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REVIEW ARTICLE

Phosphoinositides: multipurpose cellular lipids with emerging roles in cell death

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Abstract

Phosphorylated phosphatidylinositol lipids, or phosphoinositides, critically regulate diverse cellular processes, including signalling transduction, cytoskeletal reorganisation, membrane dynamics and cellular trafficking. However, phosphoinositides have been inadequately investigated in the context of cell death, where they are mainly regarded as signalling secondary messengers. However, recent studies have begun to highlight the importance of phosphoinositides in facilitating cell death execution. Here, we cover the latest phosphoinositide research with a particular focus on phosphoinositides in the mechanisms of cell death. This progress article also raises key questions regarding the poorly defined role of phosphoinositides, particularly during membrane-associated events in cell death such as apoptosis and secondary necrosis. The review then further discusses important future directions for the phosphoinositide field, including therapeutically targeting phosphoinositides to modulate cell death.

Facts

- Phosphoinositides are spatiotemporally enriched membrane lipids, and their turnover is dynamically, yet tightly, regulated.
- Phosphoinositides were initially characterised as second messengers for major signalling pathways.
- Phosphoinositides were later found to act as docking lipids to recruit and modulate actin cytoskeleton remodelling and membrane dynamics.
- Involvement of phosphoinositides in cell death had been poorly defined, often overshadowed by their traditional role as second messengers and regulators of cytoskeleton-membrane interactions.
- Recent reports support emerging importance of phosphoinositides in pyroptosis, necroptosis, host defense peptide-induced necrosis, NETosis and autophagic cell death.

Open questions

- Considering the importance of phosphoinositides in membrane and cytoskeleton dynamics, what are their roles in morphological changes during cell death, such as apoptosis?
- Do other lytic forms of cell death, including secondary necrosis, require phosphoinositides and what are the phosphoinositide-binding effectors?
- Can we target phosphoinositides to modulate cell death execution for therapeutic development?

Introduction

Phosphoinositides are derived from phosphatidylinositol (PI), comprising a diacylglycerol moiety linked to a D-myo-inositol ring via a phosphodiester linkage (Fig 1a). The inositol hydroxyls at positions 3, 4 or 5 are reversibly conjugated with phosphate groups, resulting in monophosphorylated [PI(3)P, PI(4)P and PI(5)P], bisphosphorylated [$PI(3,4)P_2$), $PI(3,5)P_2$ and $PI(4,5)P_2$] and trisphosphorylated [$PI(3,4,5)P_3$] derivatives. These seven interconvertible

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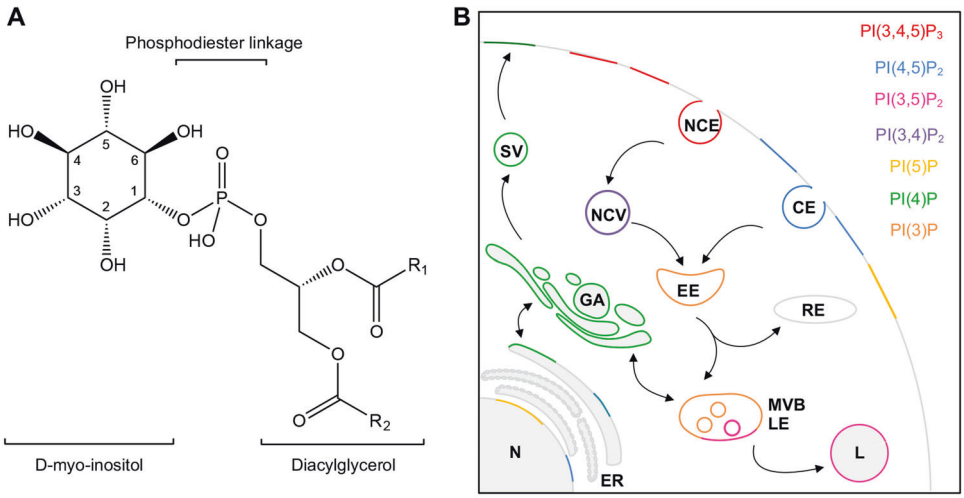

Fig. 1 Structure and cellular distribution of phosphoinositides. **a** Chemical structure of phosphatidylinositol, comprising phosphodiester-linked diacylglycerol and D-myo-inositol moieties. R1 and R2 represent any acyl (fatty acid) chain. Hydroxyl at positions 3, 4 and 5 of D-myo-inositol ring are readily phosphorylated, giving rise to seven phosphoinositides. **b** Localisation of phosphoinositides on plasma and organelle membrane. This distribution map is exemplary, only showing the cellular location where a particular phosphoinositide species is prominently found. CE clathrin-dependent endocytosis, NCE clathrin-independent endocytosis, NCV non-clathrin endocytic vesicles, EE early endosomes, RE recycling endosomes, MVB multivesicular bodies, LE late endosomes, L lysosome, ER endoplasmic reticulum, N nucleus, GA Golgi apparatus, SV secretory vesicles. Black arrow (→) indicates the progression of membrane trafficking pathway

phosphoinositide species are distinctly distributed at the cytoplasmic side of the plasma and subcellular organelle membranes where they dynamically participate in various distinct cellular processes (Fig. 1b). PI(4,5)P_2 and PI(3,4,5)P_3 are predominantly enriched at the inner leaflet, particularly accumulated at sites of active membrane-associated activities [1]. PI(4)P is located along Golgi-endosomal trafficking axis, on exocytic vesicles and also at the plasma membrane [2]. In contrast, 3-phosphorylated phosphoinositides are primarily associated with endosomal pathways: PI(3)P on the limiting membrane of early endosomes and intraluminal vesicles contained within multivescular bodies, PI(3,4)P_2 on non-clathrin endocytic vesicles, and PI(3,5)P_2 at late endolysosomal membranes [3, 4]. The precise location of PI(5)P pool(s) has not been comprehensively defined, although low levels of PI(5)P have been found in the nucleus, the plasma membrane, Golgi complex and sarco/endoplasmic reticulum [5, 6]. Under certain physiological and pathological circumstances, phosphoinositides can be synthesised at locations other than those aforementioned. For example, PI(4,5)P_2 has also been observed at sites of membrane remodelling and ruffling, the nuclear mRNA splicing factor compartment (nuclear speckles), perinuclear vesicles, focal adhesion and epithelial cell-cell junctions [7–10].

Phosphoinositide turnover at specific compartmentalised distributions is dynamically, but tightly, regulated by specific kinases and antagonistic phosphatases which add or remove phosphates from the 3, 4 or 5 positions of the myo-inositol ring. This critically allows for the rapid generation of product phosphoinositides or removal of the precursor phosphoinositides [1, 4, 11]. Typically, these enzymes are catalytically selective for specific phosphoinositide species, and generally exist as different isoforms with non-overlapping localisation. For instance, PI(4,5)P_2 is synthesised chiefly by phosphorylation of PI(4)P by either of three PI(4)P 5-kinase (PIP5K) isoforms (α, β and γ) [7–10]. These PIP5Ks are mainly localised on the plasma membrane, although distinct additional subcellular distributions have also been observed. Together, spatially restricting and temporally governing the phosphoinositide-metabolising enzymes are critical for phosphoinositide metabolic turnover at dedicated membrane compartments and to a particular stimulation.

Despite their low abundance, phosphoinositides and their metabolism are crucial to the precise spatiotemporal regulation of key cellular events, where phosphoinositides can serve as second messengers or modulate recruitment and/or activity of membrane proteins [1, 12]. These include, but are not limited to, signal transduction, cytoskeleton reorganisation, membrane dynamics, membrane and vesicular trafficking (e.g. endocytosis, phagocytosis, macropinocytosis and exocytosis). Until recently, phosphoinositide involvement in the context of cell death was largely inferred from phosphoinositide-mediated cell signalling or based on their crucial role in regulating cytoskeleton and membrane remodelling.

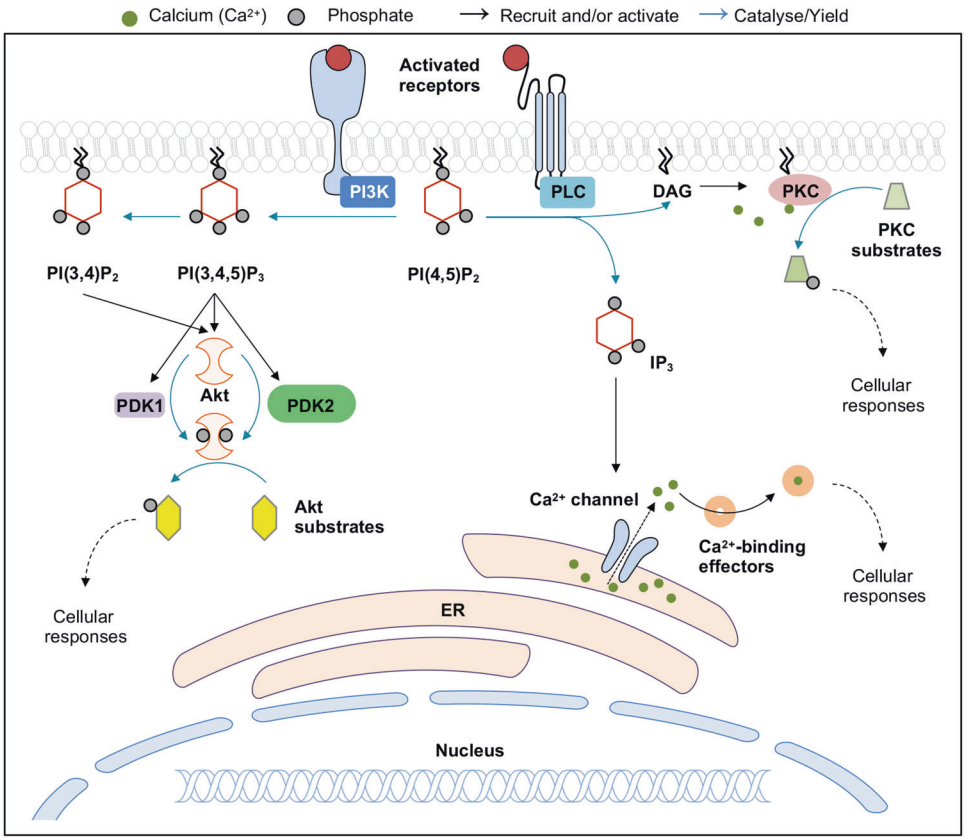

Fig. 2 Phosphoinositides as a second messengers. $PI(4,5)P_2$ mediates two major transmembrane signalling pathways: PLC-DAG-IP_3 and PI3K-Akt. Activated receptor recruits and induce class I PI3K to phosphorylate $PI(4,5)P_2$. The resultant $PI(3,4,5)P_3$ then recruits Akt and its activator kinases PDK1 and PDK2 (or mTORC2, ILK) can also to the plasma membrane, leading to complete phosphorylation of Akt. Alternatively, Akt can also be non-canonically recruited by $PI(3,4)P_2$. Subsequently, Akt regulates downstream effectors through its activated kinase activity. PLC, on the other hand, cleaves $PI(4,5)P_2$ into DAG and IP_3 upon stimulation. DAG remains at the plasma membrane to recruit and activate PKC to modulate its downstream effectors via its regulatory phosphorylation. IP_3 translocates to ER to excite calcium channels, causing calcium flux into cytoplasm, initiating calcium signalling via calcium binding effectors. PI3K phosphoinositide 3-kinases, PLC phosphoinositide-specific phospholipase C, DAG diacylglycerol, IP_3 inositol 1,4,5-trisphosphate, PKC protein kinase C, Akt protein kinase B, PDK1 phosphoinositide-dependent kinase 1, PDK2 phosphoinositide-independent kinase 2, ER endoplasmic reticulum, mTORC2 mechanistic target of rapamycin complex 2, ILK integrin-linked kinase (ILK)

Phosphoinositides in the context of cell death via classical activities

Second messengers in signal transduction

Phosphoinositides have been well-defined as second messengers of transmembrane signalling. $PI(4,5)P_2$, in particular, is an essential intermediate for two important pathways: phosphoinositide-specific phospholipase C (PLC)/diacylglycerol (DAG)/inositol-1,4,5-trisphosphate (IP_3) pathway and class I phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway (Fig. 2). Typically, stimulation of G-protein-coupled receptors and receptor tyrosine kinases leads to activation of PLC, that via its $PI(4,5)P_2$-binding Pleckstrin homology (PH) domain, hydrolyses $PI(4,5)P_2$ to generate DAG and IP_3 and thus initiating two-armed intracellular signalling cascade. DAG remains at the plasma membrane to facilitate activation and membrane localisation of protein kinase C (PKC) family members, whereas cytosolic IP_3 binds the IP_3 receptor and activates a ligand-gated calcium channel on smooth ER surface, triggering calcium release from intracellular storage [13–15]. In contrast, the PI3K/Akt signalling cascade canonically begins with the phosphorylation of $PI(4,5)P_2$ by PI3K to yield $PI(3,4,5)P_3$, an essential effector of Akt signalling [16]. The resultant $PI(3,4,5)P_3$ promotes translocation of phosphoinositide-dependent kinase 1 (PDK1) and Akt to the plasma membrane, leading to Akt phosphorylation of threonine-308 and its partial activation [16]. Complete Akt activation requires additional phosphorylation of serine-473, achievable by multiple proteins, including phosphoinositide-dependent kinase 2 (PDK2), mechanistic target of rapamycin complex (mTORC), integrin-linked kinase (ILK) or DNA-dependent protein kinase (DNA-PK) [17, 18]. Subsequently, downstream

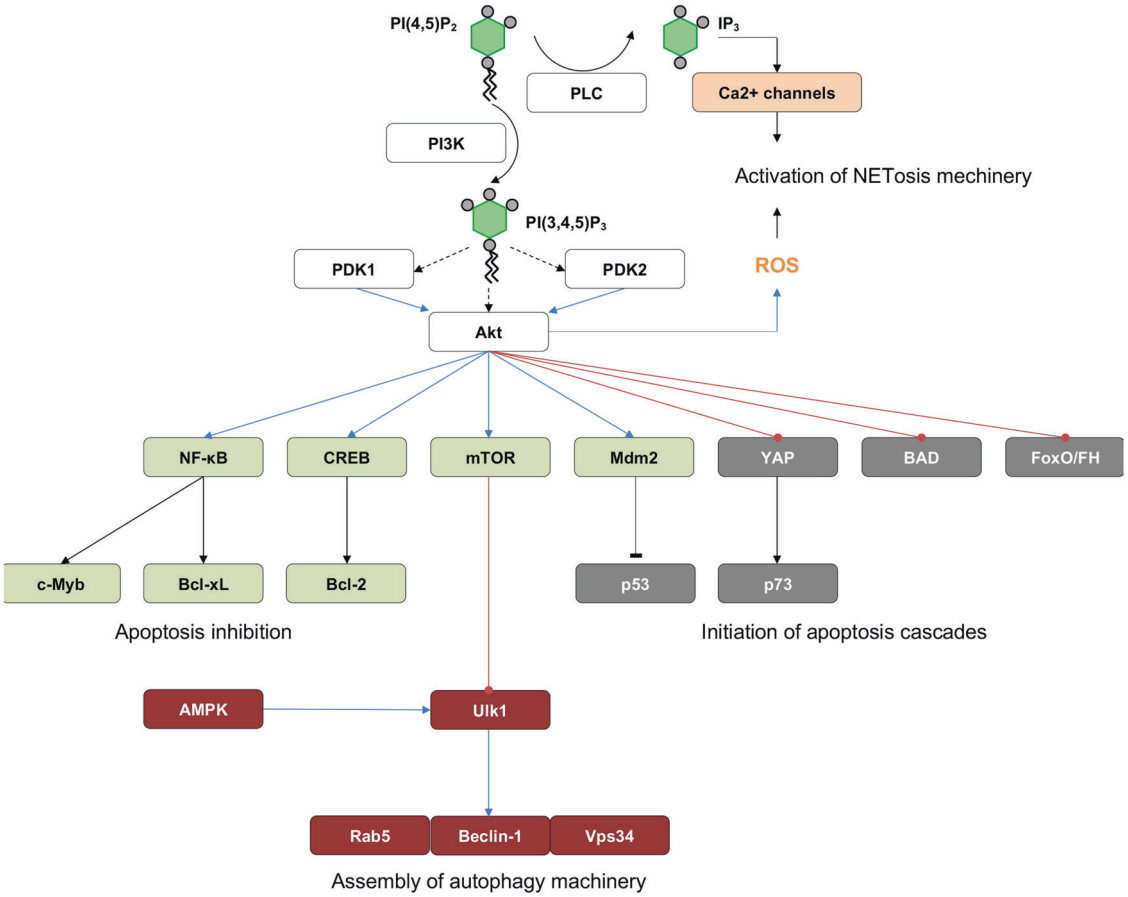

Fig. 3 Phosphoinositide-mediated signalling pathways in cell death and survival. Proteins involved in (pro-)apoptosis are in dark grey box, anti-apoptosis and survival in green box, autophagy in maroon box and NETosis in orange box. Legends for arrows/lines: kinase-catalysed PIP synthesis (curl arrow), PIP-mediated membrane recruitment (dashed arrow), direct inhibition (blunt-ended solid line), direct activation (solid arrow), stimulatory phosphorylation (blue arrow), inhibitory phosphorylation (red arrow)

transduction of DAG/PKC, IP_3/calcium and Akt signalling indispensably orchestrate many cellular and physiological processes including, but are not limited to, cell proliferation, cell growth and survival, cell division, cell migration, immune response, muscle contraction, memory and learning [19–21].

Other phosphoinositides, such as $PI(3,4)P_2$ and PI(5)P, although historically considered as intermediate products of $PI(3,4,5)P_3$ and $PI(4,5)P_2$ metabolism, respectively, have also emerged to be second messengers themselves. Challenging the exclusive $PI(3,4,5)P_3$/PI3K/Akt signalling axis, mounting evidence has suggested the importance of $PI(3,4)P_2$ in directly recruiting Akt, hence executing a distinct arm of the PI3K pathway [22]. Understanding of $PI(3,4,5)P_3$ and $PI(3,4)P_2$ cross-talk in regulating, balancing and contextualising Akt activity, however, undoubtedly requires future investigation. PI-derived PI(5)P is reportedly also an important second messenger for different cellular processes, depending on its production pathway [23–26]. PI(5)P generated by direct phosphorylation of PI, via phosphoinositide 5-kinase PIKfyve catalytic activity, is required for antiviral immunity, via interferon regulatory factor 3 (IRF3)-TANK-binding kinase (TBK1) axis [24]. In addition, PI(5)P can also be generated 'indirectly' through a complex series of steps involving class III PI3K (generating PI(3)P from PI), then PIKfyve (converting PI(3)P to $PI(3,5)P_2$) and finally phosphoinositide 3-phosphatase myotubularin-related protein-3 (MTMR3), dephosphorylating $PI(3,5)P_2$ into PI(5)P). PI(5)P produced by this indirect pathway is involved in cell migration signalling (via PI(5)P-recruited and activated exchange factors T-cell lymphoma invasion and metastasis 1 (Tiam1) and Ras-related C3 botulinum toxin substrate (Rac1)) [23, 25, 26].

The role of phosphoinositides in cell death, survival and proliferation is often deduced from the abovementioned phosphoinositide-mediated signalling pathways. For instance, the PI3K-Akt signalling pathway critically confers protection against apoptosis and autophagy, hence essential for cell survival (Fig. 3). Activated Akt, through its kinase activity, induces either inhibitory or stimulatory phosphorylation of components of the apoptotic machinery. For example, the phosphorylated nuclear Forkhead transcription

factors (FoxO/FH) are exported to cytosol and subsequently degraded via ubiquitin-proteasome-dependent pathway, resulting in the repression of FOX-activated pro-apoptotic gene expression [27, 28]. Akt also inhibits pro-apoptotic proteins, including p73-mediated apoptotic Yes-associated protein (YAP) [29] and caspase cascade-stimulating Bcl-2-associated death promoter (BAD) and caspase 9 [30]. Conversely, Akt-mediated phosphorylation positively regulates pro-survival/anti-apoptotic gene expression, via NF-κB induction (for c-Myb and Bcl-xL expression) [31] or cAMP response element binding protein (CREB) and CREB-binding protein (for Bcl-2 expression) [32, 33]. In addition, Akt indirectly inhibits p53-dependent apoptosis via apoptotic murine double minute 2 (Mdm2)-mediated degradation of p53 [34]. In addition, the PI3K-Akt signalling downregulates autophagy via activation of downstream Akt effector mTOR kinase which is a negative regulator of autophagy proteins [35].

Intriguingly, despite the well-characterised pro-survival role, PI3K-Akt as well as PLC-PKC cascades are also two major signalling pathways driving NETosis (Fig. 3), a more recently described form of programmed neutrophil death that results in the formation of neutrophil extracellular traps (NETs). Phorbol myristate acetate or microbial infection (e.g. *Leshmania amazonesis, Candida albicans*) trigger the signal transduction through PI3K and PLC, leading to reactive oxygen species release or increased intracellular calcium [36–38]. These key events in turn activate myeloperoxidase, neutrophil elastase and protein-arginine deiminase type 4 to promote chromatin decondensation and ultimately forming NETs to ensnare invading pathogens [37]. The discovery of the phosphoinositide-mediated pathways in transducing NETotic death signal has added another layer of complexity into functional pleiotropy of phosphoinositides. It remains to be determined how these pathways fine tune the opposing outcomes in different cellular contexts.

Actin cytoskeleton and membrane dynamics

Tightly regulated and precisely coordinated changes in the cell membrane and cytoskeleton are central to many aspects of cell physiology, such as membrane-associated and actin-dependent motility, membrane trafficking, cell polarity, cell signalling, cell division, and other morphogenetic processes. The physical and mechanistic links between the actin network and cell membrane are essential for these critical changes, which includes membrane detachment/attachment, actin polymerisation/depolymerisation, membrane curvature and membrane protrusion [39]. Phosphoinositides, especially $PI(4,5)P_2$ and $PI(3,4,5)P_3$, associate with multiple signal transductions, actin-binding proteins and membrane-remodelling machineries, hence they

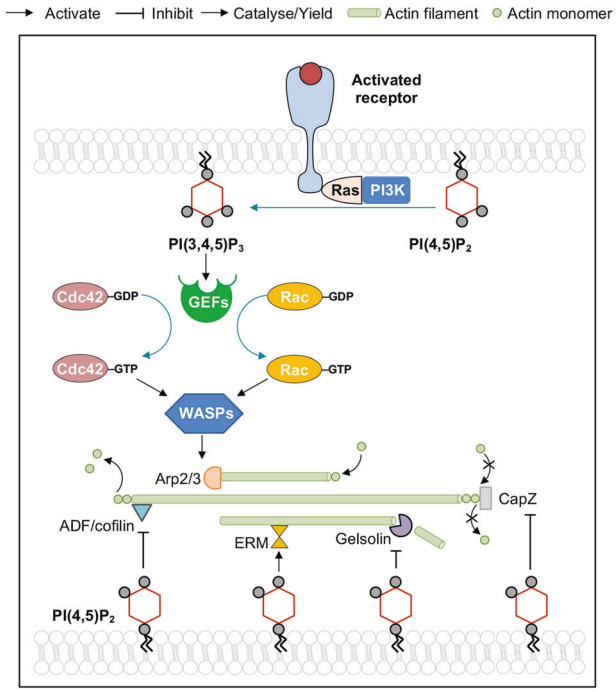

Fig. 4 Regulation of actin remodelling by phosphoinositides PI(3,4,5)P2, synthesised from PI(4,5)P2 upon receptor-mediated stimulation of PI3K via Ras GTPase, recruits GEFs to plasma membrane. GEFs then facilitate GTP loading on Rho family GTPases Cdc42 and Rac, leading to activation of actin nucleators WASPs and, subsequently, Arp2/3 complex. PI(4,5)P2 directly binds to and activates protein mediating membrane-cytoskeleton interaction (e.g. ERM proteins) or inhibits actin depolymerising factor ADF/cofilin, capping proteins (e.g. CapZ) and severing proteins (e.g. gelsolin). PI3K phosphoinositide 3-kinases, Ras Rat sarcoma GTPase, Cdc42 cell division control protein 42 homologue, Rac Ras-related C3 botulinum toxin substrate, GEF guanine nucleotide exchange factor, WASP Wiskott-Aldrich syndrome protein, Arp2/3 actin-related proteins 2/3 complex, ERM erzin, radixin and moesin, ADF actin depolymerising factor

spatiotemporally and interconnectedly control the organisation and dynamics of actin cytoskeleton and plasma membrane [11, 39].

In addition to the aforementioned phosphoinositide signalling, the actin cytoskeleton remodelling and actin-binding proteins are also controlled by various master regulators, among which are small GTPases (Fig. 4). The Rho-family small GTPases (e.g. Rac1, cell division control protein 42 homologue Cdc42), are in turn regulated by PI $(3,4,5)P_3$-recruited guanine nucleotide exchange factors (GEFs) in formation of focal adhesion complexes, extension of migratory pseudopods, or specialised actin filament assembly such as stress fibres [40, 41]. $PI(3,4,5)P_3$, and the more rigorously characterised $PI(4,5)P_2$, are also considered as direct-positive regulators of actin filament formation, by directly interacting with actin-binding proteins at cellular and subcellular organelle membranes in a local concentration-dependent manner [39, 40]. Binding to PI $(4,5)P_2$ typically leads to conformational change-associated

activation of proteins that are involved in promoting actin filament assembly such as nucleators Wiskott-Aldrich syndrome proteins (WASPs) and actin-related protein 2/3 complex (Arp 2/3), rulers, stabilisers and membrane anchoring. Conversely, the PI(4,5)P_2 interaction can inhibit actin depolymerising factor ADF/cofilin, capping (CapZ), bundlers, crosslinkers and severing proteins as well as other actin disassembly inducers, such as profilin [39]. The PI(4,5)P_2:actin-binding protein interactions are generally mediated through canonical PI(4,5)P_2 binding domains, such as PH domain and band 4.1-ezrin-radixin-moesin (FERM), or defined basic amino acid-rich clusters/motifs on their sequences [42, 43]. For instance, ERM family proteins, which directly connect the actin cytoskeleton to the plasma membrane, characteristically contain an N-terminal FERM domain and C-terminal actin-binding site. An interaction between these two domains effectively establishes autoinhibition, rendering these proteins inactive. The subsequent binding of PI(4,5)P_2 to the FERM domain leads to their activation by exposing the actin-binding site [44]. In contrast, gelsolin binds PI(4,5)P_2 through two basic amino acid-rich regions which also overlap with actin-binding sites, resulting in the blocking of its actin binding, and thus actin-severing and filament end-capping activity [45].

Dynamic membrane remodelling and generation of membrane curvatures are also mechanistically associated with phosphoinositide-mediated actin reorganisation. For example, mainly PI(4,5)P_2 and PI(4)P) binding activity of certain actin-associated proteins, such as Bin-Amphiphysin-Rvs (BAR) domain superfamily, epsin/ATP180 NH_2-terminal homology (ENTH/ANTH) domain family and dynamin, directly influences membrane remodelling including membrane protrusions or invaginations by manipulating phosphoinositide-rich membranes [46, 47]. The binding of BAR domain-containing proteins, such as amphiphysin 2, to phosphoinositides promotes their membrane binding and bending to create rigid scaffolds and affects the degree of intrinsic curvature and mechanistic variability [46–49]. Similarly, phosphoinositide binding via ENTH domains also enables membrane interactions of epsins as well as induce folding of distorted N-terminal extension into an amphipathic α-helix, an important structure for ENTH-mediated membrane bending/deformation [50, 51]. The interaction between dynamin and PI(4,5)P_2 through its PH domain not only allows its membrane translocation, but also mediates membrane scission via PI(4,5)P_2 clustering [52].

Through the modulation of membrane and cytoskeleton dynamics, phosphoinositides could be involved in morphological changes of dying cells, such as membrane blebbing (formation of circular membrane bulges) [39, 53]. The membrane blebs are locally disrupted cortical cytoskeleton-plasma membrane interaction and/or increased internal hydrostatic pressure, modulable by PI(4,5)P_2-regulated ERM family proteins [39, 53]. Hence, elevated plasma membrane PI(4,5)P_2 concentration suppresses bleb formation by strengthening actin-membrane interaction, whilst its depletion causes membrane blebbing due to the loss of adhesion energy between plasma membrane and cytoskeleton [39]. *Pseudomonas aeuginosa* phospholipase-like cytotoxin ExoU hydrolyses PI(4,5)P_2 acutely causes disrupted focal adhesion, disorganised cytoskeleton structure, membrane blebbing and eventually cell lysis due to loss of membrane integrity [54, 55]. Similar membrane-bleb-associated cell death was also reported for *Gambierdiscus toxicus* maitotoxin, allegedly due to its PI(4,5)P_2 phosphodiesterase activity [56, 57]. Membrane blebbing is also a hallmark of apoptotic cell death, formed upon Rho kinase (ROCK1)-dependent actin-myosin contraction through phosphorylation of myosin light chain [53]. Cortical contraction-induced increase in cytoplasmic hydrostatic pressure and associated disruption of ERM-mediated membrane-cytoskeleton interaction are believed to be the driving forces of bleb formation during apoptosis [58–60]. However, the role of phosphoinositides, including PI(4,5)P_2, in apoptotic bleb formation remains largely speculative. Furthermore, as apoptotic blebs go through cycles of formation, expansion and retraction, a dynamic machinery to modulate membrane-actin cortex network should be in place [60]. The trigger and progression of such machinery is still relatively unknown. As crucial players in regulating membrane and cytoskeleton dynamics, it will be interesting to decipher the importance of phosphoinositides in the apoptotic bleb-cycling machinery.

Emerging roles of phosphoinositides in directly facilitating cell death

Considering that phosphoinositides crucially mediate numerous cellular processes, one would expect they would have greater and a more direct influence on cell death than merely being second messenger lipids of PI3K-Akt signalling or modulating membrane and actin dynamics. However, only recently have phosphoinositides been recognised for their roles in regulating cell death. A number of impactful studies have reported their essential functions in mediating the execution of lytic cell death (pyroptosis, necroptosis and defensin-induced necrosis), autophagic cell death, and NETosis (Fig. 5), whilst a few others have limitedly addressed this in the context of apoptosis.

Autophagic cell death

Autophagy, also known as autophagocytosis, is a destructive mechanism to degrade and recycle unnecessary or dysfunctional cellular components [61, 62]. Autophagy is

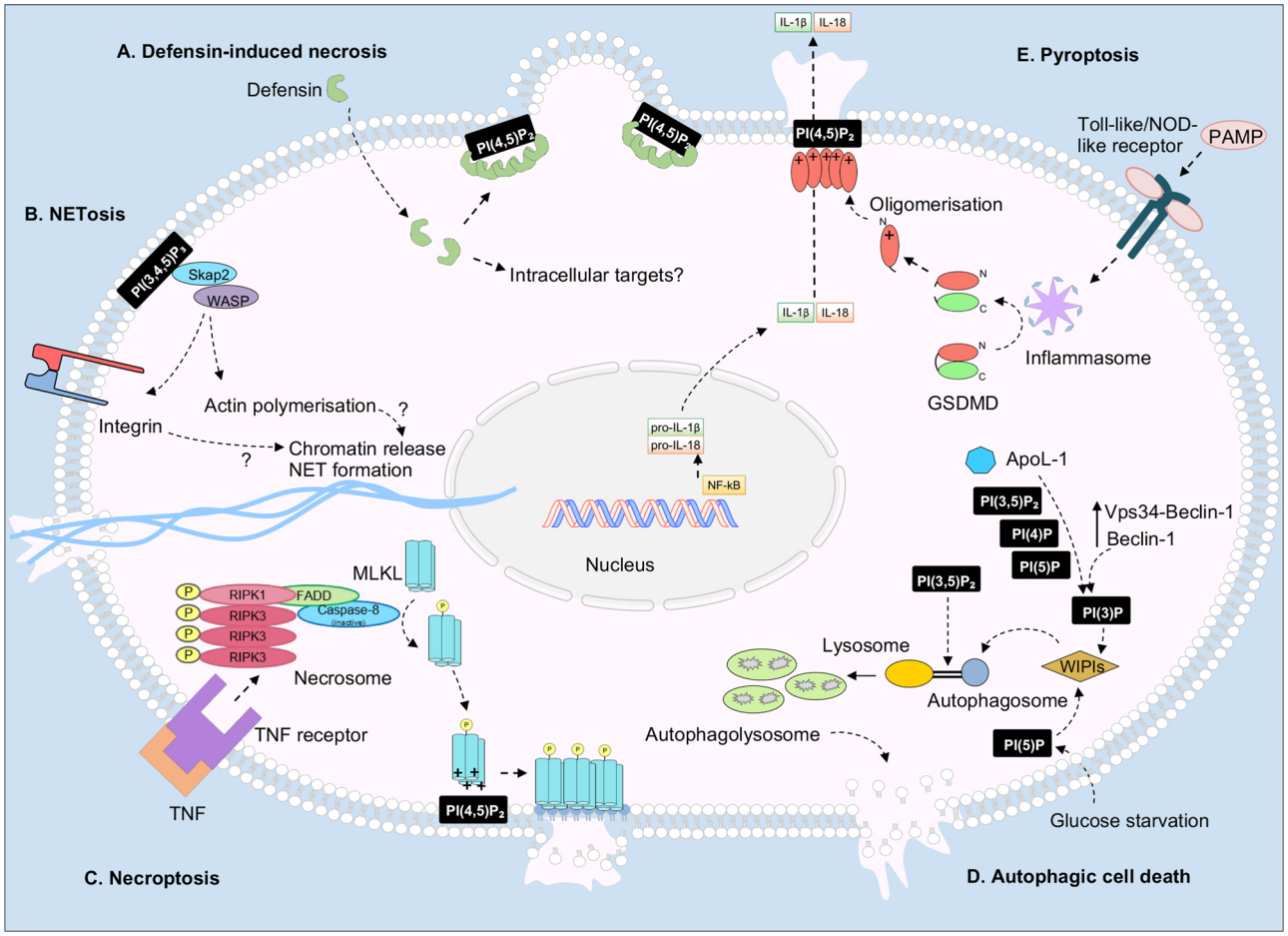

Fig. 5 Emerging roles of phosphoinositides in cell death. **a** Defensin-induced necrosis. Defensins internalise into target cells, then bind to $PI(4,5)P_2$ and oligomerise, leading to bleb-associated membrane permeabilisation and cell lysis. **b** Suicidal NETosis. $PI(3,4,5)P_3$ binding of Src kinase-associated phosphoprotein-2 (Skap2) liberates its active conformation to recruit Wiskott-Aldrich syndrome protein (WASP) for activation of integrins and actin polymerisation, which triggers expulsion of neutrophil extracellular traps (NETs). **c** Necroptosis. Tumour necrosis factor (TNF) binds to TNF receptor to trigger the formation of the necrosome multiplex, which phosphorylates MLKL and induces its conformational change to expose a $PI(4,5)P_2$-binding domain. Upon $PI(4,5)P_2$ binding, MLKL is recruited to membrane and forms lytic pore to induce membrane rupture. **d** Autophagic cell death. Lethal autophagy, or autophagic cell death (ACD), occurs upon autophagy overactivation, glucose starvation or death-specific inducer (e.g. lipid-binding ApoL-1). Activation of autophagy effectors (e.g. WIPIs) via PI(3)P- or PI(5)P-mediated pathway leads to the formation of autophagosomes, which are fused with lysosomes, possibly via $PI(3,5)P_2$-dependent manner to form autophagolysosomes. Membrane rupture and accumulation of autophagolysosomes are some typical ACD characteristics. **e** Pyroptosis. Binding of pathogen-associated molecular patterns (PAMPs) to receptors activate the inflammasome to cleave the N-terminus of GSDMD (GSDMD-Nter) from its C-terminus. Through interaction with $PI(4,5)P_2$, GSDMD-NT relocates to the plasma membrane and oligomerises to form lytic membrane pores, causing the release of interleukin 18 (IL-18) and IL-1b

often characterised by the formation of cytoplasmic autophagic vesicles (autophagosomes) that sequester disposed constituents targeted for digestion in autolysosomes [63, 64]. Physiologically, the tight regulation of autophagy is required for proper cellular homoeostasis. Autophagy also provides a defense mechanism by killing ingested bacteria [65, 66]. Under certain developmental, pathophysiological (e.g. post-ischaemic injury, starvation) or chemical (e.g. vintexin treatment) conditions, autophagy can result in autophagic cell death (ACD) [67–69]. Typical characteristics of ACD include membrane rupture, numerous autophagolysosomes, and enlargement of major cellular organelles [70–72]. Mechanistically, ACD is the lethal outcome of disturbed autophagic homoeostasis via excessive self-consumption of cellular content, uncontrolled mitophagy and autosis (Na^+/K^+-ATP-dependent ACD) [68, 69].

ACD strictly depends on autophagy machinery, which requires the production of PI(3)P by Vps34 kinase complexes, disruptions of which, demonstrated by genetic interference and pharmacological inhibitions, inhibit the autophagic pathway [73–77]. PI(3)P fundamentally provides docking platforms on the ER cytoplasmic membrane for autophagy effectors, and possibly mediates the fusion of autophagosomes and lysosomes to form autolysosomes [77]. Particularly in humans, PI(3)P is reported to specifically

recruit Autophagy-linked FYVE protein (Alfy), WD-repeat domain phosphoinositide-interacting proteins (WIPIs) and FYVE domain-containing protein 1 (DFCP1) that sequesters target cargo to the autophagic machinery and forms an isolation compartment, eventually maturing into autophagosomes [78–80]. Similar to most phosphoinositide-regulating processes, PI(3)P turnover in autophagy by phosphatidylinositol 3-kinase Vps34-Beclin-1 and phosphatase Jumpy is critical for the initiation and termination of autophagy [74, 81]. Non-canonically, autophagy can be executed via Vps34-independent (hence PI(3)P-independent) pathways, mediated by PIKfyve-synthesised PI(5)P via known PI(3)P effectors, such as WIPI proteins. The non-canonical PI(5)P-dependent pathway can compensate the absence of PI(3)P, e.g. due to defective Vps34, or overrule PI(3)P in particular circumstances, such as glucose starvation [82, 83]. It also been reported that PI(3,5)P_2 is required for completion of basal autophagy, particularly in mouse models of neurodegeneration. PI(3,5)P_2-deficient mice exhibit neuronal loss and accumulation of autophagy intermediates, possibly due to the lack of proper PI(3,5)P_2-governed autophagosome-lysosome fusion and/or lysosome function [83, 84].

A shift from basal autophagy to ACD has been linked with hyperactivation of Vps34-Beclin-1, overexpression of Beclin-1, downregulation of the molecular brakes of Beclin-1, hyperactivation of non-canonical autophagic pathways and certain death-specific factors (e.g. apolipoprotein 1 ApoL-1) [67, 68, 85]. ApoL-1, a BH3-only lipid-binding protein, can induce ACD, speculatively via disruption of phosphatidic acid-mTOR interaction and inhibition mitochondrial cardiolipin-associated apoptosis [85]. However, in this study, the protein-lipid overlay assay also indicated that ApoL-1 binds to phosphoinositides, particularly PI(3)P, PI(4)P, PI(5)P, PI(3,5)P_2 at comparable affinity to PA and CL [85]. Whether the binding of ApoL-1 to phosphoinositides alters the lipid homoeostasis leading to ACD as suggested by the authors [85], or a more sophisticated phosphoinositide-associated mechanism of ApoL-1-induced ACD, requires further elucidation.

NETosis

Suicidal NETosis is a novel form of neutrophil cell death, characterised by the formation of NETs that follows DNA decondensation and plasma membrane rupture [86, 87]. NET release forms a novel host defense mechanism against invading microbial pathogens. Though the execution and regulation of NETosis are still not fully understood, it is emerging that PI(3,4,5)P_3-mediated recruitment and activation of NETotic effectors, including Src kinase-associated phosphoprotein-2 (Skap2), WASPs and integrins [88]. While initial studies showed that Skap2 adopts an autoinhibitory conformation in steady-state neutrophils, upon stimulation, interactions with PI(3,4,5)P_3 via its PH domain induces a switch to the active conformation [89]. Then, Skap2 can form a complex with WASPs and translocate to the inner leaflet of the plasma membrane, leading to the downstream activation of integrins [88, 89]. In *Skap2*$^{-/-}$ neutrophils reconstituted with a Skap2 R140M mutant, which has been previously shown to impair PI(3,4,5)P_3 binding to the PH domain [89], both Skap2 and WASPs are unable to translocate to the plasma membrane and, consequentially, resulting in failed NETosis. These findings suggest the importance of direct PI(3,4,5)P_3-mediated membrane recruitment in directing NETosis.

Pyroptosis

In order to respond to bacterial infection and danger signals, cells can undergo pyroptosis, a programmed cell death associated with membrane lysis and inflammatory cytokine release [90]. During pyroptosis, activated caspase-1 or caspase-11 cleaves the effector protein gasdermin D (GSDMD), liberating its N-terminal (GSDMD-Nter) from the autoinhibitory C-terminal domain (GSDMD-Cter) [91–93]. The GSDMD-Nter then relocates to the plasma membrane, through its preferential interaction with PI(4,5)P_2. In fact, GSDMD-Nter harbours clusters of positively charged and hydrophobic residues, a common feature observed in PI(4,5)P_2-binding motifs, within its α1 helix and the β1–β2 loop [94]. Upon PI(4,5)P_2 binding, GSDMD-Nter promptly inserts into plasma membrane, oligomerises and forms large transmembrane pores to trigger inflammatory cytokine (interleukins 18 and 1β) release and, subsequently, membrane lysis [93, 95]. A recent time-lapse atomic force microscopy study has highlighted the crucial role of PI(4,5)P_2 throughout GSDMD assembly progress, not only governing its membrane targeting step but also accelerating membrane insertion and ensuring correct pore topology [94].

Necroptosis

Necroptosis is another inflammatory and lytic form of programmed cell death, occurring upon tumour necrosis factor receptor stimulation and caspase inhibition [96, 97]. Unlike pyroptosis, necroptosis is however distinctively characterised by caspase independence, membrane damage and release of inflammatory damage-associated molecular patterns [96, 97]. The GSDMD counterpart for necroptosis is mixed lineage kinase-like (MLKL) pseudokinase, structurally consisting of a N-terminal bundle (NB) domain fused by a brace region to a C-terminal pseudokinase domain [96, 97]. During necroptosis, MLKL is phosphorylated by a multiprotein complex, called necrosome,

leading to its transition from dormant monomeric to brace-mediated and activated oligomeric confirmation [98]. The MLKL oligomer is initially recruited to the plasma membrane through its low-affinity PI(4,5)P_2 binding provided by clusters of basic residues within NB domain [99, 100]. Subsequent robust membrane association progresses as higher-affinity PI(4,5)P_2 binding sites are exposed and displace the brace [100]. Mutation of basic residues at the putative binding sites suggests PI(4,5)P_2 interaction is particularly essential for the lytic activity. However, it remains to determine how PI(4,5)P_2-bound MLKL causes membrane rupture, possibly by its membrane destabilisation, pore formation or osmosis imbalance induced by its cation channel-like activity [98–101].

Defensin-induced necrosis

In addition to pyroptosis and necroptosis, defensin-induced necrosis represents a parallelly novel form of PI(4,5)P_2-dependent lytic cell death. Defensins are small cationic peptides of host innate immunity displaying broad-spectrum antimicrobial and immunomodulatory activities [102]. Additionally, recent studies have reported on membranolytic effect of ornamental tobacco defensin NaD1 [103], tomato defensin TPP3 [104] and human β-defensin 3 (HBD-3) [105] via PI(4,5)P_2-mediated membrane permeabilisation. At sub-micromolar concentrations, these defensins promptly enter mammalian cell followed by binding to PI(4,5)P_2 at inner membrane leaflet, thus destabilising the plasma membrane, causing bulge-like membrane structures (blebs) and eventually cell lysis. Supported by site-directed mutagenesis and crystallographic studies, the PI(4,5)P_2 interaction was mapped to analogous cysteine-flanked, β-strand-spanning, cationic clusters (^{33}HCSKILRR^{40} in NaD1, ^{37}HCSKLQRK^{42} in TPP3 and ^{32}KCSTRGRK^{39} in HBD-3), which resemble the K/R-X(3,7)-K-X-K/R-K/R motif found in nuclear PI(4,5)P_2-binding proteins [106]. Strikingly, NaD1 can form an arch-shaped oligomer with PI(4,5)P_2, stabilised by an extensive network of protein-protein and protein-lipid interactions. The oligomerisation propensity may increase NaD1 avidity for PI(4,5)P_2, and resultant tight NaD1:PI(4,5)P_2 complex would efficiently sequester PI(4,5)P_2, hence disrupting plasma membrane [103]. Interestingly, the membranolytic defensins show a great specificity towards tumour cells, likely due to certain morphological changes of plasma membranes upon tumour transformation to influence robust growth, motility, invasion and metastasis, as opposed to normal cells. These may include increased negatively charged phospholipid, increased membrane surface area and possibly increased phosphoinositide levels [105]. More recently, the oligomeric defensin:PI(4,5)P_2 complex has also been reported for human β-defensin 2 (HBD-2). Though structurally and mechanistically different to the abovementioned defensins, the HBD-2:PI(4,5)P_2 complex is also be crucial for *C. albicans* membrane permeabilisation, directly related to its antifungal property [107]. These findings accentuate an interspecies conserved PI(4,5)P_2-dependent mechanism among membrane-targeting innate immune molecules, mediating the necrosis of altered-self (such as tumour cells) and invading pathogens.

Apoptosis and secondary necrosis

Some early studies demonstrated a direct, yet opposing, action of phosphoinositides in mediating apoptosis, a non-lytic, non-inflammatory, caspase-dependent programmed cell death. PI(4,5)P_2 acts as a direct inhibitor of apoptosis initiator caspases 8 and 9 and their common effector caspase 3, independently of PI3K and Akt [108]. Increased PI(4,5)P_2 levels by PIP5Kα overexpression exacerbates apoptotic suppression; and PIP5Kα is actually cleaved by caspase 3 in vitro at a consensus cleavage site, mutation of which prevents PIP5Kα inactivation and enhances apoptosis in vivo [108]. In contrast, PI(4,5)P_2, in a concentration-dependent fashion, essentially activates cation channel $P2X_7$ current upon ATP stimulation, and indispensably induces $P2X_7$-mediated cell death, particularly apoptosis, in endothelial cells, T cells and macrophages [109]. It is uncertain why such discrepancy exists for the role of phosphoinositides in apoptosis, although cell-type dependency could be part of the explanation. To this point, however, the direct involvement of phosphoinositides in apoptosis remains largely underexplored despite apoptosis being the most well-studied cell death program.

Recent breakthroughs in apoptosis demonstrate that apoptosis is associated with three finely tuned downstream events with distinct membrane morphologies including: (i) the previously discussed membrane blebbing, (ii) formation of thin apoptotic membrane protrusion causing bleb separation and (iii) protrusion fragmentation to form membrane-bound extracellular vesicles, called apoptotic bodies [110–112]. The entire process, collectively known as apoptotic cell disassembly, is an important physiological or pathophysiological phenomenon downstream of apoptosis. Resultant apoptotic bodies mediate efficient phagocytic removal of apoptotic cells as well as aid intercellular communication [110]. In line with the suggestion earlier, the role of membrane-actin dynamics-regulating phosphoinositides, particularly PI(4,5)P_2 and PI(3,4,5)P_3, in mediating morphological changes during apoptotic cell disassembly definitely require further investigation.

Intriguingly, in the events of impaired clearance, apoptotic cells can progress into secondary necrosis due to progressive loss of plasma membrane integrity. Though

long regarded as an unregulated progress, recent reports suggest caspase 3-mediated cleavage of gasdermin E (GSDME, also known as DFNA5) predisposing apoptosis to membrane rupture via similar mechanisms to MLKL and GSDMD [113, 114]. GSDME knockout apoptotic cells fail to lyse, but instead extensively fragment to form apoptotic bodies. Once cleaved N-terminal fragment of GSDME translocates to the plasma membrane and induces membrane permeabilisation [113, 114]. Though detailed membrane recruitment and interaction of GSDME is unknown, based on its relatedness to GSDMD, one can logically propose a role for phosphoinositides in GSDME-induced secondary necrosis.

Exploiting phosphoinosititdes to therapeutically modulate cell death

With phosphoinositides emerging to critically integrate into an array of cell death pathways, developing phosphoinositide-targeting therapeutics could be useful in modulating cell death pathways, whether it be an inhibiting or promoting cell death. Phosphoinositide-binding cell death effectors (such as the defensins, GSDMD and MLKL) or related molecular mimetics could be exploited in the future to combat a range of diseases related to both microbial pathogens and cancer by inducing cell death through phosphoinositide interactions. This could prove an effective way of overcoming the ability of some cells, particularly in cancer, to evade programmed cell death, as direct treatment with effector molecule would enable bypass of the upstream signalling pathways.

In contrast, the opposite effect could be achieved by targeting phosphoinositides with therapeutics designed to prevent interactions with cell death effector molecules. This approach would be relevant in disease settings associated with unwanted cell death and inflammation (such as in autoimmune diseases), as reducing cell death could be beneficial. For example, in multiple sclerosis, where unwarranted pyroptosis contributes to neuroinflammation and demylenation, preventing the translocation of GSDMD-Nter to the plasma membrane by blocking $PI(4,5)P_2$ could be useful in treating the disease [115]. It is therefore possible that other cell death-associated diseases, e.g. neurodegenerative diseases, can be limited by blocking autophagic cell death can using PI(3)P and PI(5)P-sequestering agents.

Concluding remarks

The last few years have seen a remarkable increase in reports of phosphoinositide-facilitated cell death, emphasising the importance of phosphoinositides in many important aspects of life and death within the cell. Direct PI(3)P, $PI(3,5)P_3$, $PI(4,5)P_2$ and $PI(3,4,5)P_3$ function in mediating autophagic, lytic and NETotic forms of cell death essentially contribute to the homoeostasis, development and host defense mechanisms in many disease settings by preventing pathogen/tumour cell growth and alerting host immune response. These seminal findings however require additional in vivo evidence to strengthen the emerging importance of phosphoinositides in the context of cell death. Future investigations should also further delineate the death effector-phosphoinositide interactions and how such interactions molecularly execute different forms of cell death. It is worth studying how these pathways differentially are employed and/or cross-talk in different immune cells and against various infections/altered-self. There may also other cell death settings possibly recruiting phosphoinositides, particularly $PI(4,5)P_2$, for their enactment including apoptotic cell disassembly, secondary necrosis and necrosis by other lytic host defense peptides and molecules such as venoms. Taken together, phosphoinositides, in particular $PI(4,5)P_2$, may have potential as novel targets for combatting infection and cancer. The ability of $PI(4,5)P_2$ to orchestrate lytic cell death through the recruitment and activation of death effectors is indeed an attractive feature to manipulate cell death for therapeutic benefit.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. Nature. 2006;443:651–7.
2. Dickson EJ, Jensen JB, Hille B. Golgi and plasma membrane pools of PI(4)P contribute to plasma membrane PI(4,5)P2 and maintenance of KCNQ2/3 ion channel current. Proc Natl Acad Sci USA. 2014;111:E2281–90.
3. Sbrissa D, Ikonomov OC, Fu Z, Ijuin T, Gruenberg J, Takenawa T, et al. Core protein machinery for mammalian phosphatidylinositol 3,5-bisphosphate synthesis and turnover that regulates the progression of endosomal transport. Novel Sac phosphatase joins the ArPIKfyve-PIKfyve complex. J Biol Chem. 2007;282:23878–91.
4. Cullen PJ, Carlton JG. Phosphoinositides in the mammalian endo-lysosomal network. Subcell Biochem. 2012;59:65–110.
5. Sarkes D, Rameh LE. A novel HPLC-based approach makes possible the spatial characterization of cellular PtdIns5P and other phosphoinositides. Biochem J. 2010;428:375–84.
6. Grainger DL, Tavelis C, Ryan AJ, Hinchliffe KA. The emerging role of PtdIns5P: another signalling phosphoinositide takes its place. Biochem Soc Trans. 2012;40:257–61.

7. van den Bout I, Divecha N. PIP5K-driven PtdIns(4,5) P2 synthesis: regulation and cellular functions. J Cell Sci. 2009; 122(Pt 21):3837–50.
8. Ling K, Bairstow SF, Carbonara C, Turbin DA, Huntsman DG, Anderson RA. Type I gamma phosphatidylinositol phosphate kinase modulates adherens junction and E-cadherin trafficking via a direct interaction with mu 1B adaptin. J Cell Biol. 2007; 176:343–53.
9. Giudici ML, Lee K, Lim R, Irvine RF. The intracellular localisation and mobility of Type Igamma phosphatidylinositol 4P 5-kinase splice variants. FEBS Lett. 2006;580:6933–7.
10. Gericke A, Leslie NR, Losche M, Ross AH. PtdIns(4,5)P2-mediated cell signaling: emerging principles and PTEN as a paradigm for regulatory mechanism. Adv Exp Med Biol. 2013;991:85–104.
11. Balla T. Phosphoinositides: tiny lipids with giant impact on cell regulation. Physiol Rev. 2013;93:1019–137.
12. Falkenburger BH, Jensen JB, Dickson EJ, Suh BC, Hille B. Phosphoinositides: lipid regulators of membrane proteins. J Physiol. 2010;588(Pt 17):3179–85.
13. Berridge MJ, Irvine RF. Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature. 1984;312: 315–21.
14. Meldrum E, Parker PJ, Carozzi A. The PtdIns-PLC superfamily and signal transduction. Biochim Biophys Acta. 1991;1092: 49–71.
15. Katan M. The control of inositol lipid hydrolysis. Cancer Surv. 1996;27:199–211.
16. Rameh LE, Cantley LC. The role of phosphoinositide 3-kinase lipid products in cell function. J Biol Chem. 1999;274:8347–50.
17. Osaki M, Oshimura M, Ito H. PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis. 2004;9:667–76.
18. Hemmings BA, Restuccia DF. PI3K-PKB/Akt pathway. Cold Spring Harb Perspect Biol. 2012;4:a011189.
19. Yu JS, Cui W. Proliferation, survival and metabolism: the role of PI3K/AKT/mTOR signalling in pluripotency and cell fate determination. Development. 2016;143:3050–60.
20. Heras-Sandoval D, Perez-Rojas JM, Hernandez-Damian J, Pedraza-Chaverri J. The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. Cell Signal. 2014;26: 2694–701.
21. Cain RJ, Ridley AJ. Phosphoinositide 3-kinases in cell migration. Biol Cell. 2009;101:13–29.
22. Li H, Marshall AJ. Phosphatidylinositol (3,4) bisphosphate-specific phosphatases and effector proteins: a distinct branch of PI3K signaling. Cell Signal. 2015;27:1789–98.
23. Oppelt A, Lobert VH, Haglund K, Mackey AM, Rameh LE, Liestol K, et al. Production of phosphatidylinositol 5-phosphate via PIKfyve and MTMR3 regulates cell migration. EMBO Rep. 2013;14:57–64.
24. Kawasaki T, Takemura N, Standley DM, Akira S, Kawai T. The second messenger phosphatidylinositol-5-phosphate facilitates antiviral innate immune signaling. Cell Host Microbe. 2013;14: 148–58.
25. Haugsten EM, Oppelt A, Wesche J. Phosphatidylinositol 5-phosphate is a second messenger important for cell migration. Commun Integr Biol. 2013;6:e25446.
26. Viaud J, Lagarrigue F, Ramel D, Allart S, Chicanne G, Ceccato L, et al. Phosphatidylinositol 5-phosphate regulates invasion through binding and activation of Tiam1. Nat Commun. 2014; 5:4080.
27. Zhang X, Tang N, Hadden TJ, Rishi AK. Akt, FoxO and regulation of apoptosis. Biochim Biophys Acta. 2011;1813: 1978–86.
28. Tzivion G, Dobson M, Ramakrishnan G. FoxO transcription factors; regulation by AKT and 14-3-3 proteins. Biochim Biophys Acta. 2011;1813:1938–45.
29. Basu S, Totty NF, Irwin MS, Sudol M, Downward J. Akt phosphorylates the Yes-associated protein, YAP, to induce interaction with 14-3-3 and attenuation of p73-mediated apoptosis. Mol Cell. 2003;11:11–23.
30. Cardone MH, Roy N, Stennicke HR, Salvesen GS, Franke TF, Stanbridge E, et al. Regulation of cell death protease caspase-9 by phosphorylation. Science. 1998;282:1318–21.
31. Kane LP, Shapiro VS, Stokoe D, Weiss A. Induction of NF-kappaB by the Akt/PKB kinase. Curr Biol. 1999;9:601–4.
32. Du K, Montminy M. CREB is a regulatory target for the protein kinase Akt/PKB. J Biol Chem. 1998;273:32377–9.
33. Pugazhenthi S, Nesterova A, Sable C, Heidenreich KA, Boxer LM, Heasley LE, et al. Akt/protein kinase B up-regulates Bcl-2 expression through cAMP-response element-binding protein. J Biol Chem. 2000;275:10761–6.
34. Ogawara Y, Kishishita S, Obata T, Isazawa Y, Suzuki T, Tanaka K, et al. Akt enhances Mdm2-mediated ubiquitination and degradation of p53. J Biol Chem. 2002;277:21843–50.
35. Kim J, Kundu M, Viollet B, Guan KL. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol. 2011;13:132–41.
36. Papayannopoulos V. Neutrophil extracellular traps in immunity and disease. Nat Rev Immunol. 2018;18:134–47.
37. DeSouza-Vieira T, Guimaraes-Costa A, Rochael NC, Lira MN, Nascimento MT, Lima-Gomez PS, et al. Neutrophil extracellular traps release induced by Leishmania: role of PI3Kgamma, ERK, PI3Ksigma, PKC, and [Ca2+]. J Leukoc Biol. 2016;100:801–10.
38. Zawrotniak M, Bochenska O, Karkowska-Kuleta J, Seweryn-Ozog K, Aoki W, Ueda M, et al. Aspartic proteases and major cell wall components in Candida albicans trigger the release of neutrophil extracellular traps. Front Cell Infect Microbiol. 2017;7:414.
39. Saarikangas J, Zhao H, Lappalainen P. Regulation of the actin cytoskeleton-plasma membrane interplay by phosphoinositides. Physiol Rev. 2010;90:259–89.
40. Egami Y, Taguchi T, Maekawa M, Arai H, Araki N. Small GTPases and phosphoinositides in the regulatory mechanisms of macropinosome formation and maturation. Front Physiol. 2014;5:374.
41. Benard V, Bohl BP, Bokoch GM. Characterization of rac and cdc42 activation in chemoattractant-stimulated human neutrophils using a novel assay for active GTPases. J Biol Chem. 1999;274:13198–204.
42. Toker A. The synthesis and cellular roles of phosphatidylinositol 4,5-bisphosphate. Curr Opin Cell Biol. 1998;10:254–61.
43. Kabachinski G, Yamaga M, Kielar-Grevstad DM, Bruinsma S, Martin TF. CAPS and Munc13 utilize distinct PIP2-linked mechanisms to promote vesicle exocytosis. Mol Biol Cell. 2014;25:508–21.
44. Barret C, Roy C, Montcourrier P, Mangeat P, Niggli V. Mutagenesis of the phosphatidylinositol 4,5-bisphosphate (PIP(2)) binding site in the NH(2)-terminal domain of ezrin correlates with its altered cellular distribution. J Cell Biol. 2000;151: 1067–80.
45. Yu FX, Sun HQ, Janmey PA, Yin HL. Identification of a polyphosphoinositide-binding sequence in an actin monomer-binding domain of gelsolin. J Biol Chem. 1992;267:14616–21.
46. Lemmon MA. Membrane recognition by phospholipid-binding domains. Nat Rev Mol Cell Biol. 2008;9:99–111.
47. Gallop JL, McMahon HT. BAR domains and membrane curvature: bringing your curves to the BAR. Biochem Soc Symp. 2005;72:223–31.

48. Wang Q, Kaan HY, Hooda RN, Goh SL, Sondermann H. Structure and plasticity of Endophilin and Sorting Nexin 9. Structure. 2008;16:1574–87.
49. Frost A, Perera R, Roux A, Spasov K, Destaing O, Egelman EH, et al. Structural basis of membrane invagination by F-BAR domains. Cell . 2008;132:807–17.
50. Ford MG, Mills IG, Peter BJ, Vallis Y, Praefcke GJ, Evans PR, et al. Curvature of clathrin-coated pits driven by epsin. Nature. 2002;419:361–6.
51. Itoh T, Koshiba S, Kigawa T, Kikuchi A, Yokoyama S, Takenawa T. Role of the ENTH domain in phosphatidylinositol-4,5-bisphosphate binding and endocytosis. Science. 2001;291:1047–51.
52. Bethoney KA, King MC, Hinshaw JE, Ostap EM, Lemmon MA. A possible effector role for the pleckstrin homology (PH) domain of dynamin. Proc Natl Acad Sci USA. 2009;106:13359–64.
53. Zhang Y, Chen X, Gueydan C, Han J. Plasma membrane changes during programmed cell deaths. Cell Res. 2018;28:9–21.
54. Sato H, Frank DW. Intoxication of host cells by the T3SS phospholipase ExoU: PI(4,5)P2-associated, cytoskeletal collapse and late phase membrane blebbing. PLoS ONE. 2014;9:e103127.
55. Tyson GH, Hauser AR. Phosphatidylinositol 4,5-bisphosphate is a novel coactivator of the Pseudomonas aeruginosa cytotoxin ExoU. Infect Immun. 2013;81:2873–81.
56. Bernard V, Laurent A, Derancourt J, Clement-Durand M, Picard A, Le Peuch C, et al. Maitotoxin triggers the cortical reaction and phosphatidylinositol-4,5-bisphosphate breakdown in amphibian oocytes. Eur J Biochem. 1988;174:655–62.
57. Estacion M, Schilling WP. Maitotoxin-induced membrane blebbing and cell death in bovine aortic endothelial cells. BMC Physiol. 2001;1:2.
58. Lorentzen A, Bamber J, Sadok A, Elson-Schwab I, Marshall CJ. An ezrin-rich, rigid uropod-like structure directs movement of amoeboid blebbing cells. J Cell Sci. 2011;124(Pt 8):1256–67.
59. Wickman G, Julian L, Olson MF. How apoptotic cells aid in the removal of their own cold dead bodies. Cell Death Differ. 2012;19:735–42.
60. Charras GT, Hu CK, Coughlin M, Mitchison TJ. Reassembly of contractile actin cortex in cell blebs. J Cell Biol. 2006;175:477–90.
61. Takeshige K, Baba M, Tsuboi S, Noda T, Ohsumi Y. Autophagy in yeast demonstrated with proteinase-deficient mutants and conditions for its induction. J Cell Biol. 1992;119:301–11.
62. Ichimura Y, Kirisako T, Takao T, Satomi Y, Shimonishi Y, Ishihara N, et al. A ubiquitin-like system mediates protein lipidation. Nature. 2000;408:488–92.
63. Nara A, Mizushima N, Yamamoto A, Kabeya Y, Ohsumi Y, Yoshimori T. SKD1 AAA ATPase-dependent endosomal transport is involved in autolysosome formation. Cell Struct Funct. 2002;27:29–37.
64. Mizushima N, Ohsumi Y, Yoshimori T. Autophagosome formation in mammalian cells. Cell Struct Funct. 2002;27:421–9.
65. Ogawa M, Yoshimori T, Suzuki T, Sagara H, Mizushima N, Sasakawa C. Escape of intracellular Shigella from autophagy. Science. 2005;307:727–31.
66. Gutierrez MG, Master SS, Singh SB, Taylor GA, Colombo MI, Deretic V. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell . 2004;119:753–66.
67. Bhardwaj M, Paul S, Jakhar R, Khan I, Kang JI, Kim HM, et al. Vitexin confers HSF-1 mediated autophagic cell death by activating JNK and ApoL1 in colorectal carcinoma cells. Oncotarget. 2017;8:112426–41.
68. Bialik S, Dasari SK, Kimchi A. Autophagy-dependent cell death - where, how and why a cell eats itself to death. J Cell Sci. 2018;131:18.
69. Liu Y, Shoji-Kawata S, Sumpter RM Jr., Wei Y, Ginet V, Zhang L, et al. Autosis is a Na+,K+-ATPase-regulated form of cell death triggered by autophagy-inducing peptides, starvation, and hypoxia-ischemia. Proc Natl Acad Sci USA. 2013;110:20364–71.
70. Tsujimoto Y, Shimizu S. Another way to die: autophagic programmed cell death. Cell Death Differ. 2005;12(Suppl 2):1528–34.
71. Liu Y, Levine B. Autosis and autophagic cell death: the dark side of autophagy. Cell Death Differ. 2015;22:367–76.
72. Green DR, Levine B. To be or not to be? How selective autophagy and cell death govern cell fate. Cell . 2014;157:65–75.
73. Takatsuka C, Inoue Y, Matsuoka K, Moriyasu Y. 3-methyladenine inhibits autophagy in tobacco culture cells under sucrose starvation conditions. Plant Cell Physiol. 2004;45:265–74.
74. Juhasz G, Hill JH, Yan Y, Sass M, Baehrecke EH, Backer JM, et al. The class III PI(3)K Vps34 promotes autophagy and endocytosis but not TOR signaling in Drosophila. J Cell Biol. 2008;181:655–66.
75. Eskelinen EL, Prescott AR, Cooper J, Brachmann SM, Wang L, Tang X, et al. Inhibition of autophagy in mitotic animal cells. Traffic. 2002;3:878–93.
76. Blommaart EF, Krause U, Schellens JP, Vreeling-Sindelarova H, Meijer AJ. The phosphatidylinositol 3-kinase inhibitors wortmannin and LY294002 inhibit autophagy in isolated rat hepatocytes. Eur J Biochem. 1997;243:240–6.
77. Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, et al. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol. 2008;182:685–701.
78. Walker S, Chandra P, Manifava M, Axe E, Ktistakis NT. Making autophagosomes: localized synthesis of phosphatidylinositol 3-phosphate holds the clue. Autophagy. 2008;4:1093–6.
79. Jeffries TR, Dove SK, Michell RH, Parker PJ. PtdIns-specific MPR pathway association of a novel WD40 repeat protein, WIPI49. Mol Biol Cell. 2004;15:2652–63.
80. Filimonenko M, Isakson P, Finley KD, Anderson M, Jeong H, Melia TJ, et al. The selective macroautophagic degradation of aggregated proteins requires the PI3P-binding protein Alfy. Mol Cell. 2010;38:265–79.
81. Vergne I, Roberts E, Elmaoued RA, Tosch V, Delgado MA, Proikas-Cezanne T, et al. Control of autophagy initiation by phosphoinositide 3-phosphatase Jumpy. EMBO J. 2009;28:2244–58.
82. Vicinanza M, Korolchuk VI, Ashkenazi A, Puri C, Menzies FM, Clarke JH, et al. PI(5)P regulates autophagosome biogenesis. Mol Cell. 2015;57:219–34.
83. Hasegawa J, Strunk BS, Weisman LS. PI5P and PI(3,5)P2: minor, but essential phosphoinositides. Cell Struct Funct. 2017;42:49–60.
84. Ferguson CJ, Lenk GM, Meisler MH. Defective autophagy in neurons and astrocytes from mice deficient in PI(3,5)P2. Hum Mol Genet. 2009;18:4868–78.
85. Wan G, Zhaorigetu S, Liu Z, Kaini R, Jiang Z, Hu CA. Apolipoprotein L1, a novel Bcl-2 homology domain 3-only lipid-binding protein, induces autophagic cell death. J Biol Chem. 2008;283:21540–9.
86. Dabrowska D, Jablonska E, Garley M, Ratajczak-Wrona W, Iwaniuk A. New aspects of the biology of neutrophil extracellular traps. Scand J Immunol. 2016;84:317–22.
87. Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, et al. Neutrophil extracellular traps kill bacteria. Science. 2004;303:1532–5.

88. Boras M, Volmering S, Bokemeyer A, Rossaint J, Block H, Bardel B, et al. Skap2 is required for beta2 integrin-mediated neutrophil recruitment and functions. J Exp Med. 2017;214: 851–74.
89. Swanson KD, Tang Y, Ceccarelli DF, Poy F, Sliwa JP, Neel BG, et al. The Skap-hom dimerization and PH domains comprise a 3'-phosphoinositide-gated molecular switch. Mol Cell. 2008;32: 564–75.
90. Fink SL, Cookson BT. Apoptosis, pyroptosis, and necrosis: mechanistic description of dead and dying eukaryotic cells. Infect Immun. 2005;73:1907–16.
91. Shi J, Zhao Y, Wang K, Shi X, Wang Y, Huang H, et al. Cleavage of GSDMD by inflammatory caspases determines pyroptotic cell death. Nature. 2015;526:660–5.
92. Kayagaki N, Stowe IB, Lee BL, O'Rourke K, Anderson K, Warming S, et al. Caspase-11 cleaves gasdermin D for non-canonical inflammasome signalling. Nature. 2015;526:666–71.
93. Ding J, Wang K, Liu W, She Y, Sun Q, Shi J, et al. Pore-forming activity and structural autoinhibition of the gasdermin family. Nature. 2016;535:111–6.
94. Liu Z, Wang C, Rathkey JK, Yang J, Dubyak GR, Abbott DW, et al. Structures of the Gasdermin D C-terminal domains reveal mechanisms of autoinhibition. Structure. 2018;26:778–84 e3.
95. Liu X, Zhang Z, Ruan J, Pan Y, Magupalli VG, Wu H, et al. Inflammasome-activated gasdermin D causes pyroptosis by forming membrane pores. Nature. 2016;535:153–8.
96. Wang H, Sun L, Su L, Rizo J, Liu L, Wang LF, et al. Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3. Mol Cell. 2014;54:133–46.
97. Sun L, Wang H, Wang Z, He S, Chen S, Liao D, et al. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. Cell . 2012;148:213–27.
98. Cai Z, Jitkaew S, Zhao J, Chiang HC, Choksi S, Liu J, et al. Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. Nat Cell Biol. 2014; 16:55–65.
99. Dondelinger Y, Declercq W, Montessuit S, Roelandt R, Goncalves A, Bruggeman I, et al. MLKL compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. Cell Rep. 2014;7:971–81.
100. Quarato G, Guy CS, Grace CR, Llambi F, Nourse A, Rodriguez DA, et al. Sequential engagement of distinct MLKL phosphatidylinositol-binding sites executes necroptosis. Mol Cell. 2016;61:589–601.
101. Xia B, Fang S, Chen X, Hu H, Chen P, Wang H, et al. MLKL forms cation channels. Cell Res. 2016;26:517–28.
102. Phan TK, Lay FT, Hulett MD. Importance of phosphoinositide binding by human beta-defensin 3 for Akt-dependent cytokine induction. Immunol Cell Biol. 2018;96:54–67.
103. Poon IKH, Baxter AA, Lay FT, Mills GD, Adda CG, Payne JA, et al. Phosphoinositide-mediated oligomerization of a defensin induces cell lysis. eLife. 2014;3:e01808.
104. Baxter AA, Richter V, Lay FT, Poon IK, Adda CG, Veneer PK, et al. The tomato defensin tpp3 binds phosphatidylinositol (4,5)-bisphosphate via a conserved dimeric cationic grip conformation to mediate cell lysis. Mol Cell Biol. 2015;35:1964–78.
105. Phan TK, Lay FT, Poon IK, Hinds MG, Kvansakul M, Hulett MD. Human beta-defensin 3 contains an oncolytic motif that binds PI(4,5)P2 to mediate tumour cell permeabilisation. Oncotarget. 2016;7:2054–69.
106. Lewis AE, Sommer L, Arntzen MO, Strahm Y, Morrice NA, Divecha N, et al. Identification of nuclear phosphatidylinositol 4,5-bisphosphate-interacting proteins by neomycin extraction. Mol Cell Proteom. 2011;10:M110 003376.
107. Jarva M, Phan TK, Lay FT, Caria S, Kvansakul M, Hulett MD. Human beta-defensin 2 kills Candida albicans through phosphatidylinositol 4,5-bisphosphate-mediated membrane permeabilization. Sci Adv. 2018;4:eaat0979.
108. Mejillano M, Yamamoto M, Rozelle AL, Sun HQ, Wang X, Yin HL. Regulation of apoptosis by phosphatidylinositol 4,5-bisphosphate inhibition of caspases, and caspase inactivation of phosphatidylinositol phosphate 5-kinases. J Biol Chem. 2001; 276:1865–72.
109. Zhao Q, Yang M, Ting AT, Logothetis DE. PIP(2) regulates the ionic current of P2X receptors and P2X(7) receptor-mediated cell death. Channels. 2007;1:46–55.
110. Atkin-Smith GK, Poon IKH. Disassembly of the dying: mechanisms and functions. Trends Cell Biol. 2017;27: 151–62.
111. Atkin-Smith GK, Tixeira R, Paone S, Mathivanan S, Collins C, Liem M, et al. A novel mechanism of generating extracellular vesicles during apoptosis via a beads-on-a-string membrane structure. Nat Commun. 2015;6:7439.
112. Jiang L, Tixeira R, Caruso S, Atkin-Smith GK, Baxter AA, Paone S, et al. Monitoring the progression of cell death and the disassembly of dying cells by flow cytometry. Nat Protoc. 2016;11:655–63.
113. Rogers C, Fernandes-Alnemri T, Mayes L, Alnemri D, Cingolani G, Alnemri ES. Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. Nat Commun. 2017;8:14128.
114. Wang Y, Gao W, Shi X, Ding J, Liu W, He H, et al. Chemotherapy drugs induce pyroptosis through caspase-3 cleavage of a gasdermin. Nature. 2017;547:99–103.
115. McKenzie BA, Mamik MK, Saito LB, Boghozian R, Monaco MC, Major EO, et al. Caspase-1 inhibition prevents glial inflammasome activation and pyroptosis in models of multiple sclerosis. Proc Natl Acad Sci USA. 2018;115: E6065–E74.