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Gasdermin D pores hitch a ride: extracellular vesicles spread pyroptosis

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Abstract

Pyroptosis is a highly immunogenic cell death due to the release of damage-associated molecular patterns and pro-inflammatory cytokines such as IL-1β and IL-18. A recent study published in *Cell* by Wright and colleagues uncovered a novel mechanism in which extracellular vesicles released from pyroptotic cells serve as carriers of functional gasdermin D pores to propagate pyroptosis to bystander cells, providing valuable insights into the process of bystander cell death and opening up potential therapeutic avenues.

Pyroptosis is a unique and highly inflammatory form of programmed lytic cell death that not only plays a crucial role in host defense and immune regulation but also contributes to pathological conditions such as autoimmune diseases, inflammation and cancer.[1-5] Unlike apoptosis, which is immunologically silent, pyroptosis leads to the release of damage-associated molecular patterns (DAMPs), cytokines and/or other inflammatory mediators to alert the immune system. These signals recruit immune cells and activate the immune system, and when not properly shut down, contribute to disease initiation and progression. Pyroptosis is primarily mediated by the inflammasome, a multiprotein complex that senses pathogenic or cellular danger signals, leading to the activation of caspase-1 or other inflammatory caspases.[5,6] Caspase-1 then cleaves gasdermin D (GSDMD) into an N-terminal domain (GSDMD-NT) and a C-terminal domain. GSDMD-NT binds acidic lipids, oligomerizes and inserts into the plasma membrane, forming pores that disrupt cellular integrity and inducing pyroptosis.[3,4] Notably, GSDMD undergoes S-palmitoylation — a crucial post-translational modification regulating its membrane localization, activation and function.[7]

While the mechanisms governing pyroptosis within individual cells are relatively well understood, less is known about whether and how pyroptosis spreads between cells and in tissues. Traditional views suggest that pyroptosis is a cell-autonomous process that remains confined to cells in which inflammasomes are activated. However, a new study by Wright

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COMPETING INTERESTS

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and colleagues[8] has now demonstrated that pyroptosis can propagate from pyroptotic cells to bystander cells of different types in vitro and in vivo via extracellular vesicles (EVs), significantly expanding our understanding of inflammatory cell death and its consequences (Fig. 1).

The authors began their investigation by coculturing wild-type (WT) mouse bone marrow-derived macrophages (BMDMs) with *Nlrp3*$^{-/-}$ BMDMs. They demonstrated that nigericin-stimulated and inflammasome-activated WT pyroptotic cells could induce cell death in neighboring *Nlrp3*$^{-/-}$ BMDMs that are resistant to inflammasome activation. Further investigation using an inflammasome-independent system with immortalized *Gsdmd*$^{-/-}$ BMDMs expressing inducible GSDMD-NT (iBMDM-iGSDMD-NT) demonstrated that Dox-induced pyroptosis in these cells caused bystander death of cocultured WT BMDMs. Time-lapse microscopy and imaging flow cytometry showed that upon Dox induction, iBMDM-iGSDMD-NT cells and cocultured WT BMDMs sequentially became positive for DRAQ7 — a fluorescent DNA dye that marks lytic cell death — confirming the propagation of pyroptosis to bystander cells.

In an *E. coli* infection model in vivo, WT mice infected intraperitoneally (i.p.) with Alexa Fluor 647-LPS-labeled *E. coli* exhibited bystander cell death in uninfected (AF647-LPS^{-}) peritoneal cells. Additionally, transferring membrane dye CellBrite488-labeled pyroptotic macrophages into the peritoneal cavity of WT mice resulted in death of bystander cells. Similarly, transferring FITC-LPS-electroporated BMDMs into *Gsdmd*$^{-/-}$ mice induced pyroptotic death in bystander cells. Thus, pyroptosis can spread to other cells in vivo.

The authors then investigated whether pyroptosis spreads in a contact-dependent or -independent manner. In transwell experiments, pyroptotic iBMDM-iGSDMD-NT cells in the upper chamber induced pyroptosis in bystander BMDMs in the lower chamber, confirming that pyroptosis could spread without direct contact. Supernatants from pyroptotic cells were also able to induce death in naive BMDMs and RAW macrophages, indicating that a secreted factor was responsible for the spread of pyroptosis. Using size exclusion chromatography, the authors identified a fraction of pyroptotic supernatants that contain EVs and strongly induced cell death. Electron microscopy confirmed the presence of GSDMD pores on these EVs released by pyroptotic cells. Pyroptotic EVs were shown to kill WT BMDMs, HeLa, and HEK293T cells. EV depletion experiments revealed that removing EVs abolished the lytic activity of pyroptotic supernatants, establishing that EVs were necessary and sufficient to mediate the death of bystander cells.

Finally, the role of pyroptotic EVs in mediating bystander pyroptosis and inflammation was confirmed in vivo. Intraperitoneal injection of pyroptotic EVs into *Gsdmd*$^{-/-}$ mice resulted in death of peritoneal cells with elevated plasma LDH levels and tissue damage, supporting the in vivo relevance of EV-mediated bystander pyroptosis. Additionally, *Gsdmd*$^{-/-}$ mice primed with low-dose LPS exhibited exacerbated inflammation when injected with pyroptotic EVs relative to PBS treatment, highlighting the inflammatory potential of pyroptotic EVs in vivo. EVs carrying active GSDMD pores propagate pyroptosis by transferring functional GSDMD pores to bystander cell membranes and inducing cell death.

EVs, including exosomes and microvesicles, are critical mediators of intercellular communication by transporting proteins, lipids, and nucleic acids between cells.[9] This study revealed that EVs from pyroptotic cells carry cleaved, pore-forming GSDMD, amplifying inflammatory signaling and propagating cell death. Notably, these EVs can travel significant distances in tissue cultures and in vivo, suggesting that pyroptosis extends beyond immediate neighbors to distant microenvironments. This finding provides key insights into how localized inflammation escalates into systemic damage, with implications for autoimmune diseases and cancer. Targeting EV-mediated pyroptosis may offer novel therapeutic strategies to control inflammation.

While this study provides strong evidence for EV-mediated pyroptosis propagation, several key questions remain. The precise molecular mechanisms governing EV fusion and GSDMD activation in recipient cells require further elucidation. Although GSDMD pores were identified as essential components, the study did not fully clarify how EVs are transplanted to recipient cells and whether it is by specific membrane fusion. Additionally, the potential contributions of other bioactive molecules within pyroptotic EVs — such as inflammatory cytokines, lipids, or RNA — remain unexplored. Since recent studies have shown that NINJ1 oligomers can be released into the culture supernatant[10] and that GSDMD pore-containing EVs likely also contain NINJ1, it may be interesting to ask whether NINJ1 also plays a role in EV-mediated propagation of pyroptosis. Moreover, Dox-induced GSDMD-FL expression also caused significant cell death shown by multiple assays, indicating that iGSDMD-FL may undergo palmitoylation and pore formation as we showed previously.[7] Further investigation is needed to determine whether palmitoylated GSDMD-FL pores can also facilitate the spread of pyroptosis to bystander cells.

The physiological and pathological relevance of EV-mediated pyroptosis in humans remains unclear, as most findings stem from in vitro and murine models. Future studies using patient-derived samples or organoid models are needed to assess its role in human diseases like sepsis, autoimmunity, and cancer. Additionally, the impact of EV-mediated pyroptosis on immune cell dynamics and tissue microenvironments needs further investigation, particularly in determining whether this process exacerbates or regulates inflammation over time.

In conclusion, the study by Wright et al. unveils a paradigm-shifting mechanism in cell death and inflammation,[8] showing that EVs propagate pyroptosis, amplifying inflammation and tissue damage. This discovery has profound implications for inflammatory diseases, suggesting that targeting EV-mediated GSDMD-NT transfer could offer novel therapeutic strategies. As research advances, these findings will inspire further exploration of the complex and dynamic interplay between cell death, inflammation, and disease.

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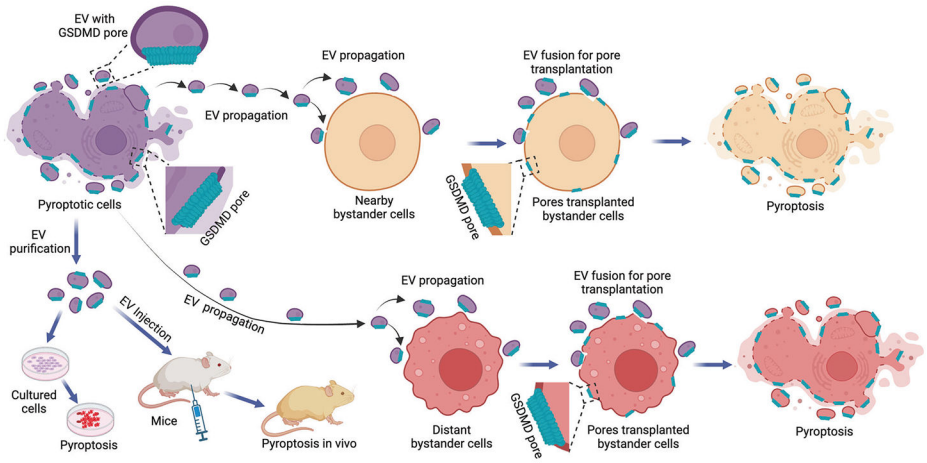

Fig. 1. EVs shuttle GSDMD pores and drive pyroptosis to bystander cells.

Pyroptotic cells initially form GSDMD pores and release EVs loaded with these pores. These EVs disseminate to both nearby and distant bystander cells, where they fuse with the plasma membrane, delivering GSDMD pores. This transfer triggers pyroptosis and DAMP release in recipient cells in vitro and in mice.