PYROPTOSIS

Jack of all trades inhibits inflammation (in sober people)

Caspase-cleaved gasdermin D forms pores in cellular membranes, thus executing proinflammatory cell death by pyroptosis. Disulfiram — a drug used to treat chronic alcoholism — is now found to be an inhibitor of pore formation, which may therapeutically counteract exacerbated inflammation in sepsis and beyond.

Florian I. Schmidt and Eicke Latz

Pathogenic microbes or sterile cell damage can trigger the assembly of cytosolic inflammasomes, typically nucleated by one of several known inflammasome sensors or receptors, in specialized sentinel cells. Inflammasomes coordinate the activation of proinflammatory caspases, which cleave proinflammatory cytokines such as interleukin (IL)-1β and IL-18, as well as the effector molecule gasdermin D (GSDMD). Proteolytic processing allows the N terminus of GSDMD (GSDMD-N) to oligomerize and to insert into cellular membranes, forming pores that ultimately kill the cell and facilitate the release of the activated proinflammatory cytokines. These events trigger a multitude of desired antimicrobial processes, but they can also result in pathological inflammatory conditions. Using a compound library of more than 3,750 molecules along with quantification of GSDMD-mediated liposome leakage, Hu et al. identify disulfiram as a potent inhibitor of GSDMD pore formation, with the potential to inhibit sepsis in mouse models[1].

The long sought-after effector of inflammasome-mediated cell death, GSDMD, was finally identified in 2015, leading to the surprising identification of an entire family of pore-forming proteins[2,3]. These cell death executioner proteins share a homologous N terminus that is rendered inactive by a C-terminal regulatory domain. The regulatory domains of GSDMD and GSDME are lost after cleavage by proinflammatory and proapoptotic caspases, respectively[4], while GSDMB is processed by granzyme A[5]. The activation mechanisms as well as the physiological roles of the other family members remain elusive.

Cleavage of GSDMD by caspase-1, as well as by caspase-4/5 in humans or caspase-11 in mice, triggers a furious type of cell death described as pyroptosis. Pore formation is followed by water influx and the loss of membrane potential, swelling of cells and, ultimately, the disruption of cellular membranes. This results in the release of cytosolic content, including molecules that are larger than the diameter of GSDMD pores. While typically tightly controlled, aberrant inflammasome activation is linked to an increasing number of pathological conditions, including atherosclerosis, gout, diabetes, Alzheimer's disease and sepsis. Inhibition of the two major downstream proinflammatory cytokines, IL-1β and IL-18, is a successful treatment strategy for many diseases[6].

Yet animal models of lethal inflammation mediated by gain-of-function mutants of NLRP3 demonstrate cytokine-independent immunopathology, suggesting that GSDMD-mediated cell death can also directly contribute to disease manifestation[7]. Consistent with this, GSDMD was recently shown to be critical for antimicrobial cell death by NET (neutrophil extracellular traps)-osis[8]. Overall, the universal role of GSDMD in pyroptosis and other cell death pathways makes GSDMD a desirable drug target.

Structures of full-length gasdermins and of pores formed by the N terminus of murine GSDMA3 (GSDMA3-N) indicate that the gasdermin N terminus undergoes substantial conformational rearrangements after cleavage, including the conversion of α-helices into β-sheets that ultimately line the pore formed by oligomers of around 27 gasdermin N-terminal domains[9,10]. It remains unclear whether gasdermins oligomerize in a prepore formation at the membrane before all monomers insert into the lipid bilayer in a concerted fashion, as indicated by an interesting intermediate protein assembly observed in an earlier study[9], or whether monomeric N termini insert into membranes one by one to assemble into growing arcs or pores, as

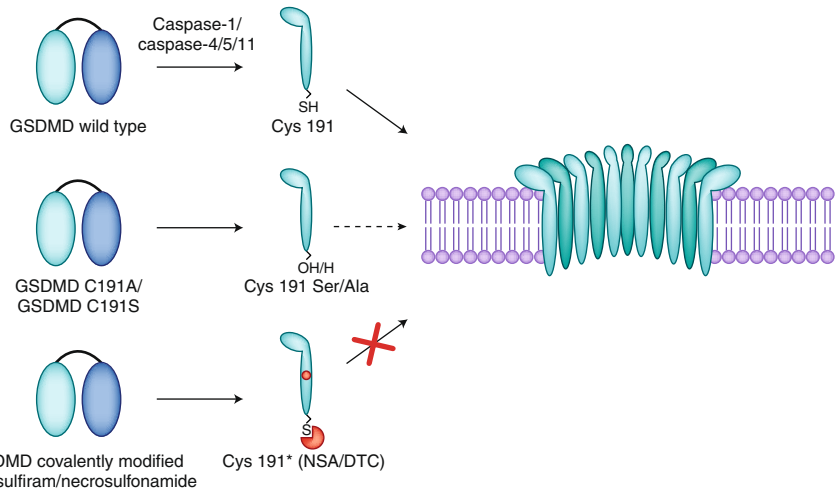

Fig. 1 | Mechanism of GSDMD inhibition. GSDMD pore formation, cell death by pyroptosis and sepsis are inhibited by modification of cysteine 191 (Cys 191*) with thiol-reactive disulfiram, leading to a dithiodiethylcarbamoyl (DTC) adduct. Likewise, necrosulfonamide (NSA) adducts at the same residue also inhibit GSDMD pore formation. Modification of other cysteine residues (symbolized by the red circle) cannot be ruled out in the applied experimental settings. Cysteine 191 is not conserved in other gasdermin family members, and its mutation to serine or alanine has only partial effects on the activity of GSDMD. This suggests that the bulky adducts, rather than the loss of reactive cysteines, may be responsible for the observed effect of disulfiram.

observed by atomic force microscopy studies[11]. Better tools to interfere with GSDMD function may thus also aid in completing our mechanistic understanding of GSDMD pore formation in the future.

In a biochemical screening setup, Hu et al. reconstituted caspase-11-induced GSDMD pore formation in artificial vesicles (liposomes) filled with Tb^{3+}. Tb^{3+} released from liposomes through GSDMD pores forms fluorescent complexes with the chelator dipicolinic acid, which was used as a readout for a screen of 3,752 compounds. These efforts led to the identification of disulfiram as an inhibitor of GSDMD. Disulfiram is an approved drug for the treatment of alcohol addiction, as it inhibits aldehyde dehydrogenase, leading to the accumulation of acetaldehyde after alcohol consumption, resulting in dose-dependent general malaise. Further analysis showed that disulfiram-derived dithiodiethylcarbamoyl (DTC) covalently modified cysteine 191 of human GSDMD (Fig. 1). This proposed mechanism of action is in line with the known thiol-reactive behavior of disulfiram, which also covalently inhibits caspases, aldehyde dehydrogenase in the catabolism of ethanol, and NPL4 in stress response pathways relevant to cancer[12]. Stabilization of reactive DTC with Cu^{2+} cations improved the inhibitory potential of the drug. At the chosen concentrations, disulfiram in tissue culture did not inhibit caspase-1 processing, GSDMD cleavage or necroptosis.

Solvent-exposed cysteine residues of GSDMD, including cysteine 191 that is modified by disulfiram, have already been recognized as essential for pore formation. Indeed, the same cysteine can be modified by necrosulfonamide (NSA), which also inhibits pyroptosis and oligomerization of GSDMD[13]. Disulfide-bonded dimers and oligomers of GSDMD-N had previously been resolved by non-reducing SDS-PAGE and were thus used as readouts for GSDMD-N oligomerization[14]. This led to the speculation that disulfide bond formation may be an intricate part of pore assembly. Yet predicted structural models of the GSDMD-N pore based on the GSDMA3-N pore do not indicate any cysteine residues suitably positioned for intermolecular (or intramolecular) disulfide bonds[10]. It therefore remains unclear whether GSDMD pores or their intermediates contain genuine disulfide bonds. Artificial disulfide bond formation during lysis or denaturation had initially been ruled out by alkylating free cysteines before lysis[14], but the observed disulfide-bonded species of GSDMD vary with experimental conditions. Cysteine 191 is not conserved in other gasdermin family members, and its alkylation by iodoacetamide or its mutation to alanine had either no effect or a less pronounced effect on its activity[13]. The leucine residue in the corresponding position of GSDMA3-N forms part of the tip of the β-sheet structure that inserts into the membrane for pore formation. Hence, it is possible that modification of cysteine 191 with bulkier adducts interferes with membrane insertion and thus pore opening of GSDMD. Future experiments will have to determine whether the thiol group of cysteine 191 — predicted to be the most reactive cysteine of the molecule — plays a functional role in pore formation or whether this is a serendipitous vulnerability of GSDMD that can be exploited for potential therapeutic intervention. The approach used by Hu et al. involved reconstituting GSDMD pores in nanodiscs. This method is typically used to gain structural insights into a protein in the context of its surrounding lipids, raising the hope that further structural studies of GSDMD-N pores by the involved laboratories are forthcoming.

Due to its reactive thiol group, disulfiram is known to inhibit several cysteine-containing enzymes[12]. Hence, it is crucial to demonstrate specificity for inhibiting a particular pathway at the chosen concentration. In tissue culture, the authors could show that disulfiram does not inhibit caspase-1 or necroptosis at the tested levels. In mouse models of sepsis, the applied amount of 50 mg kg^{-1} disulfiram could reduce or inhibit the lethality of sepsis, depending on the dose of LPS. In combination with copper gluconate, disulfiram had even partially protective effects when administered after the onset of sepsis, the more realistic time window for treatment of patients with sepsis. When allometrically scaled to the body surface, the applied dose corresponds to 284 mg per day in humans, that is, 4.7 mg kg^{-1} in a 60 kg human. This is within the dose range of 250–500 mg disulfiram per day that is used to treat alcoholism[15].

The benefit of disulfiram as a new treatment option for GSDMD-mediated immunopathology is that the drug has been in clinical use to treat alcohol addiction for more than 30 years. Recent trials have shown combination therapy with copper can be used to treat certain types of cancer. Despite its potentially broad activity against cysteine-containing proteins, the drug seems to be well tolerated if no alcohol has been consumed. It will be interesting to test whether the mutation of cysteine 192 (corresponding to human cysteine 191) to serine in murine GSDMD, which allows partial pore formation and pyroptosis in vitro, still facilitates LPS-mediated sepsis in vivo. As this activity is expected to be insensitive to thiol-reactive compounds, it will be possible to test the on-target activity of disulfiram in mouse models using this mutant. Reassuringly, Hu et al. could show that drug treatment did not alter inflammasome-independent inflammatory responses and priming. Beneficial effects were also observed in caspase-1 knockout mice, suggesting that the observed effects are not caused by inhibition of canonical inflammasomes (the mouse model of sepsis relies on caspase-11 and GSDMD). Given that many inflammasome sensors are tasked with the detection of cell invasion by pathogens, it is conceivable that blockage of pyroptosis could increase the risk for microbial infections. While no such side effects are described, this aspect should be monitored in the case of long-term treatment of chronic inflammatory diseases.

In summary, Hu et al. have conducted an elegant biochemical screen to identify a potential, clinically approved candidate for the treatment of GSDMD-mediated pathology, including chronic inflammatory diseases (in combination with abstinence). ❐

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Competing interests

E.L. is a cofounder of IFM Therapeutics. F.I.S. is a scientific consultant of IFM Therapeutics.