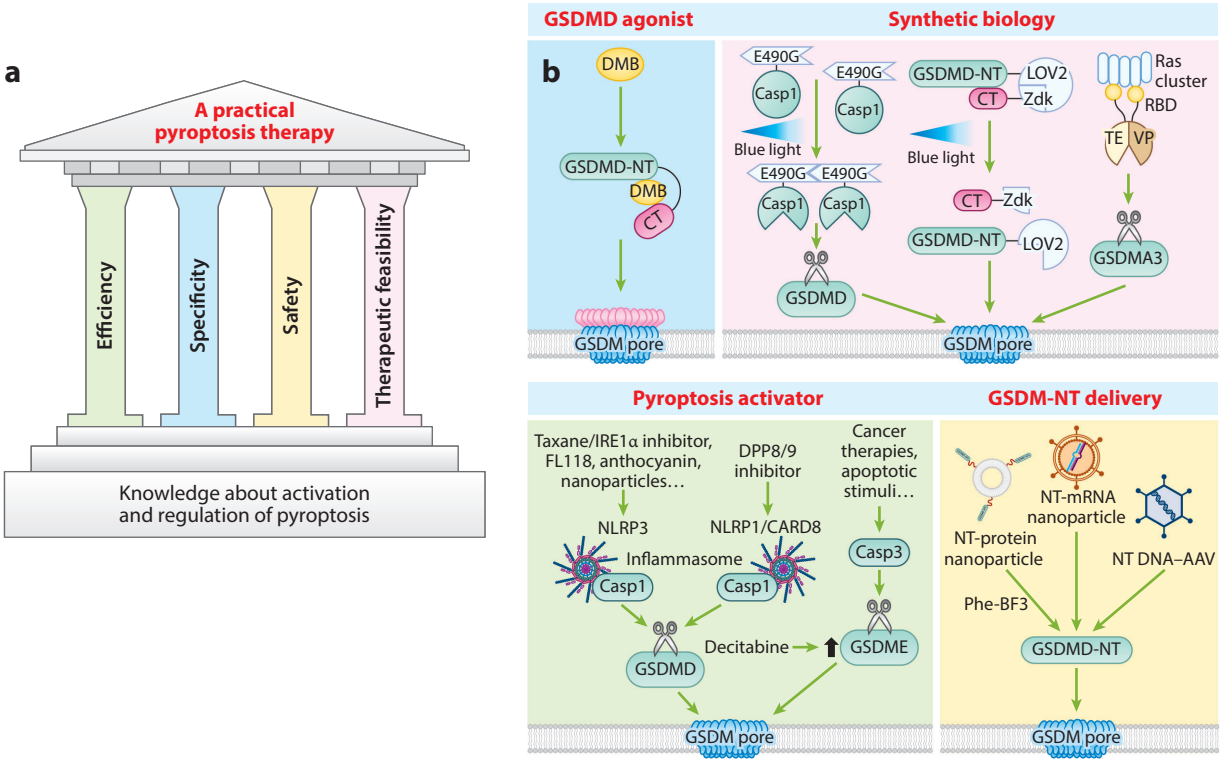


Figure 4

Representative strategies to activate pyroptosis in tumors. (*a*) A practical pyroptosis-based cancer therapy should be efficient, specific, safe, and therapeutically feasible. A better understanding of the activation and regulation of pyroptosis will guide therapeutic design. (*b*) Strategies proposed to activate pyroptosis in tumors. A GSDMD agonist DMB activates pyroptosis (95) by relieving the autoinhibition of full-length GSDMD. A few examples of pyroptosis activators in tumor cells include Val-boroPro, which activates NLRP1 and CARD8 inflammasomes (181), and the camptothecin analog FL118, the fruit pigment anthocyanin (177), IRE1α inhibitors in combination with taxane (184), and nanoparticles (178–180), all of which activate NLRP3-mediated pyroptosis. In addition, epigenetically silenced *GSDME* can be derepressed by decitabine (106, 107). GSDME-mediated pyroptosis can also be activated by radiotherapy, chemotherapy, targeted cancer drugs, and killer lymphocyte attack (10, 11, 13, 50, 54, 79, 106–111, 185, 186). Ways to deliver GSDM-NT to tumor cells to induce pyroptosis have also been proposed. GSDMA3-NTs linked by a silyl ether to nanoparticles can be released by Phe-BF3-mediated desilylation specifically in tumor cells (52). GSDMD-NT can also be expressed in tumor cells using recombinant AAV (187), and GSDMB-NT-encoding mRNA can be delivered intratumorally via lipid nanoparticles (188). Synthetic biological strategies could also be used. The effector domains of inflammatory caspases fused to light-sensitive cryptochrome 2 E490G (189) can be oligomerized and activated by blue light to cut GSDMD and trigger pyroptosis (190). In another example, the GSDMD-NT and -CT fragments were fused to LOV2 and Zdk, and optogenetic dissociation of LOV2–Zdk released GSDMD-NT from the inhibitory GSDMD-CT to trigger pyroptosis (192). Another study took advantage of Ras clustering in Ras-dependent tumor cells to engineer a tumor-specific TEVP by joining RBD-fused split TEVP to cleave GSDMA engineered with a TEVP cleavage site (152). Abbreviations: AAV, adeno-associated virus; CARD8, caspase recruitment domain–containing protein 8; Casp1, caspase-1; CT, C-terminal domain; DMB, 6,7-dichloro-2-methylsulfonyl-3-*N*-tert-butylaminoquinoxaline; DPP8/9, dipeptidyl peptidase 8/9; GSDM, gasdermin; IRE1α, inositol-requiring enzyme 1α; NLRP1, nucleotide-binding oligomerization domain–like receptor family pyrin domain–containing protein 1; NT, N-terminal domain; Phe-BF3, phenylalanine trifluoroborate; RBD, Ras-binding domain; TEVP, tobacco etch virus protease. Panel *a* adapted from images created in BioRender; Zhang Z. 2025. **https://BioRender.com/251704j**. Panel *b* adapted from images created in BioRender; Zhang Z. 2025. **https://BioRender.com/vmo7gn1**.

recruitment domain–containing protein 8) inflammasomes and GSDMD-mediated pyroptosis in most human acute myeloid leukemia cell lines tested; this activation suggests that Val-boroPro might be useful for treating acute myeloid leukemia (181). However, DPP8 and DPP9 inhibitors also activate pyroptosis in B cells, $CD34^+$ hematopoietic stem cells (181), and resting T cells (182), and this broad activation could cause treatment-associated toxicity.

The NLRP3 inflammasome, which can be activated by numerous stimuli that disrupt the integrity of the cell membrane, has been extensively implicated in inflammatory and autoimmune disease and cancer (183). Several compounds have been reported to trigger NLRP3-mediated pyroptosis in tumor cells, including the camptothecin analog FL118 and the fruit pigment anthocyanin (177). A recent study also suggested that inhibition of the ER stress sensor IRE1α (inositol-requiring enzyme 1α) allows taxane to induce NLRP3-dependent pyroptosis in TNBC cells, sensitizing TNBC to chemo-immunotherapy (184). NLRP3-mediated pyroptosis can also be activated by multiple types of nanoparticles (178–180).

GSDME is activated by radiotherapy and chemotherapy drugs, including cisplatin, etoposide, topotecan, doxorubicin, actinomycin D, and mitoxantrone, that activate caspase-3, which can then cleave and activate GSDME and trigger pyroptosis (54, 185). GSDME can also be activated by other "apoptotic" stimuli, including targeted therapy drugs (50, 186) and killer lymphocyte attack (10–13). However, activation of GSDME-mediated pyroptosis relies on *GSDME* expression, which is epigenetically silenced in most tumors. Decitabine restores *GSDME* expression and pyroptosis in cultured tumor cells and in mouse tumors (54, 139). Treatment with decitabine and chemotherapy drugs encapsulated into nanoparticles increased *GSDME* expression in tumors, activated GSDME-mediated pyroptosis, and enhanced the efficacy of chemotherapy in mouse cell line models of lung and breast cancers (106, 107).

6.3. Delivery of N-Terminal Fragments of Gasdermins

Another approach to activate pyroptosis in tumors to get around their low GSDM expression is the delivery of an activable full-length GSDM, an already cleaved GSDM, or the corresponding mRNA selectively to tumor cells in vivo. Because of the high toxicity of GSDM-NTs, the development of these strategies will need tumor-selective delivery, GSDM activation, or both to be clinically useful. A strategy to turn off GSDM activation in vivo if inflammation gets out of hand may also be advisable. In one illustrative example, tumor-bearing mice were treated with nanoparticles that were linked by a silyl ether to GSDMA3-NT (in the presence of GSDMA3-CT to prevent spontaneous GSDMA3-NT oligomerization into pores) and then with a PET cancer imaging agent, phenylalanine trifluoroborate (Phe-BF3), which concentrates in tumor cells and has desilylation activity. Phe-BF3 concentrated in mouse 4T1 and EMT6 TNBCs and desilylated the silyl ether link to release GSDMA3-NT into the tumor cells and thereby trigger pyroptosis selectively in the tumor (52). Although pyroptosis was triggered only in approximately 15% of tumor cells, the tumor was controlled, and this control was immune mediated. The tumor response was blocked by anti-IL-1. The treated tumors showed increased infiltrating NK and T cells and fewer infiltrating immunosuppressive MDSCs, neutrophils, and macrophages. The nanoparticle–Phe-BF3 combination therapy also synergized with anti-PD-1. Although the mice did not show obvious toxicity, further investigation of safety and antitumor effectiveness in more physiological models and other tumor types is needed. In another study, GSDMD-NT was expressed by recombinant adeno-associated virus in tumor cells to induce pyroptosis and antitumor immunity (187). To reduce leaky expression and cytotoxicity to the viral producer insect cell lines, GSDMD-NT expression was driven by a mammalian promoter, and the insertion of the *GSDM* gene in the vector was inverted, with syntropic double Lox sites that could only be reverted and activated by

the coexpression of Cre recombinase. Another study delivered GSDMB-NT-encoding mRNA in lipid nanoparticles intratumorally into B16 and 4T1 tumors to induce pyroptosis and partially control tumor growth (188). These proof-of-concept studies show that delivery of GSDM protein or mRNA might be an effective component of tumor immunotherapy, but the development of these approaches into useful drugs will require a lot more work.

6.4. Synthetic Biological Strategies

Synthetic biological strategies, including optogenetic tools, have also been used to induce pyroptosis. These strategies require access to the tumor, which is not always practical. Cryptochrome 2 E490G from *Arabidopsis thaliana* oligomerizes after exposure to blue light (189). When this protein is fused to the effector domains of inflammatory caspases, the caspases can be activated by blue light to cut GSDMD and trigger pyroptosis in various cells and models (190). A light-inducible dissociation LOVTRAP system has also been used to induce pyroptosis. LOV2 interacts with Zdk in the dark but dissociates rapidly after photostimulation (191). When the GSDMD-NT and -CT fragments are fused to LOV2 and Zdk, light exposure dissociates GSDMD-NT from the inhibitory CT to trigger pyroptosis (192). Synthetic protein circuits have also been designed to manipulate apoptosis and pyroptosis (152). GSDMA, engineered with a tobacco etch virus protease (TEVP) cleavage site in its linker region, was coexpressed with TEVP to induce pyroptosis. A sensor for active Ras was used to achieve cancer cell selectivity. Briefly, the TEVP sequence was split into two fragments, and each half was fused to a Ras-binding domain (RBD). In Ras-dependent tumors, active Ras clusters at the cell membrane, binds to both RBD–TEVP fragment fusion proteins, and thereby reconstitutes the active protease only in Ras-activated cells to cleave engineered GSDMA and trigger pyroptosis. Thus, pyroptosis occurred selectively in Ras-dependent tumor cells. Although these systems are elegant, they have mostly been tested in cultured cell lines. More physiologically relevant in vivo tests in cancer-bearing mice are required to evaluate the therapeutic feasibility of these approaches.

7. CONCLUDING REMARKS AND FUTURE PERSPECTIVES

Pyroptosis plays dual roles in regulating tumors. Pyroptosis in a tumor is highly immunogenic and triggers an inflammatory cell death that can augment antitumor immunity by sounding a danger alarm that recruits immune cells to tumors and activates the full functionality and memory of TILs. However, chronic inflammation also promotes tumorigenesis by activating oncogenic transcription factors and enhancing tumor angiogenesis, proliferation, invasiveness, and immune evasion. In addition to inflammasome-activated, GSDMD-dependent pyroptosis, other GSDMs and proteases can trigger inflammasome-independent pyroptotic pathways. Sorting out the tumor subtypes and stages in tumor development wherein pyroptosis activates or inhibits tumors is still a work in progress and is critical for developing therapeutic strategies that can harness pyroptosis to promote immune tumor control without enhancing tumorigenesis. One of the key advantages of the induction of pyroptosis in a tumor—and, more generally, of immunotherapy—is that the therapy can work even if the initial therapy triggers an immune alarm in only a small proportion of tumor cells. Unlike cytotoxic chemotherapy, which requires immediate elimination of virtually all cancer cells to avoid relapse and drug resistance, an immune danger signal in a small number of tumor cells can be amplified by the recruitment and activation of tumor-infiltrating immune cells that can develop into effector and memory cells. Most importantly, we need to figure out how to harness pyroptosis to induce effective antitumor immunity but avoid treatment-associated toxicity. Several pyroptosis-activating strategies have been reported to kill tumor cells and activate tumor-specific immune protection; however, their efficiency, specificity, and safety

in mouse models that resemble human cancer need to be evaluated. A better understanding of how pyroptosis can be triggered and regulated should increase the chance of designing practical therapies.

DISCLOSURE STATEMENT

J.L. is a cofounder of Ventus Therapeutics with stock options, which might be perceived as affecting the objectivity of this review.

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