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AUTHOR'S VIEWS

Gasdermins: novel mitochondrial pore-forming proteins

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ABSTRACT

Gasdermin proteins have been extensively characterized for their ability to form necrotic pores in the plasma membrane, however, their interactions with other organelles have yet to be described. We recently demonstrated that some gasdermin proteins can also permeabilize the mitochondria to augment apoptotic signaling which may be important in the context of cancer and hearing loss.

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The gasdermin superfamily, composed of gasdermin A (GSDMA), gasdermin B (GSDMB), gasdermin C (GSDMC), gasdermin D (GSDMD), gasdermin E (GSDME/DFNA5), and Pejvakin (PJVK/DFNB59), is a novel group of proteins with recently discovered pore-forming activity. All members except Pejvakin adopt an autoinhibited conformation whereby the C-terminal domain masks a lipid-binding moiety in the N-terminal necrotic domain.[1] Activation of the pore-forming activity of these proteins requires removal of their C-terminal inhibitory domain. In GSDMD and GSDME this is achieved by proteolytic processing with inflammatory caspases[2] during inflammasome activation and caspase-3[3] during apoptosis, respectively. The liberated pore-forming N-terminal domain translocates to the plasma membrane, homooligomerizes, and forms membrane-spanning pores leading to pyroptotic/necrotic cell death.[4] Previous studies showed that GSDMA, GSDMC, GSDMD, and GSDME are often downregulated in cancer cell lines and primary tumors compared to normal tissues, while their enforced expression in cancer cell lines leads to suppression of colony formation and growth.[5,6] Specifically *GSDME/DFNA5* gene is often silenced in breast, gastric, and colorectal cancer due to promoter hypermethylation, and this downregulation is correlated with an increased risk of breast cancer metastasis.[6–8] While the caspase-3-liberated necrotic activity of GSDME has recently been extensively characterized,[3] this function does not fully explain its ascribed putative tumor suppressor activity, as tumor suppressors typically act upstream of caspase-3 activation and apoptosis.

Our recent work demonstrates that activated gasdermin proteins can promote apoptosis by also forming pores in the mitochondria.[9] We showed that cancer cell lines deficient in GSDME expression display reduce kinetics and magnitude of caspase-3/-7 activation in response to diverse apoptotic stimuli including the glucocorticoid triamcinolone acetonide, ultraviolet (UV) irradiation, serum starvation, etoposide, and tumor necrosis factor α (TNFα) which could be rescued upon re-expression of wild-type (WT) GSDME, but not a pore-forming deficient mutant harboring a T6E mutation. By setting up *in vitro* cleavage reactions with purified mitochondria, we demonstrated mechanistically that caspase-3-cleaved WT GSDME, but not GSDME T6E, causes mitochondrial membrane permeabilization and release of proapoptotic factors like cytochrome c (cyt c; Figure 1). Furthermore, expression of the necrotic N-terminus of GSDME (GSDME-N), but not full-length (FL) GSDME or the GSDME-N T6E mutant in 293T cells, induces the release of cyt c from the mitochondria to similar levels as the known mitochondrial pore-forming protein BCL2-associated X (Bax) protein. As GSDME-N also forms pores in the plasma membrane, this cyt c is ultimately released outside of the cell whereas it is retained in the cytosol when released by Bax. Consistent with a role for GSDME in mitochondrial permeabilization and cyt c release during apoptosis, deletion of GSDME in the acute lymphoblastic leukemia cell line CEM-C7 significantly reduces cyt c release from the mitochondria in response to UV irradiation and TNFα treatment.

To determine the order in which GSDME-N permeabilizes the mitochondria and plasma membrane, we performed a time-lapse microscopy experiment to examine the distribution of the mitochondrially localized high-temperature-regulated A2 (HtrA2/Omi) protein upon GSDME-N expression. Our results showed that upon GSDME-N expression, HtrA2/Omi is released into the cytosol before cells show any features of swelling or necrosis demonstrating that GSDME-N first permeabilizes the mitochondria and then ruptures the plasma membrane. We also showed that GSDME-N-mediated release of cyt c and HtrA2/Omi from the mitochondria activates caspase-3/-7 and generates a positive feedback loop that amplifies GSDME cleavage. Interestingly, this mitochondrial pore-forming function is also conserved in GSDMA and GSDMD as expression of their active N-terminal fragments in HEK293T cells is sufficient to activate caspase-3/-7 and

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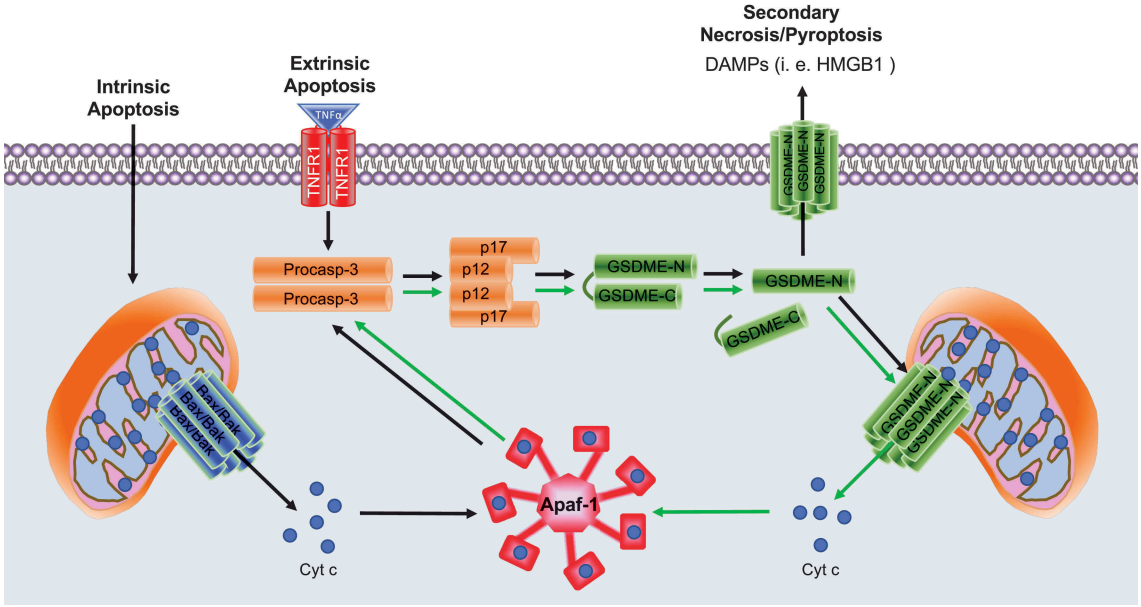

Figure 1. The N-terminus of Gasdermin E permeabilizes the plasma and mitochondrial membranes. Activation of the intrinsic apoptotic pathway by glucocorticoids, ultraviolet (UV) irradiation, etoposide, or serum starvation or the extrinsic apoptotic pathway by tumor necrosis factor (TNFα) leads to activation of caspase-3 which cleaves gasdermin E (GSDME) liberating the pore-forming ~30 kDa N-terminal fragment (GSDME-N) that translocates to and permeabilizes the mitochondria. The GSDME-N mitochondrial pores release cyt c which augment activation of the apoptotic protease activating factor 1 (Apaf-1) apoptosome, and positive feedback on caspase-3 activation and GSDME cleavage (green arrows). After permeabilizing the mitochondria GSDME-N then permeabilizes the plasma membrane causing secondary necrosis/pyroptosis, which allows the release of proinflammatory damage-associated molecular pattern (DAMP) molecules like high mobility group box 1 (HMGB1).

release cyt c from the mitochondria. The mitochondrial pore-forming function of GSDMD-N may mechanistically link activation of the inflammasome-mediated pyroptotic pathway to that of the apoptotic pathway. Indeed, macrophages deficient in GSDMD expression display little caspase-3/-7 activation and mitochondrial cyt c release in response to activation of the noncanonical inflammasome by intracellular lipopolysaccharide (LPS).0

The pore-forming functions of gasdermins likely explain their ability to act as tumor suppressors. In addition to being significantly downregulated in breast, gastric, and colorectal cancers, we show that melanoma tumors deficient in GSDME expression grow more quickly than WT tumors when grafted onto mice. These proteins may function to suppress tumor growth by a number of different mechanisms. First, mitochondrial pore-formation may lower the threshold for activation of the apoptotic program by accelerating and enhancing the release of proapoptotic mitochondrial proteins, thus making it easier to kill tumor cells by the variety of stresses that they encounter as they continuously grow and divide. Second, mitochondrial outer membrane permeabilization (MOMP) has been shown to engage nuclear factor (NF)-κB activation, which is a key immunogenic determinant essential for anti-tumor immunity and tumor regression.[10] Third, gasdermins may promote tumor suppression by virtue of their plasma membrane pore-forming function and induction of secondary necrosis. Certain chemo- and radiotherapies are known to promote immunogenic cell death which enhances tumor destruction by activating $CD8^+$ killer T-cells that target tumor cells. This form of cell death requires the release of adjuvants like high mobility group box 1 (HMGB1) which we show are released through GSDME plasma membrane pores during GSDME-induced secondary necrosis.[9] It is, therefore, possible that the gasdermins also function as tumor suppressors by stimulating immunogenic cell death and enhancing T-cell-mediated tumor cell killing. Future studies should determine which of these mechanisms is most critical for tumor suppression and how it can be manipulated to improve therapeutic outcomes. In conclusion, our work uncovers a novel function of gasdermin family members in forming pores in the mitochondria and suggests a new mechanism by which they may function as tumor suppressors. This work paves the way for future clinical studies on the role of the gasdermins in cancer progression and provides a promising, novel therapeutic target.

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