Cell death

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# Cleavage-independent GSDME activation by UVC

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Gasdermins (GSDMs) are mediators of cell death that trigger membrane lysis. A study shows that full-length GSDME induces pyroptosis after ultraviolet irradiation, involving GSDME PARylation that releases autoinhibition and lipid reactive oxygen species that promote pore formation. This study adds insights on how GSDMs can be activated non-canonically.

Gasdermin family proteins (GSDMA, GSDMB, GSDMC, GSDMD, GSDME and GSDMF) are well-established executors of pyroptosis[1], a form of programmed cell death characterized by cell swelling, lysis and release of pro-inflammatory cytokines. Canonically, after activation by cleavage, the N-terminal domain of GSDMs (GSDM-NT) oligomerizes to form pores in the plasma membrane, disrupting cellular integrity[2]. For GSDMD, this activation is now known to require a lipid modification, *S*-palmitoylation at Cys191, which facilitates GSDMD-NT membrane translocation[3–6]. Additionally, reactive oxygen species (ROS) enhance palmitoylation to enable even full-length GSDMD (FL-GSDMD) to form pores by overcoming autoinhibition and promoting membrane localization[3], which may be considered a non-canonical form of GSDMD activation.

In this issue of *Nature Cell Biology*, Zhou et al.[7] discovered a non-canonical form of FL-GSDME activation, triggered by ultraviolet (UV) radiation-induced DNA damage. UV radiation is a serious environmental stressor consisting of UVA, UVB and UVC. Zhou et al.[7] adopted the highly toxic stressor UVC, previously used to study other forms of cell death, to probe immunological signalling pathways. Interestingly, under high UVC dosage, they found that pyroptosis could be preferentially induced in cervical carcinoma cells and a broad range of other cancer cell lines. The addition of inhibitors for necroptosis, autophagy and copper-dependent death (cuproptosis) did not affect this cell death. Beyond its importance as a physiological phenomenon, a robust understanding of the mechanism of this pyroptosis pathway may allow us to suppress undesired cell growth as a cancer therapy.

Zhou et al.[7] found that cleavage of GSDMA, GSDMB, GSDMC, GSDMD and GSDME was not detected during UVC-induced pyroptosis. Knockdown of these GSDMs revealed that only GSDME was involved in UVC-induced pyroptosis. Treatment with pan-caspase inhibitors and mutations of GSDME at the caspase-3 cleavage site did not have any effects, suggesting that GSDME-specific caspase-3 was not involved and that FL-GSDME induces pyroptosis[7].

As UVC induces ROS, the authors wanted to determine if UVC-induced ROS are associated with FL-GSDME-mediated pyroptosis. Treatment with ferrostatin-1 or deferoxamine, inhibitors that suppress lipid ROS, substantially reduced UVC-induced GSDME pyroptosis. By contrast, total ROS inhibitors, *N*-acetyl cysteine or glutathione, did not downregulate subsequent pyroptosis, suggesting that lipid ROS, rather than total ROS, serve as initiators of UVC-induced pyroptosis. Meanwhile, knockdown of GSDME did not affect lipid ROS levels, indicating that lipid ROS increase triggered by UVC occurs as an upstream mediator of FL-GSDME activation[7].

Because ROS can enhance *S*-palmitoylation to activate GSDMD[3], the authors conducted tests with inhibitors of *S*-palmitoylation, *S*-nitrosylation and *S*-sulfhydration or antibodies specific for *S*-glutathionylation. However, none of these post-translational modifications were identified on GSDME after UVC irradiation[7]. Instead, Zhou et al.[7] revealed that UVC irradiation induced time-sensitive oxidative oligomerization of GSDME. Lipid ROS appeared to promote intermolecular disulfide bond formation between two evolutionarily conserved residues, Cys156 and Cys180 of GSDME, as detected by mass spectrometry under non-reducing conditions, which were determined to be the key sites responsible for oligomerization[7]. The interaction was weakened in GSDME$^{C156S}$ and GSDME$^{C180S}$ mutants, and almost abolished with simultaneous mutation of both sites, compared to wild-type FL-GSDME.

The authors used a fluorescent dye and identified the spatial colocalization of TOM20–BFP, a fluorescently labelled mitochondrial outer membrane protein, and lipid ROS, suggesting the potential involvement of mitochondria in processes triggered by UVC. The authors noticed that UVC irradiation induced a mitochondrial morphological transition from linear and granular to circular structures, indicative of mitochondrial fission. This phenomenon could be reversed with knockdown of key fission-related proteins (MFF and DRP1). Elevated levels of mitochondrial ROS (mito-ROS) were also detected after UVC irradiation. Blocking mitochondrial fission decreased mito-ROS levels and downstream events, whereas treatment of cells with mito-ROS inhibitors decreased UVC-induced increase in lipid ROS. These data suggest an upstream role of mitochondrial dynamics on mitochondrial oxidative stress and subsequent lipid ROS increase, GSDME oxidation and UVC-induced pyroptosis. This conclusion was confirmed by treatment with the lipid ROS inhibitor ferrostatin-1, which did not affect mito-ROS levels[7].

Zhou et al.[7] went on to establish another signalling molecule involved in the pathway — cytochrome *c* (cyt *c*). Knockdown of cyt *c* or holocytochrome *c*-type synthase, which catalyses maturation and activation of cyt *c*, led to reduced UVC-induced lipid ROS levels and diminished GSDME oxidation. However, no effect of cyt *c* knockdown was observed on UVC-induced mitochondrial fission or mito-ROS levels, suggesting that cyt *c* acts upstream of lipid ROS and downstream of mitochondrial events after UVC irradiation. Cyt *c* is commonly involved in the activation of caspase-9, which triggers caspase-3 for GSDME cleavage[8,9]. However, under high UVC exposure, no activation of caspase-9 or caspase-3 was detected and GSDME was not cleaved. Instead, mitochondrial cardiolipin was identified as another important participant downstream of cyt *c*. Studies have shown that cyt *c* has

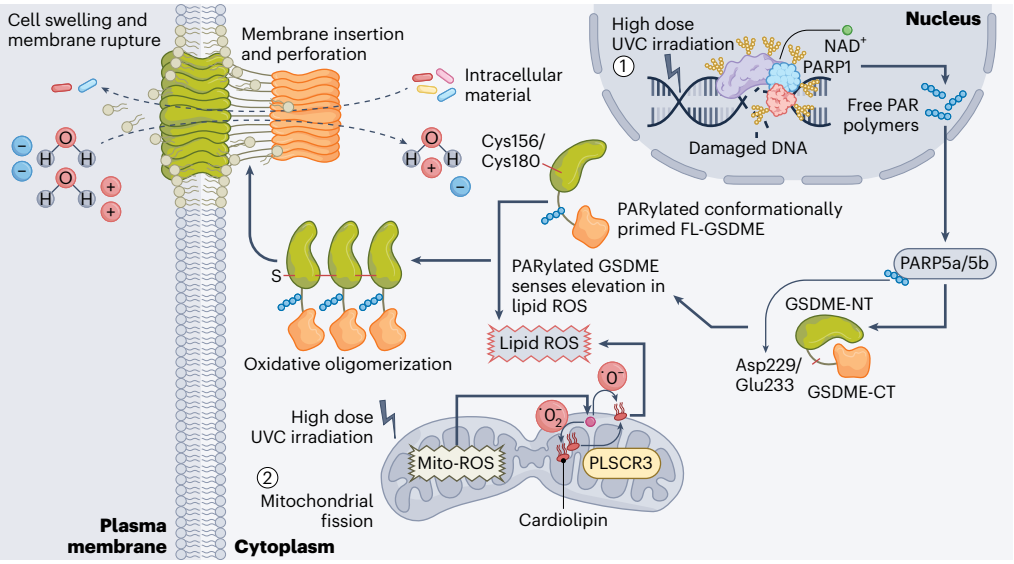


**Fig. 1 | Mechanism involving FL-GSDME-induced pyroptosis at high doses of UVC irradiation, independent of proteolytic cleavage.** After UVC irradiation, two parallel signalling pathways are activated. (1) In the nucleus, DNA damage induced by UVC hyperactivates PARP1 and promotes extensive formation of PAR polymers, which are released to the cytosol. PAR interacts directly with and activates PARP5a/5b, which catalyses the PARylation of cytosolic GSDME at Asp229 and Glu233 (near the hinge region). Conformational change in the PARylated GSDME disrupts the intramolecular autoinhibition of the GSDME N terminus on the C terminus. (2) In the mitochondria, mito-ROS are triggered in a mitochondrial-fission-dependent manner, which promotes the oxidation of cardiolipin by cytochrome *c*, found in the intermembrane space. Oxidized cardiolipin is translocated from the inner to the outer membrane by PLSCR3. This promotes the increase in lipid ROS levels in the cytosol, which is sensed by the conformationally primed GSDME. The exposed N-terminal domain (which possesses intrinsic membrane-pore-forming activity) of FL-GSDME undergoes disulfide-bond-dependent oxidative oligomerization, which forms pre-pores. Fully formed pores then target and insert into the plasma membrane for perforation, eventually leading to cell swelling and pyroptosis.

peroxidase activity and can catalyse cardiolipin oxidation[10]. Notably, this activity was increased after UVC irradiation but decreased through treatment with mito-ROS inhibitors or knockdown of MFF and DRP1 to inhibit mitochondrial fission. The authors confirmed that UVC irradiation led to the oxidation of cardiolipin, as observed by the decrease in fluorescence intensity of 10-nonyl acridine orange, a probe that recognizes unoxidized cardiolipin. Furthermore, knockdown of CRLS1, which is involved in cardiolipin synthesis, reduced generation of lipid ROS and oxidation of GSDME. Zhou et al.[7] provided evidence that GSDME is present at the mitochondrial outer membrane, as shown by TurboID proximity labelling. The translocation of oxidized cardiolipin from the inner to the outer mitochondrial membrane by phospholipid scramblase 3 (PLSCR3)[11,12] is important for, and thus could directly mediate, UVC-induced GSDME oxidation and pyroptosis[7].

UVC irradiation is also known to induce DNA damage[13]. Poly(ADP-ribose) polymerases (PARPs) are sensors hyperactivated by DNA damage[14], which catalyse poly(ADP-ribosyl)ation (PARylation) of their substrates using $NAD^+$ as a donor for ADP-ribose[15]. Consistently, UVC irradiation increased total PARylation (total-PAR) levels, detected with pretreatment using poly(ADP-ribose) hydrolase inhibitors. By contrast, pretreatment with specific inhibitors of PARPs, rucaparib for PARP1/2/3 and olaparib for PARP1/2, reduced total-PAR. Moreover, the authors showed that PARP1 enzyme activity, not PARP2, is involved in UVC-induced PARylation, as PARP1 knockout or mutation of the PARP1 active site (E998Q) reduced total-PAR. PARP1 remained localized in the nucleus after UVC irradiation and was incapable of interacting directly with cytoplasmic GSDME. Instead, PARP1 elevated the level of total free PAR polymers, which were released into the cytoplasm. The predominantly cytoplasmic PARP5a/5b were implicated as the enzymes that PARylated GSDME, as their inhibition by K756 ablated UVC-induced GSDME PARylation, but did not affect total-PAR. In vitro assays showed a direct association between PARP5a/5b, free PAR and GSDME. Additionally, the authors mapped the potential PARylation sites to Asp229 and Glu233, as the $GSDME^{D229/E233A}$ mutant exhibited diminished UVC-induced PARylation, oligomerization and plasma membrane targeting[7].

By mutating three loop regions at the interface between the C-terminal and N-terminal domains of GSDME to part of the Flag tag sequence, DDDDK, followed by anti-Flag immunoprecipitation, the authors found that UVC irradiation induced a conformational change in GSDME, shown by different accessibilities of the peptides in the three regions. Treatment with PARP1 and PARP5a/5b inhibitors and mutations at the PARylation sites reduced this GSDME conformational shift after UVC irradiation, suggesting that GSDME PARylation triggered a structural release of GSDME autoinhibition. Owing to this conformational change, UVC irradiation also increased the binding of GSDME to phosphatidylinositol (4)-phosphate and phosphatidic acid in a lipid strip assay and facilitated direct interaction with liposomes from bovine liver lipid extracts[7].

Interestingly, this pyroptotic pathway can be stimulated with a low dose of UVC and several DNA-damaging reagents to induce PARylation when co-treated with lipid-ROS-inducing agents to promote GSDME oxidative oligomerization. Under physiological conditions, this may be presented as a combinatorial antitumour therapy. Zhou et al.[7] realized such potential and tested its efficacy in nude mice bearing HeLa-cell-driven xenograft tumours, and C57BL/6J

mice bearing GSDME-overexpressing melanoma B16 tumours. Combined intraperitoneal administration of a DNA-damaging agent and a lipid-ROS-inducing agent showed the most substantial synergistic suppression of tumour growth, which is dependent on the expression of functional GSDME. FL-GSDME-mediated pyroptosis was also shown to demonstrate antitumour immunogenicity. Immune cells isolated from tumour tissue exhibited improved activation status, with increased filtration of perforin$^{+}$CD8$^{+}$ T cells, interferon (IFN)$^{+}$CD8$^{+}$ T cells and IFN$^{+}$ natural killer cells[7].

In summary, Zhou et al.[7] offered a compelling pathway in which UVC irradiation activates FL-GSDME for pore formation and pyroptosis due to GSDME PARylation, lipid ROS generation and oligomerization of GSDME through two cysteine residues (Fig. 1). One particular discrepancy is that GSDME structure modelling predicts that intermolecular distances among these cysteine residues are too far for disulfide bonding. It is also unclear if other signalling mechanisms can converge on this pathway and whether alternative forms of post-translational modifications are involved. In addition, it remains an open question how this pathway can crosstalk with other types of cell death and host immune defence. The validity of this pathway in other gasdermins, cell types and organisms also awaits further investigation. Nonetheless, Zhou et al.[7] have brought to light a pyroptosis process by FL-GSDME, which can be induced and manipulated by UVC irradiation, DNA damage and lipid ROS. This study may inspire the discovery of more physiological targets that promote or even inhibit cell death.

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## Competing interests

H.W. is a cofounder of Ventus Therapeutics. The other authors declare no competing interests.