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Gasdermin-mediated pyroptosis confers anticancer immunity

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ABSTRACT

Gasdermin (GSDM)-mediated pyroptosis, a form of immunogenic cell death, has emerged as a mechanism capable of conferring anticancer immunity. GSDM activation is central to inducing cancer cell pyroptosis (CCP) and is a crucial link to anticancer immunity. Using a bispecific antibody, we triggered GSDMB-mediated pyroptosis in gastric cancer cells, significantly improving tumor cell eradication and promoting immune cell infiltration and activation in the tumor microenvironment. This innovative strategy presents a highly promising pathway for the targeted activation of GSDM, thereby inducing CCP and ultimately amplifying the effectiveness of cancer immunotherapy. Consequently, this commentary explores the broad-ranging impacts and prospects associated with inducing GSDM-mediated pyroptosis in the context of anticancer immunity, further underscoring the potential challenges and future directions related to the employment of GSDM-mediated pyroptosis in cancer immunotherapy.

Introduction

Pyroptosis, regulated by activated gasdermin (GSDM) proteins, is a form of immunogenic cell death known for its strong inflammatory responses.[1] It falls under the category of immunogenic cell death and has the potential to enhance anticancer immunity. Evidence suggests that pyroptosis-induced tumor elimination involves activating and boosting immune cell cytotoxicity, which may improve the efficacy of cancer immunotherapy.[1–4]

The cleavage and activation of GSDM are pivotal events driving pyroptosis. Members of the GSDM family, including GSDMA, GSDMB, GSDMC, GSDMD, and GSDME, all share a common structure comprising an N-terminal pore-forming domain and a C-terminal regulatory domain. Cleavage at specific sites by caspases or granzymes releases N-terminal fragments that translocate to cell and mitochondrial membranes, forming transmembrane pores. This process promotes the release of inflammatory cytokines like interleukin (IL)-1β and IL-18 and triggers the influx of water and sodium ions, resulting in cell swelling and eventual dissolution. GSDM proteins can be cleaved by granzymes secreted by immune cells, establishing a crucial connection between pyroptosis and anticancer immunity.[1 3 4]

We recently utilized a bispecific antibody, IBI315, designed to target both programmed death (PD)-1 and HER2, to trigger GSDMB-mediated pyroptosis in HER2$^+$ gastric cancer cells. This approach substantially improves tumor cell eradication, fosters immune cell infiltration, and activates immune responses in the tumor microenvironment.[2] This innovative strategy holds promise for selectively activating GSDMB, inducing cancer cell pyroptosis (CCP), and enhancing cancer immunotherapy's effectiveness. Therefore, this commentary offers a comprehensive review of recent research on GSDM protein-mediated CCP and its implications for anticancer immunity, providing fresh insights into cancer immunotherapy.

GSDM PROTEINS AND ANTICANCER IMMUNITY

GSDMA

A recent study demonstrated targeted delivery of murine Gsdma3 (N-terminal domain), the human GSDMA homolog, into tumor cells using nanoparticles (NPs).[5] This delivery method involved cleaving NP-delivered Gsdma3 with 3-fluorobenzenesulfonyl-l-phenylalanine boronic acid (Phe-BF3), generating active GSDM proteins that induce CCP. Combining NP-Gsdma3 and Phe-BF3 led to potent anti-breast cancer effects, marked by increased CD3$^+$ T cell infiltration in the tumor microenvironment and reduced CD4$^+$FOXP3$^+$ regulatory T cell (Treg) infiltration. Notably, the efficacy of NP-Gsdma3 and Phe-BF3 co-administration depended on T cells. Further analysis revealed that, although pyroptosis occurred in less than 15% of tumor cells in this group, it was sufficient to nearly eradicate the entire tumor. These findings indicate that CCP can activate infiltrating immune cells within the tumor, maximizing tumor cell destruction.

GSDMB

Zhou and colleagues demonstrated that cytotoxic immune cells induce cell pyroptosis,

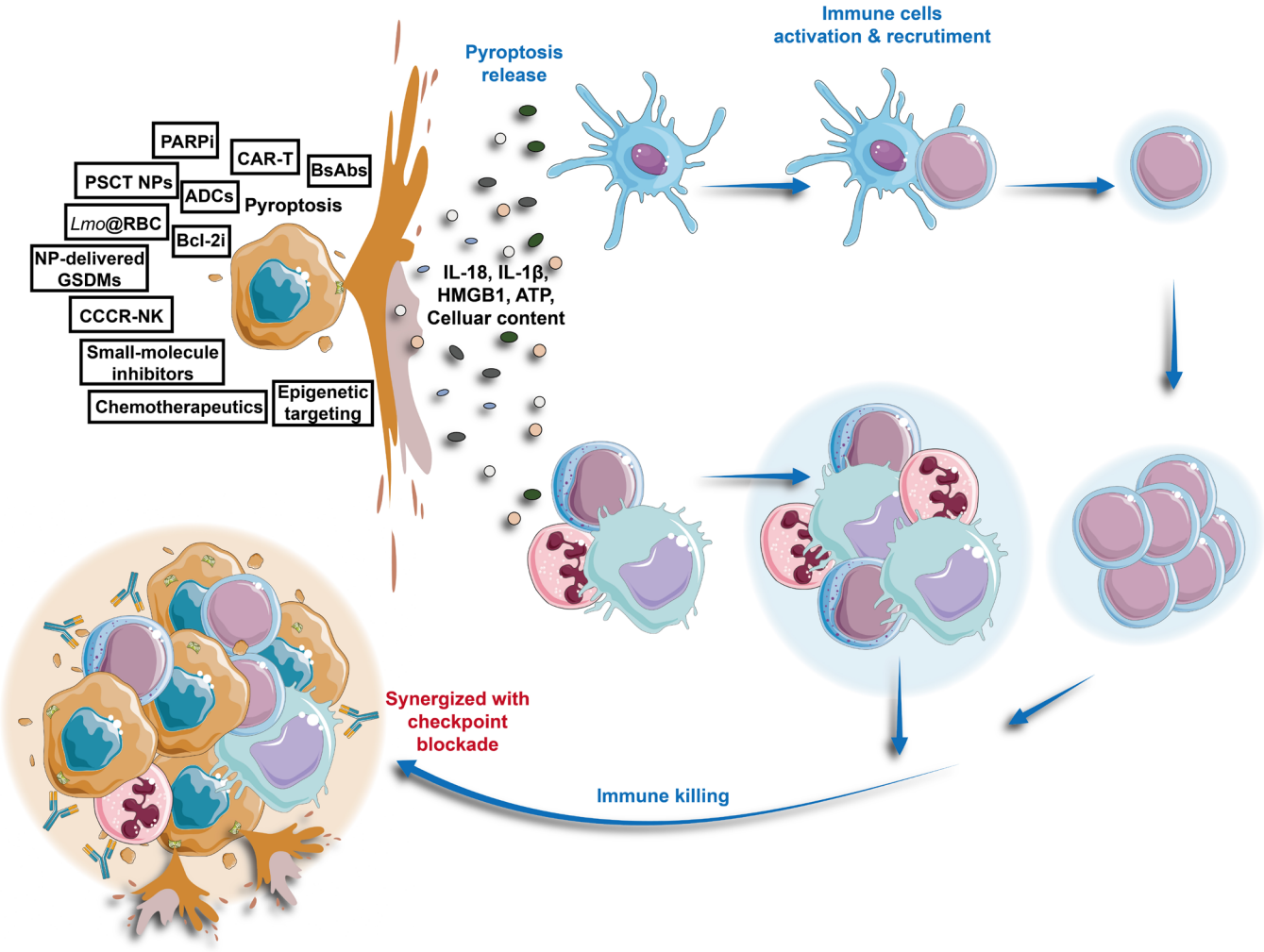

Figure 1 Gasdermin-mediated pyroptosis confers anticancer immunity. Various GSDM-targeting approaches induce cancer cell pyroptosis, leading to the release of inflammatory factors and subsequent activation and infiltration of immune cells in the tumor microenvironment. This orchestrated sequence of events fosters anticancer immunity. Combining these strategies with immune checkpoint inhibitors synergistically enhances cancer immunotherapy efficacy. ADCs, antibody-drug conjugates; ATP, adenosine triphosphate; BcL-2i, BcL-2 inhibitors; BsAbs, bispecific antibodies; CAR-T, chimeric antigen receptor T cell; CCCR-NK, co-stimulatory converting receptor-NK cell; GSDM, gasdermin; HMGB1, high mobility group box1; IL-18, interleukin-18; IL-1β, interleukin-1β; *Lmo*, Listeria monocytogenes; *Lmo*@RBC, *Lmo* encapsulated with red blood cell (RBC) membranes; NP-delivered GSDMs, nanoparticle-delivered gasdermins; PARPi, poly (ADP-ribose) polymerase inhibitors; PSCT NPs, phospholipid-coated sodium citrate nanoparticles.

mediated by the cleavage of GSDMB by granzyme A from these immune cells.[3] Additionally, GSDMB expression in colon cancer cells significantly amplifies the efficacy of anti-PD-1 antibodies. Importantly, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) produced by activated cytotoxic lymphocytes can substantially increase GSDMB expression. Hence, immune checkpoint inhibitors have the potential to boost CCP.[3]

Consistently, we improved the treatment effectiveness for HER2-positive gastric cancer using the bispecific antibody IBI315, targeting both PD-1 and human epidermal growth factor receptor 2 (HER2). Our results reveal that IBI315's antitumor effects hinge on GSDMB cleavage induced by granzyme A from $CD8^+$ T cells.[2] Importantly, the cell-free supernatant generated by IBI315-induced cell pyroptosis activates T cells, stimulating IFN-γ secretion. Consequently, IFN-γ from activated T cells increases GSDMB expression in tumor cells, establishing a positive feedback loop that enhances T cell activation and pyroptotic tumor cell destruction. In conclusion, these findings confirm granzyme A's role in GSDMB cleavage and its mediation of cell pyroptosis. They also highlight how cytotoxic lymphocytes can deliver granzyme A to GSDMB-expressing cancer cells, promoting antitumor immunity.

GSDMB can be cleaved by cytotoxic lymphocyte-derived granzyme A, inducing pyroptosis in tumor cells. Additionally, GSDMB expression can be boosted by IFN-γ and TNF-α released from cytotoxic lymphocytes. This simultaneous increase in GSDMB expression within tumor cells enhances the effectiveness of tumor immunotherapy. In our analysis of existing immunotherapy cohorts, we

identified significant potential for GSDMB as a biomarker in predicting the efficacy of immunotherapy. Notably, GSDMB demonstrated superior performance compared with traditional indicators such as microsatellite instability (MSI), PD-L1, and tumor mutational burden (TMB).[2] Consequently, GSDMB emerges as a promising predictive biomarker for assessing outcomes in tumor immunotherapy. Furthermore, the physical proximity of the GSDMB gene to the ERBB2 gene (encoding the HER2 protein) on the same genetic locus explains the frequent elevation of GSDMB expression in HER2-positive gastric cancer.[2] This helps clarify the robust response of HER2-positive gastric cancer, characterized by genomic stability and a low mutation burden, to anti-PD-1 therapy.[6] Consequently, we recommend assessing GSDMB expression in tumors to facilitate patient stratification and improve the precision of immunotherapeutic interventions. GSDMB exists in five isoforms, and currently, only isoform 3/4, which includes exon 6 encoding 13 critical amino acids in the N-terminus, has been observed to induce pyroptosis when cleaved.[7] Additionally, several Single-nucleotide polymorphisms (SNPs) do not impact the protein coding sequence but influence GSDMB transcript levels. The splice variant rs11078928, deleting the entire exon 6, eliminates the pyroptotic activity of the GSDMB protein.[8] Consequently, we hypothesize that assessing the expression of GSDMB with exon 6 (isoform 3/4) holds more significance for predicting the prognosis of tumor immunotherapy. However, further experimental validation is required.

GSDMC

Poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi) treatment induces GSDMC/caspase-8-mediated CCP and enhances cytotoxic $CD8^+$ T cell infiltration in the tumor microenvironment. Remarkably, IFN-γ can also stimulate GSDMC expression, thereby augmenting the cytotoxicity of PARPi and T cells.[9] Additionally, Li *et al* engineered phospholipid-coated sodium citrate NPs designed to dissolve within tumor cells, releasing substantial citrate ions and Na^+ ions. Elevated citrate concentration within tumor cells activates the caspase-8/GSDMC pathway, inducing CCP. This strategy demonstrates notable antitumor immune responses and effectively inhibits tumor growth.[10] The findings offer fresh perspectives on leveraging metabolism modulation and shifting cell apoptosis toward pyroptosis for enhanced antitumor immunotherapy. In another study by Liu *et al*, it was observed that the facultative intracellular bacterium Listeria monocytogenes (*Lmo*), encapsulated with red blood cell (RBC) membranes (*Lmo*@RBC), could induce extensive pore-forming protein GSDMC-dependent pyroptosis, thereby enhancing the antitumor immune response.[11] These studies, employing diverse methods to activate GSDMC, consistently induced CCP and strengthened the antitumor immune response. This sheds new light on potential avenues for tumor immunotherapy.

GSDMD

Li *et al* employed a bacteria-based delivery system (VNP-GD) to introduce GSDMD into tumor cells. Subsequently, the bacteria induced the maturation of caspase-1, leading to the cleavage of GSDMD and release of the GSDMD N-terminal domain, inducing pyroptosis in the tumor cells. Their research revealed that this induced pyroptosis in tumor cells promoted dendritic cell maturation, increased the infiltration of granzyme B^+CD8^+T cells in the tumor microenvironment, and enhanced the effectiveness of the antitumor immune checkpoint inhibitor, anti-PD-1 antibody. Simultaneously, they blocked calcium influx-triggered ESCRT III-dependent membrane repair by employing a biodegradable NP-mediated sustained release of a calcium chelator (EI-NP). This approach significantly enhanced intracellularly delivered GSDMD-induced tumor pyroptosis, working synergistically to bolster the antitumor immune response.[12]

GSDME

GSDME can be activated through various anti-cancer therapies, including chemotherapies,[13 14] BcL-2 inhibitors,[15] and antibody-drug conjugates.[16] Most of these studies primarily involve the cleavage of GSDME by caspase-3. Additionally, granzyme B from immune cells has been reported to directly cleave GSDME. Zhang *et al* reported that GSDME overexpression in breast cancer cells inhibited their growth when implanted in BALB/c mice with intact immune systems. This effect was accompanied by increased infiltration of NK cells, $CD8^+$ cytotoxic T lymphocytes, and tumor-associated macrophages that engulfed tumor cells.[1] Subsequent investigations revealed that GSDME-induced tumor suppression resulted from pyroptosis triggered by GSDME cleavage and activation, with NK cells and $CD8^+$ T cells playing pivotal roles. Granzyme B was identified as the key mediator responsible for GSDME cleavage and activation.[1] Liu *et al* also demonstrated that granzyme B, originating from chimeric antigen receptor (CAR)-T cells, cleaved GSDME and activated caspase-3 in lymphoma cells.[4] Notably, another study showed that pyroptosis induced in melanoma cells by GSDME and caspase-3 led to high-mobility group box1 (HMGB1) release, directly correlating with tumor-associated T cell activation and dendritic cell infiltration.[17] These findings collectively suggest that damage-associated molecular patterns generated during CCP, such as HMGB1, activate antitumor immune cells, contributing to antitumor immune responses.[17 18]

CHALLENGES

A significant challenge in the development of cell death-based anticancer strategies is the presence of methylations, mutations or downregulation in the expression of GSDM in many tumors.[1 19] To address this issue, epigenetic modifications can be employed

to reverse the silencing of certain GSDM proteins. For instance, the demethylation of GSDME using decitabine has been shown to reactivate the protein and subsequently induce pyroptosis in tumor cells.[20] Although cancer-associated GSDME mutation is not as common as methylation, 91% of mutations lead to the functional loss of GSDME, suggesting conventional epigenetic modifications may not offer a comprehensive solution. Therefore, alternative strategies, such as nanotechnologies targeting tumor cells, which directly deliver functional GSDM proteins, offer another effective method for inducing cell pyroptosis.[5]

Another major hurdle in the development of anticancer strategies based on cell death is the immunosuppressive nature of the tumor microenvironment, characterized by the upregulation of inhibitory receptors like PD-1 on cytotoxic immune cells. Combining cell death inducers with immune checkpoint inhibitors has emerged as a promising approach to address this challenge, as evidenced by numerous studies (figure 1).[3 14] Furthermore, research has explored the fusion of a co-stimulatory conversion receptor into NK cells, which converts inhibitory PD-1 signals into activating signals, effectively enhancing the cytotoxicity of these immune cells against tumor cells and promoting greater CCP. This innovative approach offers a potential avenue for overcoming the immunosuppressive status within the tumor microenvironment.[21]

SUMMARY AND OUTLOOK

Cell pyroptosis induced by GSDM proteins, as a form of immunogenic cell death, can exert an anticancer effect by eliciting antitumor immunity. One of the greatest challenges in applying cell pyroptosis to treat tumors appears to be the downregulation and functional loss of GSDM proteins in cancer. However, advances in molecular, genetic, and epigenetic modifications, as well as improvements in GSDM protein delivery systems, are gradually addressing this dilemma. Another substantial challenge arises from the immunosuppressive milieu within the tumor microenvironment. Hence, co-administration of GSDM protein activators, triggering cell pyroptosis, along with immune checkpoint inhibitors, could further potentiate the efficacy of antitumor immunity. With further research into cell pyroptosis and GSDM proteins, it is believed that we can more effectively harness GSDM proteins as advantageous weapons against cancer.

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