

Pannexin-1 channels bridge apoptosis to NLRP3 inflammasome activation.

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Abstract

Apoptosis can promote inflammation by triggering activation of the NLRP3 inflammasome (NLR family, pyrin domain containing 3). However, the molecular mechanisms regulating these processes are ill-defined. We recently reported that pannexin-1 is required to promote NLRP3 inflammasome assembly. We further demonstrate that differential cleavage of gasdermin D (GSDMD) by apoptotic caspases regulate inflammatory cell lysis. Here, we discuss our findings, and perspectives for future studies.

Introduction

Apoptosis is a form of caspase-dependent cell death that is traditionally classified as immunologically silent. In support of this, emerging studies indicate that apoptotic caspases cleave a number of cellular proteins to inactivate innate immune signalling pathways and to promote the removal of dying cells. For example, apoptotic caspase-3 and -7 cleave the plasma membrane glycoprotein, pannexin-1, to release 'find-me' and 'eat-me' signals to enhance clearance of apoptotic cells¹. Despite these studies, exposure of innate immune cells to 'classical apoptosis activators' such as staurosporine, cycloheximide, UV treatment, FasL, SMAC mimetics and BH3 mimetics were all reported to promote inflammation by activating the NLRP3 inflammasome (NLR family, pyrin domain containing 3)^{2,3}. NLRP3 assembly activates the cysteine protease caspase-1, which processes pro-interleukin (IL)-1 β and pro-IL-18 into their mature active forms, and also cleaves the pore-forming protein gasdermin D (GSDMD) to drive a form of lytic cell death known as pyroptosis⁴. However, the molecular mechanisms by which apoptosis triggers NLRP3 assembly are ill-defined, and whether the apoptotic caspase cascade suppresses GSDMD-dependent cell lysis has not been explored in detail.

Study

Recent studies from the Alnemri and Bachovchin laboratories demonstrated that caspase-3, and to a lesser extent caspase-7, cleave human GSDMD at position D87 (D88 in mouse) in the pore-forming domain, thereby inactivating its lytic function^{5,6}. However, under which conditions GSDMD needs to get inactivated by apoptotic effector caspases had not been examined. We speculated that it might be a mechanism to restrict the NLRP3-dependent cell lysis and cytokine release during apoptosis. Indeed, we found that induction of extrinsic apoptosis in macrophages resulted in cell lysis that was in part GSDMD-dependent (pyroptosis) and in part driven by caspase-3/7 (secondary necrosis). To study the effects of caspase-3/7-driven inactivation of GSDMD, we next engineered a *Gsdmd*^{D88A} knock-in mouse, in which we replaced aspartate at position 88 of GSDMD to alanine. Confirming previous findings, we observed that murine *Gsdmd*^{D88A} cells resist caspase-3/7 cleavage.

Consistent with our hypothesis, *Gsdma*^{D88A} knock-in macrophages, where the pore-forming domain of GSDMD could not be inactivated, were indeed more susceptible to cell lysis upon extrinsic apoptosis⁷.

While investigating GSDMD processing during extrinsic apoptosis, we made the surprising observation that GSDMD processing is largely intact in *Casp-1/11*- and *Nlrp3*-deficient cells. Unexpectedly, we found that caspase-8 directly cleaves GSDMD, albeit less efficiently than caspase-1, to induce cell lysis during apoptosis, consistent with an earlier report⁸. In the non-canonical inflammasome pathway, caspase-11-driven GSDMD pores promote potassium efflux and NLRP3 assembly⁴. By contrast and in disagreement with Orning et al.⁸, we found no evidence that caspase-8-dependent GSDMD cleavage promotes NLRP3 activation. Since caspase-1 and caspase-3 activation were blunted in cells lacking receptor-interacting serine-threonine kinase 3 during apoptosis, and a recent study reported that caspase-3 and -7 drive NLRP3 inflammasome assembly following mitochondrial apoptosis⁹, we investigated whether any known caspase-3/7 substrates promote NLRP3 activation during apoptosis. In doing so, we identified pannexin-1, a channel-forming glycoprotein, as a universal driver of NLRP3 assembly during both extrinsic and intrinsic apoptosis⁷.

In conclusion, our study demonstrates the complex regulation of GSDMD by apoptotic caspases during apoptosis, and reveals pannexin-1 as the bridge between apoptosis and NLRP3 inflammasome activation.

Perspectives

Apoptosis was long regarded as an immunologically silent form of cell death, and activation of the apoptotic caspase cascade was thought to universally shut down proinflammatory signalling pathways. However, administration of tumor cells expressing Fas ligand was observed to drive IL-1 β maturation and neutrophil recruitment in mice more than 20 years ago¹⁰; and more recently, various studies reported that exposure of innate immune cells to pro-apoptotic factors triggers cell lysis and activate the NLRP3 inflammasome³. These studies indicate that the apoptotic caspase cascade perform has a dual function, either promoting or

suppressing inflammation. Our study agrees with this emerging concept and demonstrates that caspase-8 and -3 have both pro- and anti-inflammatory functions. While caspase-8 was initially classified as an apoptotic caspase, an increasing number of studies document that caspase-8 promotes inflammation, for example by driving TLR-induced cytokine production or directly maturing IL-1 β ^{2,3}. Our study adds to this emerging literature, and demonstrates that caspase-8 cleaves GSDMD to trigger cell lysis during extrinsic apoptosis. In agreement with a dual function of caspase-3/7 during apoptosis, our study revealed that caspase-3/7-dependent cleavage and inactivation of GSDMD at position aspartate 88 suppresses lytic cell death during extrinsic apoptosis; by contrast, caspase-3/7 also promote pannexin-1 activation and potassium efflux, presumably by cleaving-off the C-terminal region¹, and thus drive NLRP3 inflammasome activation during apoptosis. Since GSDMD is rapidly inactivated by caspase-3/7, and pro-IL-1 β and NLRP3 are not constitutively expressed, we assume that caspase-8-dependent GSDMD activation and pannexin-1-driven NLRP3 assembly are unlikely to drive inflammation during tissue homeostasis (Figure 1). Instead, these pathways are likely activated during specific pathogen challenge, where caspase-8 is strongly activated, and when NLRP3 and pro-IL-1 β expression are induced, as reported during *Yersinia* infection⁸. Future studies should establish the impact of caspase-3/7 and caspase-8-dependent GSDMD cleavage during host defence *in vivo*. Nevertheless, modulating pannexin-1 activity could impact the outcome of SMAC mimetic or BH3 mimetic-based cancer chemotherapy.

An unexpected result from our study is that during extrinsic apoptosis neither GSDMD nor Gasdermin E (GSDME) are required for potassium efflux-induced NLRP3 activation, although both are activated, by caspase-8 or caspase-3 respectively, and form membrane pores under these conditions. This may in part, be driven by the endosomal sorting complexes required for transport (ESCRT)-dependent repair, which removes damaged membrane areas and could act as an additional mechanism to suppress cell lysis during apoptosis. Future studies should investigate if ESCRT-dependent membrane repair suppresses NLRP3 activation during apoptosis.

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FIGURE LEGEND:

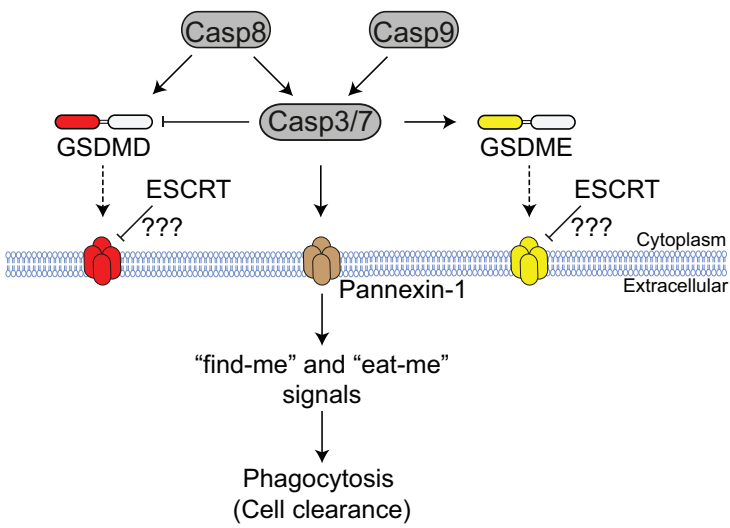
Figure 1. Immunologically silent versus inflammatory apoptosis.

(A) During apoptosis, active caspase-3 and -7 cleave and activate the plasma membrane glycoprotein pannexin-1 to enhance clearance of apoptotic cells. Caspase-8 can directly cleave Gasdermin D (GSDMD) to induce cell lysis, but this is counteracted by caspase-3/7. Caspase-3 processes Gasdermin E (GSDME) and liberates its pore-forming domain, but this does not drive cell lysis. Whether endosomal sorting complexes required for transport (ESCRT)-mediated membrane repair suppresses GSDMD or GSDME-driven pore formation during apoptosis is unknown.

(B) Infection with certain pathogens or exposure to chemotherapeutic drugs may trigger enhanced caspase-8 activation, and induce the expression of NLRP3 (NLR family, pyrin domain containing 3) and pro-interleukin (IL)-1 β . This promotes caspase-8-driven GSDMD activation and resultant cell lysis. Furthermore, pannexin-1 activation promotes NLRP3 inflammasome assembly via potassium efflux, driving IL-1 β and GSDMD processing by caspase-1.

Casp8, caspase-8; Casp9, caspase-9; Casp1, caspase-1, Casp3, caspase-3;Casp7, caspase-7

A Homeostasis
(Immunologically silent)



B Infection or chemotherapy-driven apoptosis
(Inflammatory)

