Cellular plasma membrane rupture is an active process

Plasma membrane rupture during different types of cell death was long assumed to be a passive process, induced by osmotic effects. New findings show that this is however an active process mediated by a specific membrane protein.

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Eukaryotic cells possess remarkable control mechanisms to retain functionality during their life cycle and to ensure their proper removal in the multicellular context. Among these mechanisms are multiple forms of cell death, most of which end with the rupture of the plasma cell membrane. So far, rupture of this membrane was generally thought to be a consequence of osmotic pressure, caused by uncontrolled influx of water. On page xxx in this issue of *Nature*, Kayagaki et *al.*[1] report that plasma membrane rupture is however an active process, mediated by the protein ninjurin-1. This protein exerts its effect as the final step in multiple different types of cell death.

In multicellular organisms, cells die in two possible ways. Either passively as a consequence of damage occurred to them, or in a well-regulated manner as part of a development or homeostasis program, and to eliminate malignant and infected cells[2]. The defining feature of passive cell death, which is also known as necrosis, has long thought to be the loss of plasma membrane integrity, which distinguishes it from programmed cell death. As a consequence, necrosis resembles an explosion of the cell that releases a plethora of intracellular molecules, including proteins, nucleic acids and metabolites. These act as danger signals and inform neighboring cells of the injury and induce inflammation. By contrast, apoptosis, the best studied form of programmed cell death, preserves membrane integrity to allow for an immunologically silent, non-inflammatory removal of the dead cell and tissue repair.

Over the last two decades multiple forms of programmed cell death were discovered[1] – such as pyroptosis, necroptosis and ferroptosis – which combine programmed death with passive cell lysis. Common to most of these is the formation of large protein pores in the plasma membrane[4]. These are for example the large gasdermin pores that initiate pyroptosis[5], or the MLKL channel that is assembled in necroptotic cells[6]. Formation of either of these pores is followed by osmotic swelling and finally, by plasma membrane rupture.

The mechanism underlying plasma membrane rupture has always been thought to be a passive osmotic lysis event, but now Kayagaki *et al.*, reveal that it is actually an actively regulated process. Using a forward genetic screen for pyroptosis mediators in ENU-mutagenized mice, they identified ninjurin-1 as a factor required for plasma membrane rupture. Thus far, the 16-kDa ninjurin-1 was thought to function as an adhesion factor with no direct ties to cell death[7,8], but deletion of ninjurin-1 in macrophages completely prevented membrane rupture after pyroptosis induction as measured by LDH release. Deletion also abrogated the release of other large cellular proteins such as HMGB1 into the extracellular medium, but not the secretion of IL-1 family cytokines, which are small enough to pass through the gasdermin-D pore. Ninjurin-1-deletion had also a striking effect on macrophage morphology. During pyroptosis, wild-type cells swell and form ballooning membrane protrusions, that eventually rupture[9]. *Ninj1*-deficent cells retained these balloon-like protrusions, but these never burst. The rupture is thus clearly not caused by osmosis, but by specific events that involve the protein ninjurin-1. Moreover, the data provide evidence that gasdermin-pore-induced cytokine release and cell swelling can be genetically separated from plasma membrane rupture.

While this finding alone would have already been a spectacular twist to a long-studied phenomenon, the big surprise came when studying the role of Ninjurin-1 in other cell death forms. It turned out that Ninjurin-1 not only mediated membrane rupture during pyroptosis, but also after toxin-induced permeabilization, during secondary necrosis of apoptotic cells, and upon necroptosis induction. Ninj-1 is thus a common denominator that ruptures the membrane as a final step of different cell death pathways.

But how might a small 16-kDa protein mediate such striking effects? Ninjurin-1 is a ubiquitously expressed plasma membrane protein[8], and conserved from fruit fly to man. The protein features two predicted transmembrane helices and a conserved extracellular helix that is needed for its proper function. If this helix senses a signal or serves to disrupt the membrane requires additional studies, but it appears to have an amphiphilic character similar to helices found in other membrane-penetrating proteins such as melittin, colicin or Bax.

Importantly, the reported findings will have a transformatory role on cell biology that goes beyond the function of ninjurin-1 as a common executor of death-associated lysis. The study underscores the enormous strength and resilience of the intact plasma membrane as such, and it reduces the number of non-specific events in cell biology, highlighting how stringently organisms control the fate of their cells until the very last moment. Remaining questions include what activating signal or property is sensed by ninjurin-1, and how cells prevent its accidental activation. Does ninjurin-1 require a co-factor, do other similar proteins exist, and of course what is the structure of the membrane-rupturing entity that is presumably formed by ninjurin-1? Solving these questions could lead the way to new therapeutic strategies

aimed at inhibiting ninjurin-1 or its homologs to convert necrotic death to a less inflammatory outcome.

References

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Figure legends

Figure 1. Ninjurin-1 mediates plasma membrane rupture as an endpoint to different cell death pathways. Plasma membrane rupture and necrosis is a common outcome of programmed cell death or toxin treatment. Kayagaki et *al.* [1] report that plasma membrane rupture does not happen passively as consequence of oncotic swelling, but that it is mediated by the plasma membrane protein Ninjurin-1. Execution of pyroptosis and necroptosis involves the formation of gasdermin pores or MLKL channels, respectively. These pores disrupt the electrochemical gradient and lead to oncotic swelling of the cell. Swelling is also observed after treatment with pore-forming toxins, or upon apoptosis induction, which can result in inactivation of the Na^+/K^+ ATPase. Cell swelling manifest in the formation of membrane balloons/bubbles that eventually rupture to release cytosolic content that acts as DAMPs (damage-associated molecular patterns). Plasma membrane rupture is an active process and involves he oligomerization of ninjurin-1. It remains unclear what activates ninjurin-1, but it is likely a common cellular event, such as increase cell volume.