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RESEARCH HIGHLIGHT

Gasdermins deliver a deadly punch to cancer

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The pore-forming gasdermin proteins mediate a lytic and pro-inflammatory form of cell death called pyroptosis and have been linked to the host defense against infection. Two recent studies published in *Nature* revealed that induction of pyroptosis in tumor cells promotes anti-tumor activity, highlighting gasdermins as potential new targets in cancer immunotherapy.

Gasdermins are a family of pore-forming proteins expressed in immune and non-immune cells. Cells that have been infected by pathogens or exposed to danger-associated signals undergo pyroptosis mediated by gasdermins. In this process, inflammatory caspases, apoptotic caspases and serine proteases induce proteolytic cleavage of gasdermin proteins, releasing the active N-terminal fragment.[1] The N-terminal cleavage product forms pores on the plasma membrane, which leads to pyroptosis and the release of intracellular contents, including pro-inflammatory cytokines.[2,3] This terminal event removes infected or damaged cells and contributes to the killing and clearance of pathogens.[4] The lytic and immunogenic nature of pyroptosis might be an ideal attribute for cancer immunotherapy, because lysis of cancer cells ensures their demise, and immunogenic elicitation of the immune system might further accelerate destruction of cancer cells.

Zhang et al.[5] and Wang et al.[6] investigated the possibility of harnessing the activity of gasdermin proteins for use in anti-tumor immunity (Fig. 1). Mutations in gasdermins have been identified in many types of cancers, including breast cancer, gastric cancer and colorectal cancer.[7] Zhang et al. identified that 91% (20 of 22) of the mutations in the gene encoding gasdermin E (GSDME) from cancer patients are loss of function. To investigate the role of GSDME in cancer biology, the authors genetically deleted *Gsdme* in two mouse cell lines, triple negative breast cancer EMT6 and colorectal cancer CT26, and implanted these cells in immunocompetent mice. Implanted *Gsdme*$^{-/-}$ tumors derived from both cell lines grew faster compared to control tumors expressing endogenous GSDME. In addition, the authors took advantage of two other cell lines with low endogenous amounts of GSDME (melanoma B16-F10 and triple negative breast cancer 4T1E) and engineered them to express either wild-type GSDME or GSDME carrying a F2A mutation that impairs its ability to form pores. Following implantation, cancer cells expressing wild-type GSDME were markedly impaired in their ability to proliferate compared to cells expressing the mutant form of GSDME, highlighting the importance of pore-forming activity of GSDME in the control of tumor growth.

The anti-tumor activity seen in GSDME might extend to other pore-forming gasdermins. Wang and colleagues used a bioorthogonal system which enables a controlled release of gasdermin proteins in tumor cells. The authors generated a nanoparticle (NP)-gasdermin A3 (GSDMA3) conjugate (NP-GSDMA3), which carries a linker that can be specifically desilylated by the compound phenylalanine trifluoroborate (Phe-BF_3), releasing active GSDMA3. Using this system, the authors treated mice carrying implanted 4T1 or EMT6 tumors with either NP-GSDMA3, Phe-BF_3 or in combination. Indeed, only the treatment regimen of NP-GSDMA3 and Phe-BF_3 in combination delivered over three rounds led to substantial tumor regression. Further, treatment with a single round of NP-GSDMA3 and Phe-BF_3 in combination was effective when a checkpoint inhibitor anti-PD1 antibody was administered. These data demonstrate that controlled activation of pyroptosis in tumor cells can induce anti-tumor immunity against implantable tumors, an effect that can be enhanced by checkpoint blockade.

Both research groups further profiled the cellular changes within the tumor microenvironment following pyroptosis. Zhang and colleagues observed substantially more tumor-infiltrating natural killer (NK) cells and tumor-associated macrophages in tumors engineered to express GSDME compared to that in control tumors, and that more $CD8^+$ tumor-infiltrating lymphocytes in tumors engineered to express GSDME expressed granzyme B, perforin, interferon-γ (IFN-γ) and tumor necrosis factor (TNF). In agreement with this finding, Wang and colleagues observed a marked increase in infiltrating $CD3^+$ T cells (both $CD4^+$ and $CD8^+$ subsets) and a decrease in $CD4^+$ $FOXP3^+$ T regulatory cells in tumors treated with NP-GSDMA3 and Phe-BF_3 in combination.

To further confirm the requirement of the immune system in mediating gasdermin-dependent tumor suppression, both groups performed experiments on immunodeficient mice lacking specific immune cell subsets. Zhang and colleagues found that the anti-tumor effect of GSDME was compromised in NSG (non-obese diabetic, severe combined immunodeficient, interleukin-2-receptor-γ null) mice deficient in B cells, T cells and functional NK cells. Similarly, Wang and colleagues found that athymic Nu/Nu mice lacking mature T cells had an impaired ability to execute GSDMA3-mediated tumor control. Furthermore, both groups showed that antibody-mediated depletion of $CD4^+$ T cells, $CD8^+$ T cells and/or NK cells led to impaired gasdermin-dependent suppression of tumor growth in BALB/c mice.

The importance of killer lymphocytes in gasdermin-dependent tumor suppression suggests that these cell subsets might trigger pyroptosis in cancer cells. Indeed, Zhang and colleagues showed that the human NK cell line YT induced cleavage of GSDME and pyroptosis in HeLa cells. Granzymes and perforin are normally secreted by cytotoxic lymphocytes, including NK cells, to be

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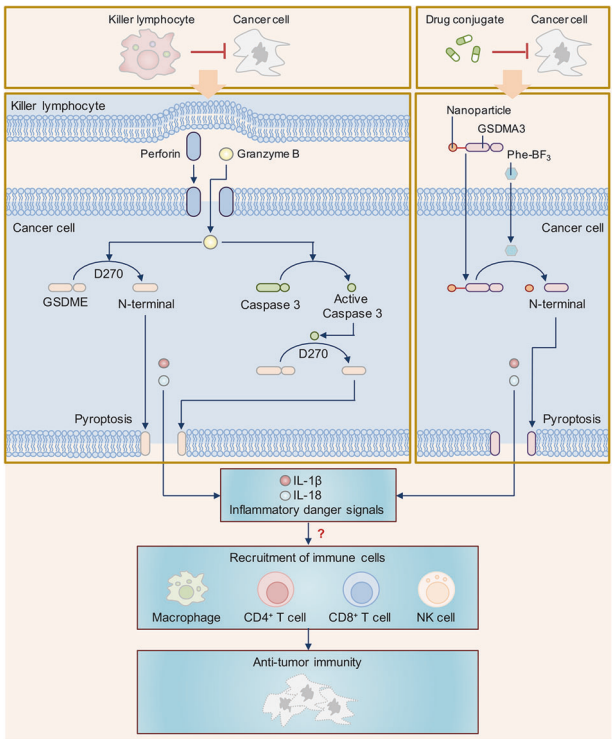

Fig. 1 Gasdermin-mediated pyroptosis induces anti-tumor immunity. Top and bottom left-hand panels: Killer lymphocytes, such as Natural Killer (NK) cells, secrete perforin and granzyme B to the target cancer cell. Granzyme B enters the cancer cell and cleaves gasdermin E (GSDME) after the site D270, or caspase-3. Active caspase-3 can also cleave GSDME after D270. Cleavage of GSDME liberates its functional N-terminal fragment (N-terminal). The N-terminal fragment of GSDME forms pores on the plasma membrane, resulting in pyroptosis of the cancer cell. Top and bottom right-hand panels: Drug conjugates can be used to promote pyroptosis in cancer cells. Nanoparticle-gasdermin A3 (NP-GSDMA3) and the compound phenylalanine trifluoroborate (Phe-BF_3) can enter the cancer cell, whereby Phe-BF_3 specifically desilylates NP-GSDMA3 and promotes the release of active GSDMA3 (N-terminal), leading to pyroptosis of the cancer cell. Pyroptosis of cancer cells further triggers the recruitment of immune cells, including macrophages, $CD4^+$ T cells, $CD8^+$ T cells and NK cells, to induce anti-tumor immunity. This recruitment event might depend on the proinflammatory cytokines IL-1β and/or IL-18, and/or inflammatory danger signal/s, but the precise signal/s are unknown (indicated by the question mark).

delivered onto target cells to induce cell death. Zhang et al. found that granzyme B specifically cleaved GSDME after D270,[5] the same site used by caspase-3 to cleave GSDME.[8] Therefore, granzyme B is an additional protease capable of activating GSDME, positioning granzyme B-secreting cytotoxic cells as a trigger of pyroptosis of target cells (Fig. 1).

The link between pyroptosis and immune cell recruitment was further investigated. The proinflammatory cytokine IL-1β is secreted primarily through the plasma membrane pores generated by gasdermins and has an established function in T cell-mediated anti-tumor immunity.[9] Wang and colleagues found that tumor regression induced by pyroptosis in response to treatment of NP-GSDMA3 and Phe-BF_3 in combination largely depended on IL-1β. In contrast, Zhang and colleagues ruled out a possible role for IL-1β in GSDME-mediated anti-tumor immunity. This inconsistency between the two studies might be owing to a putative and redundant effect of IL-18 and other pyroptosis-liberated molecules, given that Wang and colleagues reported an additional partial anti-tumor effect of IL-18 (Fig. 1).

The studies by Zhang et al.[5] and Wang et al.[6] paved the way for harnessing the functional activity of gasdermins in the fight against cancer. Several other gasdermin members have pore-forming capability and are implicated in cancer biology.[1] These proteins could be explored for use in killing cancer cells. More broadly, therapeutically activating other endogenous mammalian pore-forming proteins, such as the necroptotic effector MLKL, in cancer cells, or strategic and controlled delivery of microbial pore-forming proteins to cancer cells, might also elicit anti-tumor immunity and add to the repertoire of cancer immunotherapy. In addition to orthotopic tumor models, a future prospect is to better understand how pore-forming proteins might be delivered and activated in other cancer models, such as chemically-induced tumor models and genetically-predisposed spontaneous tumor models.[10]

REFERENCES

1. Broz, P., Pelegrín, P. & Shao, F. *Nat. Rev. Immunol.* **20**, 143–157 (2020).
2. Kayagaki, N. et al. *Nature.* **526**, 666–671 (2015).
3. Shi, J. et al. *Nature.* **526**, 660–665 (2015).
4. Miao, E. A. et al. *Nat. Immunol.* **11**, 1136–1142 (2010).
5. Zhang, Z. et al. *Nature.* **579**, 415–420 (2020).
6. Wang, Q. et al. *Nature.* **579**, 421–426 (2020).
7. Feng, S., Fox, D. & Man, S. M. *J. Mol. Biol.* **430**, 3068–3080 (2018).
8. Wang, Y. et al. *Nature.* **547**, 99–103 (2017).
9. Ghiringhelli, F. et al. *Nat. Med.* **15**, 1170–1178 (2009).
10. Pandey, A., Shen, C. & Man, S. M. *Yale J. Biol. Med.* **92**, 481–498 (2019).