RESEARCH HIGHLIGHT

A GSDMD agonist boosts specific antitumor immunity

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The beneficial role of gasdermin activation and pyroptosis induction in antitumor immunity has been implicated by several studies; however, the question of how to specifically and efficiently activate cancer cell death while minimizing toxicity to host cells remains unsolved. In a recent paper published in *Cell*, Wu and Lieberman groups identified a GSDMD agonist that can induce low-level pyroptosis in cancer cells but not in host cells, offering potential applications in cancer vaccine development and therapy in combination with checkpoint blockade.

Checkpoint blockade (CPB) and chimeric antigen receptor T cell therapies have been successfully applied to activate infiltrating T cells to treat some cancers. Yet many solid tumors, known as cold tumors, have fewer infiltrating lymphocytes and therefore do not respond efficiently to immunotherapies. Currently, many researchers are focusing on developing strategies to turn a cold tumor into a hot tumor. The idea of increasing immune cell infiltration and immune responses by promoting inflammatory tumor cell death, such as necroptosis and pyroptosis, is promising.[1]

Pyroptosis is a type of cell death which is highly immunogenic due to its lytic nature, releasing inflammatory cytokines, such as IL-1β and IL-18, and damage-associated molecular patterns, such as histones and ATP, which normally are constrained within cells. Gasdermins, such as GSDMD, are executioners of pyroptosis and are originally demonstrated to exert functions by generating N-terminal fragments (NTs) via inflammatory caspase-mediated cleavage, followed by NT oligomerization, translocation to the plasma membrane, formation of the gasdermin pores and plasma membrane rupture.[2–5] More proteins have been found to be able to cleave gasdermins, for instance, caspase-8, caspase-3, and granzymes. The activity of gasdermins is tightly regulated by post-translational modifications. Among them, K27-linked polyubiquitination (at K203 and K204 in humans, K204 and K205 in mice), oxidation (at C38, C56, C268 and C467 in humans) and palmitoylation (at C191 in humans, C192 in mice) have been reported to activate GSDMD.[6] Recently, the non-cleavable full-length GSDMD activated by reactive oxygen species (ROS)-dependent *S*-palmitoylation was found to mediate pore formation and pyroptosis, although less efficiently than palmitoylated GSDMD-NT.[7] Increasing evidence of the complex regulatory mechanisms and functions of gasdermins suggests that they are important potential drug targets in the pyroptotic signaling pathway, and that multiple manipulations could be explored to activate gasdermins to trigger tumor cell pyroptosis and stimulate antitumor immunity. To this end, one key problem to tackle is how to efficiently and specifically induce pyroptosis of cancer cells without causing harmful systemic side effects to the host.

Using a high-throughput screen system, Wu and Lieberman groups recently discovered a small-molecule agonist, quinoxaline 6,7-dichloro-2-methylsulfonyl-3-*N*-tertbutylaminoquinoxaline (DMB), that potently activates full-length GSDMD by covalently and selectively binding to GSDMD at C191, the known palmitoylation site, leading to GSDMD oligomerization and pore formation in a cleavage-independent manner.[8] Excitingly, DMB was shown to trigger pyroptosis of cancer cells and activate antitumor immunity in multiple mouse tumor models, such as the EMT6 tumors (cold triple-negative breast cancer), CT26 tumors (colorectal cancer), KP tumors (lung adenocarcinoma) and B16 tumors expressing human GSDMD (melanoma), without causing measurable systemic toxicity to the host. Mechanistically, the DMB-induced tumor killing depends on GSDMD expression in cancer cells but not in host cells, and is mediated by the host immune system. Furthermore, DMB-treated EMT6 tumor cells were verified to be effective as a tumor vaccine against secondary tumor challenge. In addition, a strong correlation between tumor size and DMB responsiveness was revealed, indicating that DMB might be particularly useful when combined with other therapies or after surgery or chemotherapy to reduce tumor burden. Importantly, at a low dose, DMB synergized with anti-PD-1 to mount effective antitumor immunity against immunologically cold, CPB-unresponsive 4T1E tumors, in which DMB or anti-PD-1 alone was ineffective, without causing obvious toxicity.[8]

Together, these findings provide a vivid scenario where full-length GSDMD that is no longer auto-inhibited is capable of inducing pyroptosis independent of GSDMD cleavage. The successful use of DMB in mice suggests that DMB, by inducing low-level pyroptosis of tumor cells, can boost antitumor immunity against multiple types of tumors and work synergistically with anti-PD-1 to suppress the growth of CPB-resistant tumors. The specificity of DMB towards tumor cells but not host cells is a crucial feature of DMB, the molecular mechanisms of which require further studies. One possible explanation is that cancer cells, by reprogramming their metabolism to meet the energetic and biosynthetic demands to proliferate, natively have a higher level of intracellular ROS than normal cells, which in combination with the palmitoylation-like full-length GSDMD–DMB complex, results in a feedforward loop[7] that can bypass inflammasome activation and GSDMD cleavage and take a shortcut to form full-length GSDMD–DMB pores, leading to cancer cell-specific pyroptosis.

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The low DMB dosage and low level of pyroptosis required to induce efficient antitumor immunity suggest that DMB is probably among the most promising compounds that could be further developed for clinical use. To advance into clinical trials, further work is required, such as evaluating potential off-target effects of DMB that might cause unexpected toxicity in human patients, as well as identifying the molecular basis and targets of the immunological memory when using DMB-treated cancer cells as a tumor vaccine. Nonetheless, this proof-of-concept study has found a simple and efficient way to convert cold tumors into hot tumors, which might provide a therapeutic approach to treat cold solid tumors.

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ADDITIONAL INFORMATION

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