Review article

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Regulated cell death pathways in kidney disease

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Abstract

Disorders of cell number that result from an imbalance between the death of parenchymal cells and the proliferation or recruitment of maladaptive cells contributes to the pathogenesis of kidney disease. Acute kidney injury can result from an acute loss of kidney epithelial cells. In chronic kidney disease, loss of kidney epithelial cells leads to glomerulosclerosis and tubular atrophy, whereas interstitial inflammation and fibrosis result from an excess of leukocytes and myofibroblasts. Other conditions, such as acquired cystic disease and kidney cancer, are characterized by excess numbers of cyst wall and malignant cells, respectively. Cell death modalities act to clear unwanted cells, but disproportionate responses can contribute to the detrimental loss of kidney cells. Indeed, pathways of regulated cell death – including apoptosis and necrosis – have emerged as central events in the pathogenesis of various kidney diseases that may be amenable to therapeutic intervention. Modes of regulated necrosis, such as ferroptosis, necroptosis and pyroptosis may cause kidney injury directly or through the recruitment of immune cells and stimulation of inflammatory responses. Importantly, multiple layers of interconnections exist between different modalities of regulated cell death, including shared triggers, molecular components and protective mechanisms.

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Key points

- Regulated cell death through either apoptosis or necrosis contributes to parenchymal cell loss in acute and chronic kidney disease and may also modulate inflammation, fibrosis and the immune response.

- Therapeutic strategies that modulate apoptosis may interfere with or promote regulated necrosis.

- Available evidence in general supports a therapeutic benefit of targeting ferroptosis or necroptosis in preclinical models of kidney disease; however, the impact of targeting pyroptosis is less clear.

- The diverse forms of regulated necrosis may be interrelated, share molecular pathways and cell defence mechanisms and coexist in the same kidney disease, affecting different cell types or the same cell type synchronously or sequentially.

- Regulated necrosis may release damage-associated molecular patterns, which induce an inflammatory response that amplifies tissue injury (necroinflammation or immunogenic cell death).

- Despite some promising preclinical data, no clinical studies have targeted regulated necrosis to prevent or treat human kidney disease.

Introduction

Kidney disease is one of the fastest growing global causes of death, with chronic kidney disease (CKD) projected to become the fifth global cause of death by 2040 (refs. [1],[2]). In addition to being a burden in its own right, CKD is intimately related to other forms of kidney disease[3–8] (Fig. 1). For instance, the risk of acute kidney injury (AKI) is increased in persons with CKD, and AKI may itself cause or accelerate the progression of CKD[9]. CKD can also be complicated by the development of acquired cystic kidney disease or renal cell carcinoma[7,8]. Many of these conditions can be characterized as disorders of cell number. For example, CKD is characterized by the progressive loss of epithelial cells, such as podocytes and tubule cells, and of endothelial cells, which causes glomerulosclerosis, tubular atrophy and capillary rarefaction, respectively, as well as the proliferation and/or recruitment of maladaptive cells such as myofibroblasts and inflammatory cells.

Novel therapeutic approaches are needed to address the suboptimal outcomes in patients with kidney disease. Pathways that target cell death pathways and therefore alter cell number may therefore represent a therapeutic approach to prevent, treat or accelerate recovery from kidney disease by limiting the loss of kidney parenchymal cells and kidney inflammation. Two main forms of cell death are known: necrosis, in which rupture of the plasma membrane releases intracellular contents, and apoptosis, a form of regulated cell death in which dying cells are rapidly engulfed by adjacent cells before the plasma membrane ruptures and intracellular contents are released into the extracellular space[10–13] (Fig. 2). Apoptosis is considered to be a non-immunogenic and non-inflammatory process that enables the orderly clearance of unwanted or damaged cells[14,15]. However, despite extensive efforts, therapeutic approaches to target apoptosis in AKI or CKD have not been successful. Contrary to the initial suggestions that necrosis is an unregulated process, several molecular programmes that result in

regulated necrosis have been identified and offer new opportunities for therapeutic intervention[16–18] (Box 1 and Supplementary Fig. 1). Evidence from preclinical models suggests that three main forms of regulated necrosis — ferroptosis, necroptosis and pyroptosis — may contribute to a range of kidney diseases (Supplementary Fig. 2). More limited evidence supports a role for mitochondrial permeability transition-regulated necrosis (MPT-RN), autosis and NETosis (derived from the term neutrophil extracellular traps (NETs)) in the pathogenesis of kidney disease[18–28]. However, much work is needed to understand the relative contribution of the diverse forms of regulated necrosis to human kidney disease, the extent to which the pathways are interconnected and the extent to which they can be targeted therapeutically. Here, we provide an overview of the relationship between necroptosis, pyroptosis, ferroptosis and apoptosis, the evidence supporting a role for these regulated pathways of necrosis in kidney disease, strategies for therapeutic targeting and research needs. Three other reviews in this issue of *Nature Reviews Nephrology* provide detailed overviews of the process of ferroptosis, necroptosis and pyroptosis in kidney disease[29–31].

Targeting cell death in kidney disease

Safety and efficacy are key requirements for the translation of any new therapy to the clinic, and depend on the extent to which the new therapy disrupts critical physiological homeostatic and adaptive processes and on the contexts in which they are used. Cell death can clear damaged or unwanted cells, and therefore has a key role in various processes, including: development, in which whole-organ structures may be transient; physiological tissue turnover; the elimination of cells that are infected, damaged or in the early stages of malignant transformation; and the elimination of cells that have accumulated in excess during repair processes[32]. Regulated cell death programmes probably evolved to protect complex organisms[32], and these protective roles should be considered when developing approaches to target cell death therapeutically. Kidney cancer cells have developed mechanisms to evade cell death[33], whereas inactivating genetic variants of the genes involved in cell death processes have long been known to contribute to the impaired deletion of self-reactive immune cells and the development of autoimmunity[34,35]. However, untimely, unwanted or excessive cell death can deplete important parenchymal cells and cause tissue injury. Moreover, certain forms of cell death can trigger inflammatory (necroinflammation) or autoimmune (immunogenic cell death) responses that can cause and/or amplify tissue injury[10,36,37].

Cell death and more specifically, regulated necrosis may represent a therapeutic target in kidney diseases, including AKI and CKD of various aetiologies, either by preventing unwanted cell death or by promoting the death of specific cell types (Fig. 1).

Preventing cell death

Therapeutic approaches that target cell death in the context of AKI and CKD should prevent aberrant kidney epithelial and endothelial cell death as well as the inflammatory and fibrotic processes that result from certain cell death modalities. However, interventions that prevent the death of parenchymal kidney cells should not interfere with the clearance of irreversibly damaged or premalignant cells[38] (Supplementary Fig. 3). Of note, some evidence suggests that papillary carcinoma may be triggered by the ischaemic necrosis of proximal tubule cells[8,39]. In addition, clear cell carcinoma may be triggered by metabolic overload of the remnant nephron's proximal tubules in CKD, highlighting that
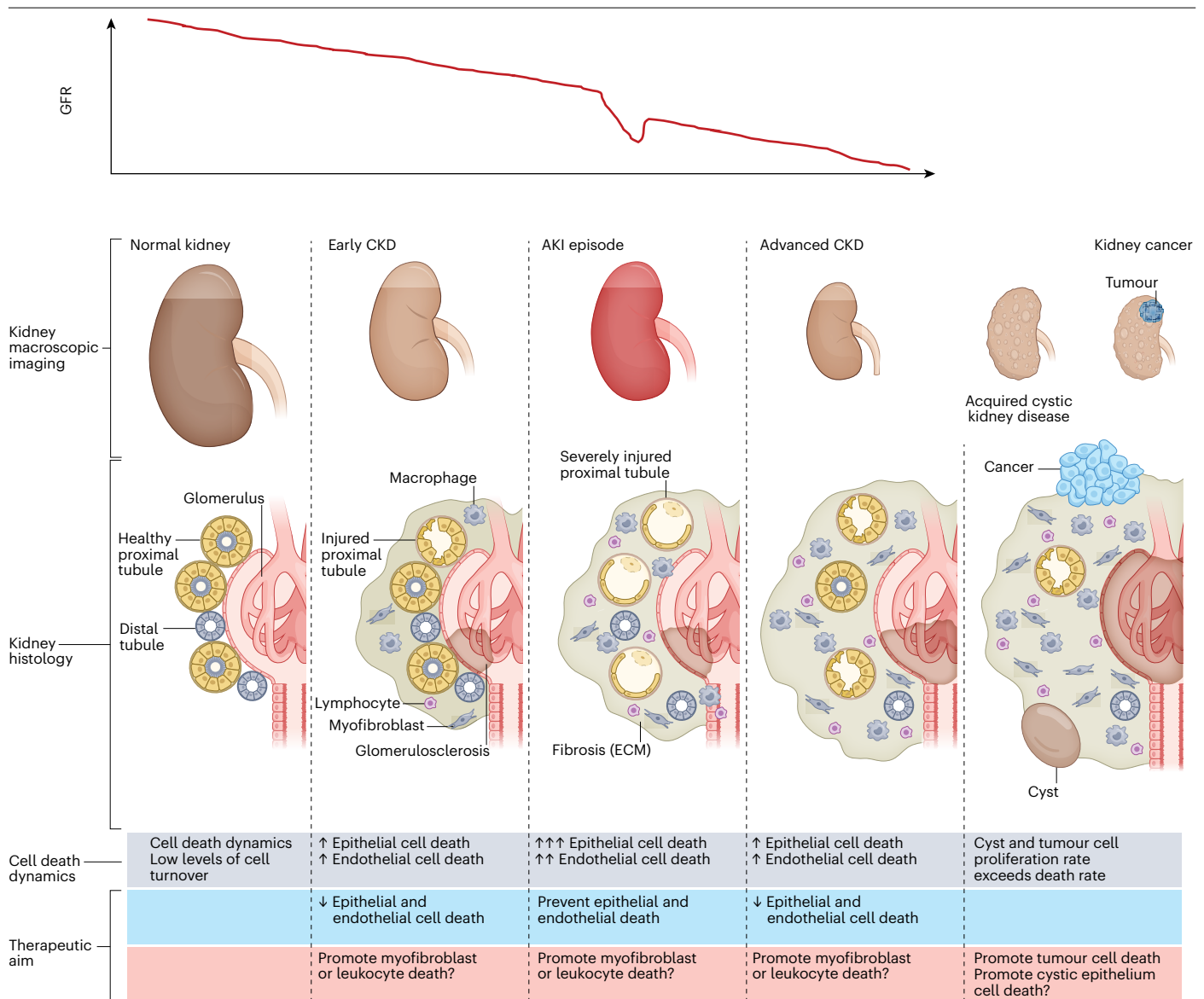
Fig. 1 | The natural history of CKD and relationship to cell number and cell death. The relationship between chronic kidney disease (CKD), acute kidney injury (AKI), acquired kidney cystic disease and kidney cancer can be conceptualized as interrelated processes, characterized by changes in cell number that are driven by the proliferation or death of key cell types. Differences in the dynamics of cell death and turnover represent potential therapeutic targets. AKI is more common in patients with prior CKD and may cause irreversible loss of kidney function or accelerate CKD progression. However, not all episodes of AKI are associated with prior CKD. Of note, AKI is not necessarily characterized by a loss of kidney epithelial cells, but cell death is common in severe episodes of AKI. Acquired kidney cystic disease is usually not clinically relevant although bleeding may occur[3]. However, acquired kidney cystic disease is a risk factor for renal cell carcinoma[7,8]. Both acquired kidney cystic disease and kidney cancer are associated with an increase in cell number. In kidney cysts, an excess number of epithelial cells allows cyst growth. Kidney cancer is characterized by an increased number of malignant epithelial cells; the goal of therapy in this context is to kill or extirpate these cells. Renal cell carcinoma may occur in individuals with or without prior CKD. However, CKD is a risk factor for renal cell carcinoma[4]. Of note, acquired cystic kidney disease renal cell carcinoma is considered a distinct renal neoplasm according to the International Society of Urological Pathology–WHO, and is associated with specific somatic mutations[5]. ECM, extracellular matrix; GFR, glomerular filtration rate.

therapies aimed at preventing tubular cell death in CKD should not interfere with antitumour surveillance mechanisms that kill cells in the early stages of tumorigenesis[8]. Thus, interventions that aim to prevent cell death should ideally differentially affect different cell types, to avoid adverse consequences[40].

Promoting cell death

Approaches to promote cell death underlie the mainstay of current anticancer therapies and are also used clinically to eliminate autoimmune lymphoid cells. A better understanding of the specific molecules that promote the survival of kidney cancer cells could enable specific

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Apoptosis

b **Regulated necrosis**
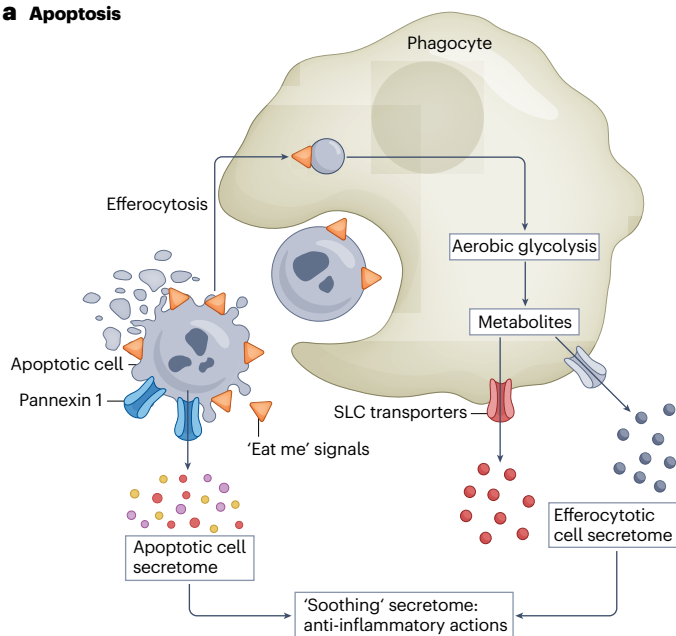
Fig. 2 | Interaction between apoptosis, regulated necrosis and inflammation.
a, The process of apoptosis clears unwanted or damaged cells during development and under homeostatic conditions. Multiple steps within the process of apoptosis convey signals to dampen inflammation. Cells that are undergoing apoptosis display cell surface molecules ('eat me' signals) that promote their rapid engulfment (efferocytosis) and clearance by adjacent cells before the plasma membrane ruptures and the pro-inflammatory contents are released[11]. Additionally, caspase-mediated opening of pannexin 1 channels at the plasma membrane of the apoptotic cell facilitates the release of an apoptotic metabolite secretome that comprises AMP, GMP, creatine, spermidine, glycerol-3-phosphate, ATP, fumarate, succinate and other compounds that suppress inflammation in adjacent cells[12]. Furthermore, efferocytosis itself is associated
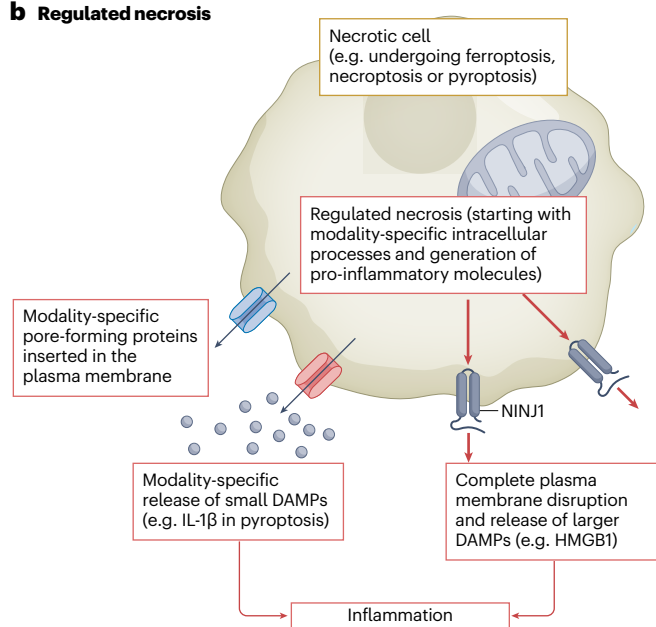
with changes in phagocyte metabolism, including a switch to aerobic glycolysis and the release of glycolytic by-products such as lactate through solute carrier family (SLC) transporters to promote anti-inflammatory responses in adjacent cells[13]. Thus, overall the processes of apoptosis and efferocytosis are associated with a complex programme that promotes the 'soothing' of adjacent cells.
b, By contrast, regulated necrosis can engage innate and adaptive immune responses at multiple steps through, for example, the lack of early engulfment, the generation of pro-inflammatory molecules (for example, IL-1β in pyroptosis) and the release of small damage-associated molecular patterns (DAMPs) through pore-forming proteins that are specific for each form of regulated necrosis. The final common pathway of these actions is disruption of the plasma membranes through the involvement of NINJ1, which allows the release of larger DAMPs.

targeting of these molecules and increase the safety of interventions. Therapeutic approaches to the promotion of cell death may also be considered to clear unwanted cells that actively contribute to kidney inflammation and fibrosis. For example, apoptosis of myofibroblast populations contributed to renal recovery following ischaemia–reperfusion injury-associated AKI (IRI-AKI)[41], whereas specific clearing of macrophages successfully halted disease progression in a model of experimental crescentic glomerulonephritis[42]. Ideally, such unwanted cells should be cleared by promoting apoptosis, given the potential adverse pro-inflammatory consequences of regulated necrosis that might amplify kidney injury by invoking a more intense inflammatory response or by triggering autoimmunity. Kidney cancer may be an exception, since promoting regulated necrosis-induced immunogenicity towards malignant cells may be beneficial[33]. In other contexts, the need to both promote and prevent cell death should be balanced. For example, efforts to prevent the death of tubule cells induced by the nephrotoxic effects of cisplatin should not interfere with the ability of cisplatin to induce the death of tumour cells.

Apoptosis

Caspase-dependent apoptosis accounts for approximately 90% of cell turnover under homeostatic conditions[43]. Its homeostatic role is closely

linked to its non-inflammatory nature[11–13] (Fig. 2). From the early 1990s to the early 2010s, the kidney and cell death literature was dominated by reports of the contribution of apoptosis to the pathogenesis of AKI and CKD[15,44]. However, apoptosis may also contribute to kidney repair by clearing excess myofibroblasts and tubule cells following their compensatory proliferation after injury[41,45].

Apoptosis has specific morphological features that can be assessed histologically (Supplementary Table 1). However, the identification of morphologically apoptotic cells in tissue sections is burdensome and hampered by their short half-life[46], and was soon replaced by easier-to-quantify histological assessment of DNA breaks as identified by terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL)[47,48] or evidence of caspase 3 activation[49]. However, TUNEL might stain necrotic cells[50] and caspase 3 activation is now known to contribute to pyroptosis through the cleavage of gasdermins[51]. Thus, older studies of apoptosis in kidney disease may not have clearly differentiated apoptosis from regulated necrosis.

The molecular regulation of apoptosis has largely been characterized[15] (Fig. 3a,b). However, and as discussed below, we now know that multiple connections exist between apoptosis and cascades of regulated necrosis (Figs. 3,4). For example, some caspases can cleave gasdermins and gasdermin amino-terminal (NT) fragments to

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regulate pyroptosis[30,51]. In addition, TNF superfamily cytokines that usually promote apoptosis by recruiting death receptor multiprotein complexes might also activate receptor-interacting serine/threonine-protein kinase 1 (RIPK1) and thereby trigger necroptosis in the context of caspase 8 inhibition (Fig. 3c,d). Moreover, compounds that induce apoptosis, such as BH3-mimetic drugs or inhibitors of anti-apoptotic BCL-2 family proteins, cooperate with inducers of ferroptosis in causing cell death[52]. In this regard, the tumour-suppressive transcription factor p53 promotes apoptosis by upregulating mediators of apoptosis, such as BAX, and downregulating anti-apoptotic molecules such as BCL-2 (refs. [53,54]), but also sensitizes cells to ferroptosis by downregulating *SLC7A11*, which encodes a subunit of the system x_c^- cystine/glutamate antiporter, and consequently decreasing cystine uptake[55] (Fig. 3e). Nephrotoxic drugs, such as cisplatin, might also induce apoptosis and ferroptosis in tubule cells, and targeting a single molecule, such as dipeptidase 1 (DPEP1), may protect from both[56].

The role of apoptosis in kidney disease is supported by studies in humans, demonstrating activation of pro-apoptotic pathways in kidney tissue, and by preclinical data, which indicate that interference with bona fide pro-apoptotic proteins is protective[15]. In an example that reached early clinical development, targeting of p53 protected cultured tubule epithelial cells from apoptosis and mice from cisplatin-induced AKI and IRI-AKI[57,58]. However, a phase III trial of teprasiran — a small interfering RNA that targets p53 — in >1,000 high-risk patients undergoing

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Box 1

Key operational definitions of cell death terms

Accidental cell death
Virtually instantaneous and uncontrollable form of cell death corresponding to the physical disassembly of the plasma membrane caused by extreme physical, chemical or mechanical cues.

Regulated cell death
Results from the activation of one or more signal transduction modules, and hence can be pharmacologically or genetically modulated (at least kinetically and to some extent). Variants of regulated cell death include programmed cell death (PCD), apoptosis and regulated necrosis.

Programmed cell death
Occurs in strictly physiological scenarios; that is, it does not relate to perturbations of homeostasis and hence does not occur in the context of failing adaptation to stress. PCD usually takes place by apoptosis

Apoptosis
Regulated cell death executed by enzymes termed caspases and characterized by specific morphological features (blebbing and cell and nuclear fragmentation, pyknosis), initial preservation of plasma membrane integrity and rapid engulfment by adjacent cells. It is considered not to promote inflammation

- **Intrinsic apoptosis**: initiated by perturbations of the extracellular or intracellular microenvironment, demarcated by mitochondrial outer membrane permeabilization, and precipitated by executioner caspases, mainly caspase 3
- **Extrinsic apoptosis**: initiated by perturbations of the extracellular microenvironment detected by plasma membrane receptors, propagated by caspase 8 and precipitated by executioner caspases, mainly caspase 3

Regulated necrosis
Regulated cell death characterized by an early loss of plasma membrane integrity and release to the extracellular space of intracellular contents that may be sensed as damage-associated molecular patterns (DAMPs) that activate innate and adaptive immune responses (that is, they may promote inflammation)

- **Autosis**: a specific instance of autophagy-dependent cell death that critically relies on the plasma membrane Na^+/K^+-ATPase
- **Ferroptosis**: initiated by oxidative perturbations of the intracellular microenvironment that is under constitutive control by glutathione peroxidase 4 (GPX4) and can be inhibited by iron chelators and lipophilic antioxidants. A key feature is peroxidation of membrane phospholipids
- **Necroptosis**: triggered by perturbations of extracellular or intracellular homeostasis that critically depends on mixed lineage kinase domain-like protein (MLKL) and receptor-interacting serine/threonine-protein kinase 3 (RIPK3), and (at least in some settings) on the kinase activity of RIPK1
- **Pyroptosis**: critically depends on the formation of plasma membrane pores by members of the gasdermin protein family, often (but not always) as a consequence of inflammatory caspase activation
- **NETotic cell death**: Reactive oxygen species (ROS)-dependent modality restricted to cells of haematopoietic derivation and associated with the extrusion of neutrophil extracellular traps (NETs; networks of extracellular material, primarily composed of DNA from neutrophils, that bind pathogens but have also been implicated in triggering autoimmune responses in anti-neutrophil cytoplasmic autoantibody vasculitis and systemic lupus erythematosus)
- **Mitochondrial permeability transition-driven necrosis**: triggered by perturbations of the intracellular microenvironment and relying on CYPD

Immunogenic cell death
Activates an adaptive immune response in immunocompetent hosts. This has been mainly linked to regulated necrosis.

Adapted from ref. 10, Springer Nature Limited.

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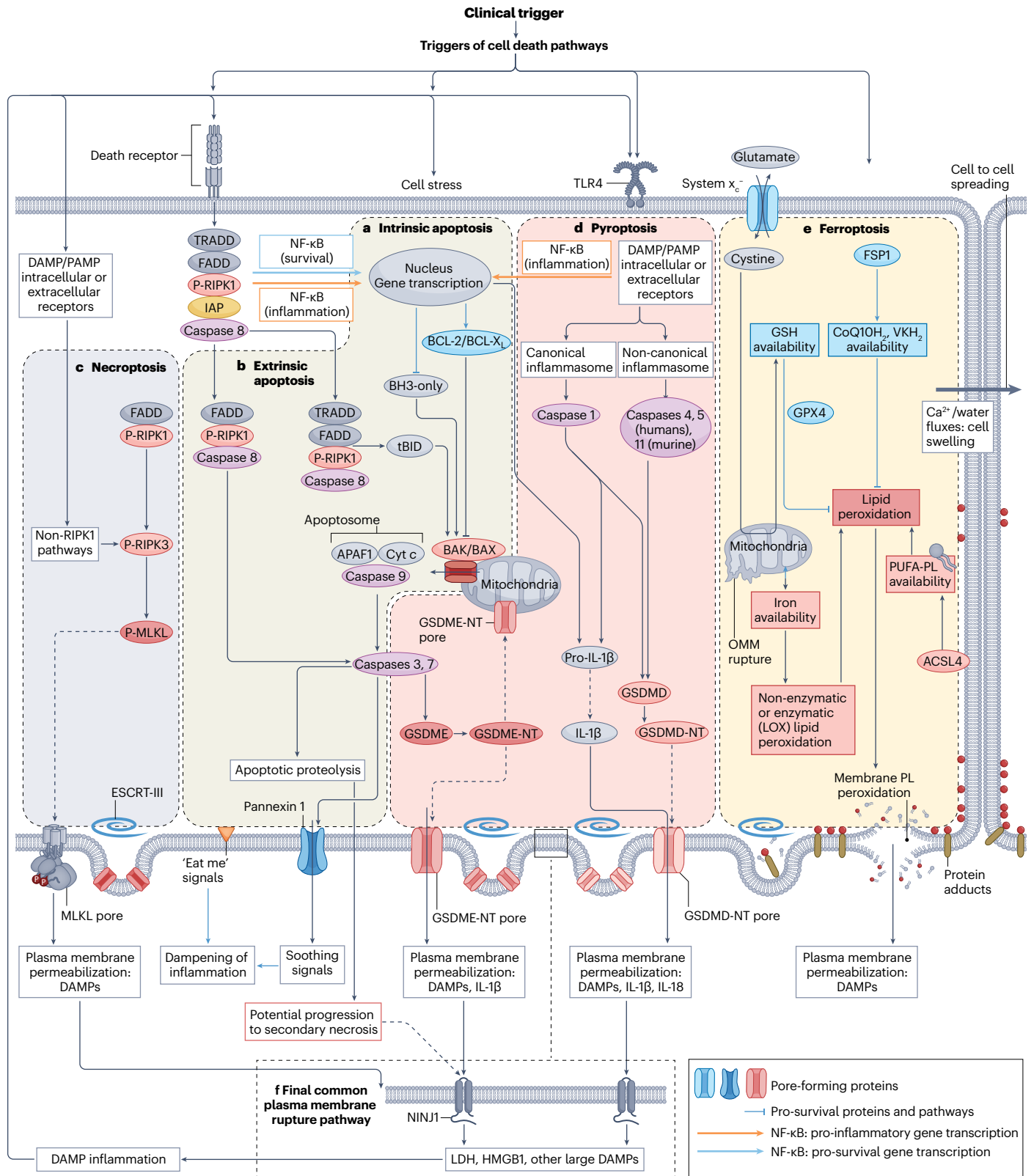
Fig. 3 | Key molecular processes and interactions between cell death pathways. Two main pathways for apoptosis are well characterized. **a**, In the intrinsic pathway, stressors or the lack of survival signals decrease the balance between pro-survival BCL-2 family proteins and pro-apoptotic BH3-only proteins, favouring the BAK/BAX-dependent mitochondrial outer membrane permeabilization, which results in the release of pro-apoptotic mitochondrial proteins, including cytochrome *c*, and leads to the formation of a multiprotein structure called the apoptosome that incorporates APAF1 and procaspase 9. This series of events results in activation of caspase 9 — an initiator caspase that subsequently activates executioner caspases such as caspase 3 and caspase 7 that contribute decisively to the dismantling of cell structures. **b**, In the extrinsic apoptosis pathway, activation of death receptors of the tumour necrosis factor receptor (TNFR) superfamily leads to activation of the initiator caspase 8, which in turn activates executioner caspases and may also process the BH3-only protein BID to tBID, which activates the mitochondrial apoptotic pathway. However, there are alternative outcomes for TNFR activation by TNF, depending on the recruitment of specific proteins to multiprotein complexes. The presence of inhibitor of apoptosis (IAP) proteins activates NF-κB to increase the transcription of anti-apoptotic proteins, although NF-κB can also induce a pro-inflammatory response. In absence of IAPs, caspase 8 is activated. Inhibition of caspase 8 may trigger necroptosis (see below). Apoptotic cells express "eat-me" signals on their surface, which promotes their rapid engulfment (efferocytosis) by adjacent cells. In addition, they secrete anti-inflammatory molecules (soothing signals). However, extensive apoptosis or decreased clearance of apoptotic cells may lead to secondary necrosis, in which the cell membrane is permeabilized through engagement of NINJ1 and damage-associated molecular patterns (DAMPs) released. **c**, Necroptosis. The core features of necroptosis are receptor-interacting serine/threonine-protein kinase 3 (RIPK3) activation by phosphorylation and the subsequent phosphorylation of mixed lineage kinase domain-like protein (MLKL). Phosphorylated MLKL oligomerizes to form pores that disrupt the plasma membrane, allowing the release of cell contents. Canonical RIPK3 activation is mediated by its interaction with the RIP homotypic interaction motif-containing proteins RIPK1, TIR domain-containing adapter molecule 1 (TICAM1) or Z-DNA-binding protein 1 (ZBP1). Binding of RIPK3 to RIPK1 occurs in response to activation of TNFRs, whereas binding of RIPK3 to TICAM1 is induced by activation of Toll-like receptors (TLRs), and binding to ZBP1 occurs in response to the presence of Z-RNA as a result of viral infection. Non-canonical RIPK3 activation occurs in response to activation of the cell membrane Na$^+$/H$^+$ exchanger 1 and increased intracellular pH (not shown). **d**, Pyroptosis. The key feature of pyroptosis is the enzymatic processing of gasdermins to amino-terminal (NT) fragments that oligomerize to assemble a plasma membrane pore, allowing the release of intracellular contents and DAMPs. The best-characterized model involves the NLRP3 (canonical) inflammasome-mediated activation of caspase 1 in macrophages, which processes pro-IL-1β to IL-1β, pro-IL-18 to IL-18 and gasdermin D (GSDMD) to GSDMD-NT. However, other inflammasomes and enzymes may process gasdermins in other cell systems and IL-1β may not be a major component of pyroptosis in cell types with low IL-1β gene expression. Additionally, gasdermins may permeabilize mitochondria, which, together with the potential for caspase 3 to cleave gasdermin E (GSDME) and caspase 8 to cleave GSDMD, provides links to apoptosis. **e**, Ferroptosis. The central event in ferroptosis is the peroxidation of plasma membrane phospholipids (PL) in an iron-dependent manner. The sensitivity of cells to ferroptosis will depend on iron availability (noting that mitochondria may serve to store iron), and on the capacity for enzymatic (for example, mediated by lipoxygenases (LOX)) or non-enzymatic lipid peroxidation as well as on the presence of cell defences against lipid peroxidation, including adequate glutathione (GSH) stores which are maintained through the entry of cystine through the system x$_c^-$ cystine/glutamate antiporter. The enzyme glutathione peroxidase 4 (GPX4) requires GSH for its antioxidant function. Additional antioxidant systems include ferroptosis suppressor protein 1 (FSP1), which maintains vitamin K (VK) and coenzyme Q10 (CoQ10; also known as ubiquinone) in a reduced state (VKH$_2$ and CoQ10H$_2$, respectively). The main consequence of ferroptosis is peroxidation of membrane PL, shown as red dots in the figure, that results in membrane protein adducts and membrane rupture. Following some triggers, ferroptosis may spread from cell to cell in a wave-like pattern via a process that involves volume shifts and calcium fluxes, as has been described in kidney tubules. **f**, Shared features between the different cell death modalities include the ability of the repair machinery, endosomal sorting complexes required for transport III (ESCRT-III), to repair membranes and the need for NINJ1 in the final step of plasma membrane fragmentation, which leads to the release of the larger DAMPs and proteins. However, NINJ1 is not required for plasma membrane rupture during ferroptosis. Of note, although all molecular pathways in the figure are represented in the same cell to emphasize the interconnections between pathways, not all cell types have the intracellular machinery or microenvironment required for all forms of regulated cell death to proceed. For pyroptosis, the increased transcription, processing and release of IL-1β have been mainly characterized in macrophages, whereas epithelial cells have a more limited capacity to release this interleukin. ACSL, long-chain fatty acid–CoA ligase 4; OMM, outer mitochondrial membrane; PAMP, pathogen-associated molecular pattern; PUFA, polyunsaturated fatty acid; PUFA-PL, PUFA-containing phospholipids.

cardiac surgery was terminated for lack of efficacy in reducing major adverse kidney-related events[59] despite promising phase II results[60].

Our improved understanding of shared molecular mechanisms between apoptosis and pathways of regulated necrosis requires a re-evaluation of the established literature on apoptosis and the kidney Unintended modulation of regulated necrosis mechanisms by interventions that aim to inhibit apoptosis may compromise their safety and efficacy; improved understanding of the interconnectivity of molecular events may optimize therapeutic approaches that target apoptosis.

Regulated necrosis

Several forms of regulated necrosis exist — each characterized by specific molecular programmes and response to inhibitors.

Necroptosis

Necroptosis is a kinase-mediated form of regulated necrosis that is characterized by the oligomerization and phosphorylation of RIPK3 and phosphorylation of the pseudokinase mixed lineage kinase domain-like protein (MLKL) (Fig. 3c). Phosphorylation of MLKL promotes its oligomerization and translocation to the plasma membrane where it executes necroptosis by inducing calcium influx, exposing phosphatidyl serine on the outer leaflet of the plasma membrane and disrupting the plasma membrane[61,62].

The best-characterized trigger for necroptosis is TNF receptor activation, which leads to the formation of RIPK1–RIPK3 complexes under conditions that prevent caspase 8 activation (Supplementary Fig. 4a). However, additional canonical and non-canonical pathways for RIPK3 activation exist that do not require RIPK1[63,64] (Fig. 3c); moreover, RIPK3 can promote inflammatory responses independent of necroptosis[65,66]. In kidney cells, inhibition of caspases prevents apoptosis, but may lead to RIPK1-mediated necroptosis, as observed in a model of cytokine-induced tubule cell death[18,67].

Inhibitors of necroptosis are termed necrostatins and — together with genetically modified mice — have been instrumental in characterizing the role of necroptosis in disease. However, early necrostatins were non-specific and, hence, caution is warranted when interpreting findings from studies that have used these tools. For example, necrostatin 1 (Nec1) — the first necroptosis inhibitor described — inhibits both

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RIPK1 and indoleamine-2,3-dioxygenase (IDO), which catabolizes tryptophan into kynurenine and modulates innate and adaptive immunity, and other enzymes[68,69]. Moreover, Nec1 may also inhibit ferroptosis induced by glutathione peroxidase 4 (GPX4) deficiency in fibroblasts in a manner that is independent of IDO and RIPK1 inhibition[70]. A more recently developed compound, Nec1f, simultaneously inhibits RIPK1 and some forms of ferroptosis in tubular cells, but has not been compared with Nec1, and its mechanism of ferroptosis inhibition is poorly understood[71]. Nec1s is a more selective and stable inhibitor of RIPK1 than Nec1, and does not inhibit ferroptosis[69,70].

Further complicating the study of necroptosis, specific RIPK3 inhibitors, such as GSK′872, also promote apoptosis by interacting with RIPK3 to activate caspase 8 via the recruitment of RIPK1, resulting in the assembly of a caspase 8–FADD–cFLIP complex independent of the effects on MLKL[72] (Supplementary Fig. 4a). This pathway is also induced by certain kinase-dead mutants of RIPK3 (ref. 72). Such crosstalk between components of necroptotic, apoptotic and ferroptotic pathways complicates assessment of the role of necroptosis in kidney injury in vivo as well as approaches for its therapeutic targeting.

Pyroptosis

Pyroptosis is a pro-inflammatory form of regulated necrosis that involves the formation of cell membrane pores by oligomers of the N-terminal fragment of gasdermins (gasdermin-NT) following gasdermin cleavage[73,74] (Fig. 3d). Pyroptosis contributes to the innate immune response and can be triggered by inflammasome-dependent and inflammasome-independent pathways. The best-characterized pyroptosis pathway occurs in macrophages, and is triggered by engagement of damage-associated molecular patterns (DAMPs) and pathogen-associated molecular patterns with the NLRP3 inflammasome, leading to activation of caspase 1. Caspase 1 consequently cleaves gasdermin D (GSDMD) to generate GSDMD-NTs that oligomerize and insert into the plasma membrane to cause membrane permeabilization[75,76]. Inflammasome and caspase 1 activation also increases processing and secretion of pro-inflammatory cytokines such as IL-1β and IL-18. Of note, the release of IL-1β through GSDMD-NT-induced pores is a key feature of macrophages undergoing pyroptosis but can also occur in viable macrophages, indicating that the role of GSDMD-NT pores in cell death and IL-1β release can be dissociated[77]. The mechanisms underlying this dissociation are unclear, but may involve the actions of defence mechanisms against membrane disruption by pore-forming proteins (such as the endosomal sorting complexes required for transport III (ESCRT-III) machinery, as described below), dynamic regulation of pore opening by local phosphoinositide circuitry, blockade of downstream NINJ1 membrane clustering, or other regulatory mechanisms[78–80]. Gasdermins can also be processed to pore-forming NT fragments by caspases other than caspase 1, including endogenous or microbial proteases (Supplementary Fig. 4b,c). For example, inflammasome-activated caspase 11 (or its human homologues caspase 4 and caspase 5) can also process GSDMD to generate GSDMD-NT[75,76] whereas caspase 3 processes gasdermin E (GSDME) to generate GSDME-NT in epithelial cells to trigger pyroptosis[81]. Neutrophil pyroptosis also contributes
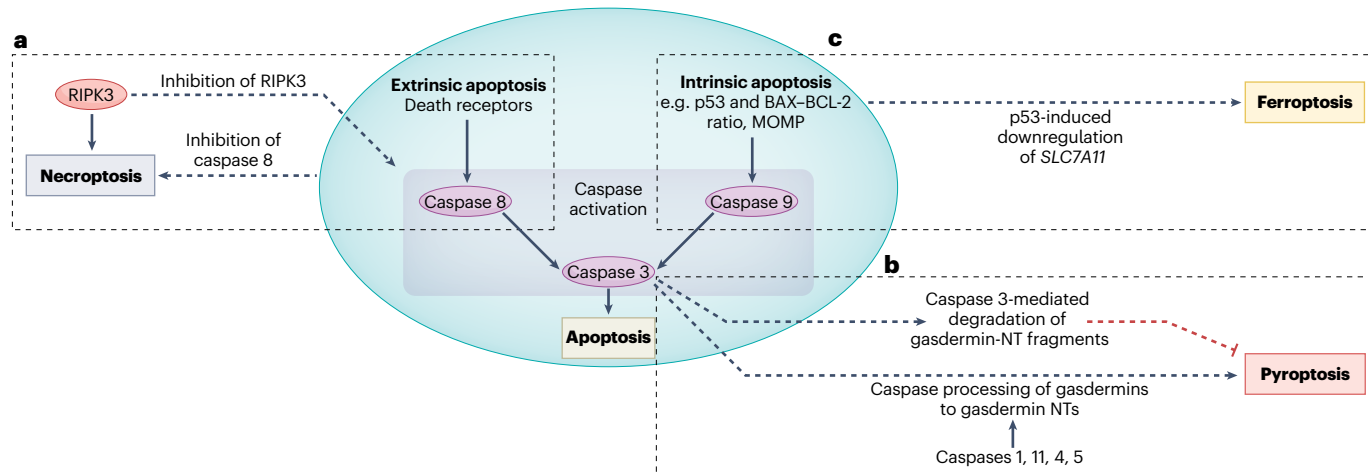
Fig. 4 | Examples of interactions between apoptosis and regulated necrosis. The molecular pathways that lead to activation of extrinsic or intrinsic apoptosis may also modulate or trigger different forms of regulated necrosis. Thus, interventions aimed at inhibiting apoptosis may trigger or modulate modalities of regulated necrosis, potentially affecting their safety and efficacy. **a**, Following the activation of death receptors, such as TNF receptor, inhibition of caspase 8 promotes necroptosis over apoptosis. Conversely, inhibitors of receptor-interacting serine/threonine-protein kinase 3 (RIPK)3 or certain kinase-dead RIPK3 mutants will prevent necroptosis but result in the assembly of multimeric protein complexes that activate caspase 8 and induce apoptotic cell death. **b**, Caspases are among the enzymes than can cleave gasdermins to generate amino-terminal (NT) fragments that oligomerize at the plasma membrane to form protein pores and trigger pyroptosis. In pyroptosis, canonical activation of the NLRP3 inflammasome results in activation of caspase 1, whereas non-canonical inflammasomes will activate murine caspase 11 (caspase 4 and caspase 5 in humans). In apoptosis, the executioner caspase 3 is activated by either caspase 8 (extrinsic apoptosis pathway) or caspase 9 (intrinsic apoptosis pathway). Caspases that are activated during apoptosis, such as caspase 8 and caspase 3 may also cleave gasdermins to gasdermin-NT fragments, as exemplified here for caspase 3. Additionally, caspase 3 may degrade NT fragments from gasdermins B and D, potentially protecting from pyroptosis. A more detailed representation of the interaction of caspases with gasdermins and gasdermin NTs is shown in Supplementary Fig. 4b. **c**, Upstream regulators of apoptosis may also modulate ferroptosis. As an example, the tumour-suppressive transcription factor p53 promotes apoptosis by upregulating mediators of apoptosis, such as BAX, and downregulating anti-apoptotic molecules such as BCL-2 (refs. 53,54), but also sensitizes cells to ferroptosis by downregulating *SLC7A11*, which encodes a subunit of the system x_c^- cystine–glutamate antiporter, and consequently decreasing cystine uptake[55]. MOMP, mitochondrial outer membrane permeabilization.

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to the process of NETosis and can be triggered by non-caspase proteolytic enzymes[82,83] (Supplementary Fig. 4c). Although gasdermins are expressed in the kidney, the contribution of pyroptosis to kidney disease is unclear despite beneficial effects of targeting the NLRP3 inflammasome[84], since inflammasome activation and the release of IL-1β are not necessarily indicative of pyroptotic cell death[85].

Ferroptosis

Ferroptosis is characterized by the iron-dependent peroxidation of membrane phospholipids and the subsequent formation of protein adducts and disruption of the plasma membrane[86,87] (Fig. 3e). Ferroptosis can be triggered by a loss of the reduction capacity of cells. Thus, cell sensitivity to ferroptosis is critically dependent on the availability of iron and on the inactivation or suboptimal function of antioxidant defences. Additionally, increased availability of lipid substrates through an increased activity of the enzyme long-chain fatty acid–CoA ligase 4 (ACSL4) favours ferroptosis. The process of lipid peroxidation can involve enzymatic pathways (for example, involving iron-containing lipoxygenases (LOXs)) or involve non-enzymatic mechanisms. The peroxidation of membrane phospholipids contributes to disruption of the outer mitochondrial membrane disruption and rupture of the plasma membrane by as-yet poorly characterized mechanisms that may include the induction of protein adducts[31,70].

The entry of cystine into cells through the system x_c^- cystine–glutamate antiporter enables the repletion of glutathione (GSH), which is required for GPX4 to catalyse the reduction of double-oxygenated phospholipids into mono-oxygenated phospholipids[70]. In parallel, ferroptosis suppressor protein 1 (FSP1) maintains vitamin K (VK) and coenzyme Q10 (CoQ10; also known as ubiquinone) in a reduced state (VKH$_2$ and CoQ10H$_2$, respectively), further enabling the reduction of phospholipids. Loss of FSP1 or GPX4 function sensitizes kidney tubule cells to ferroptosis, with one study demonstrating that acquired systemic loss of GPX4 or FSP1 sensitizes mice to AKI[71].

One interesting feature of ferroptosis is that it can rapidly propagate from cell to cell, in a wave-like manner[88]. Such synchronized necrosis has been observed in the renal tubules of models of preclinical IRI and oxalate crystal-induced AKI[23]. Synchronization of tubule cell death is not observed for necroptosis or pyroptosis, which may explain differences in the extent and distribution of kidney tubule involvement in models of AKI. However, synchronized ferroptosis may be stimulus-dependent, as was observed following a decrease in GSH availability achieved through inhibition of the system x_c^- or in the context of excess iron, but not following inhibition of GPX4 (ref. 88). Propagation of the ferroptosis-inducing signal occurs upstream of cell rupture and involves calcium fluxes and the spreading of a cell swelling effect in a lipid peroxide-dependent and iron-dependent manner[88]. In zebrafish, sublethal lipid peroxidation may also spread from cell to cell[89].

Ferroptosis may be modulated by the availability of iron or antioxidants (for example, GSH, VKH$_2$ and CoQ10H$_2$), by inhibiting enzymes that synthesize or peroxidize phospholipids or by peroxyl radical scavengers. The radical trapping antioxidants ferrostatin 1 and liproxstatin 1 have been widely used to inhibit ferroptosis, but ferroptosis may also be inhibited by Nec1, Nec1f and other non-specific compounds[31].

Interconnections between cell death pathways

Multiple layers of interconnections exist between different modalities of regulated cell death. These include molecular events that are shared between different cell death modalities, including shared triggers, final execution events and cell protective mechanisms (Figs. 3,4

and Supplementary Fig. 4), but also the possibility that one form of cell death may induce a different form of cell death in the same or other cells (Fig. 5).

Moreover, the same stimulus may induce different forms of regulated cell death, depending on the intensity of the stimulus (Fig. 5a), or on the presence of co-stimulatory factors. Outside the kidney, it has been well characterized that some stimuli (for example, viruses) can simultaneously trigger pyroptosis, apoptosis and necroptosis in a process termed PANoptosis in innate immune cells, through the generation of PANoptosome complexes that integrate components of the individual cell death pathways in a multiprotein complex that includes caspases, RIPK1, RIPK3 and ZBP1 (refs. 90,91) (Fig. 5b). Caspase 6 regulates ZBP1-mediated NLRP3 inflammasome activation and PANoptosis in a protease-independent manner[91]. However, although increased caspase 6 levels or activity have been observed in preclinical models of AKI or CKD, the role of caspase 6 in kidney disease has not been characterized[92,93]. When several cell death programmes are activated concurrently, inhibition of just one molecular pathway will not prevent cell death but rather may change the modality of cell death, as observed for different apoptosis pathways[94]. For example, MLKL deficiency in cultured fibroblasts prevented necroptosis but favoured the occurrence of ferroptosis induced by cystine deprivation, whereas deficiency of the pro-ferroptotic protein ACSL4 prevented ferroptosis but favoured the occurrence of necroptosis induced by TNF and pan-caspase inhibition[95]. Similar interconnections have been described between apoptosis and pyroptosis, and between extrinsic apoptosis and necroptosis, including in the context of cytokine-induced tubular cell death[18,30,51,67,96] (Fig. 4a,b). In the latter case, inhibition of multiple caspases by the pan-caspase inhibitor zVAD-fmk shifted the mode of cell death from apoptosis to pro-inflammatory necroptosis and increased the number of dead cells[67,96], illustrating the potential dangers of interfering with cell death pathways.

The ESCRT-III machinery facilitates membrane repair following the engagement of regulated necrosis pathways, and therefore represents a survival mechanism[62,97,98]. ESCRT-III protects cells from necroptosis, ferroptosis and pyroptosis by trapping sections of membrane that have been damaged or in which protein pore complexes have oligomerized, and shedding them into intracellular or extracellular vesicles[97]. As a consequence, knockdown of ESCRT-III sensitizes cells to regulated necrosis. Charged multivesicular body protein 1A (CHMP1A) is a component of ESCRT-III that protects cells from ferroptosis and was identified in a genome-wide association study as a risk gene for kidney disease[56].

NINJ1-mediated plasma membrane disruption seems to be a final common pathway shared by pyroptosis, necroptosis and secondary forms of necrosis when apoptotic cells are not rapidly cleared, that triggers cell lysis and the release of large DAMPs[99]. The protective effects of glycine against cell membrane rupture may be mediated by inhibition of NINJ1-mediated membrane clustering[80]. However, NINJ1 is not required for ferroptotic cell lysis[100] (Fig. 3f). Beyond NINJ1, other stimuli might trigger different forms of cell death in different cell types (Fig. 5c). The precise cell death pathways involved may depend on the nature or severity of the insult, the existence of environmental or genetic modulators of gene expression, the sensitivity of cells to potentially lethal stimuli, the specific cell types involved and the strain or species studied.

A different form of interaction takes place when a specific modality of cell death triggers cell death in other cells or cell types (Fig. 5d–f). For example, the regulated necrosis of tubule cells might release pro-inflammatory mediators and recruit innate immune cells, such as
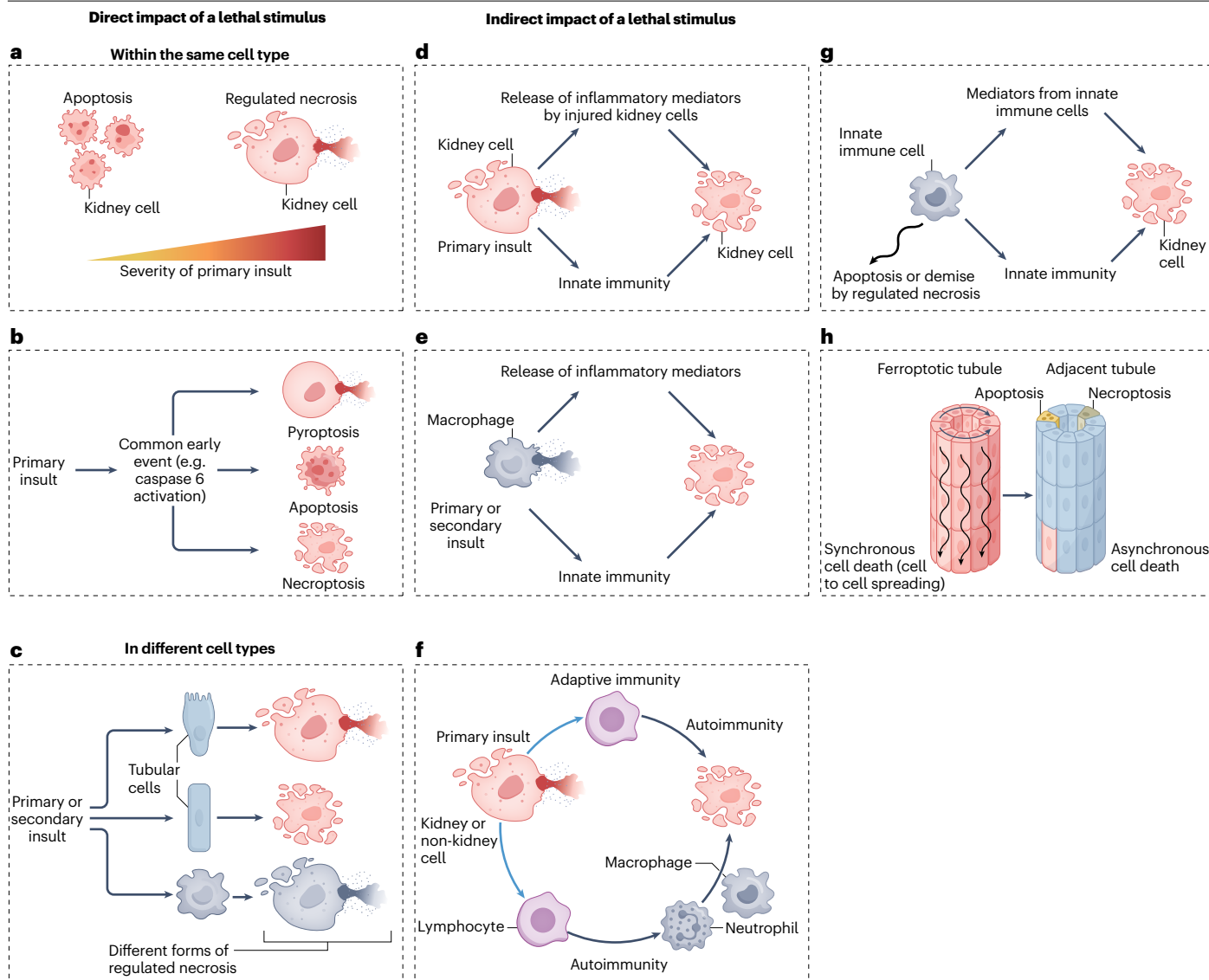
Fig. 5 | Interactions between different types of regulated necrosis: conceptual models that may explain the involvement of multiple forms of cell death in the same kidney disease. The interconnectivity of different modes of cell death suggests that multiple modes of cell death probably concurrently or sequentially coexist within the diseased kidney. The mechanisms underlying the initiation of different cell death pathways are complex and may involve various processes. Primary insults are triggers of injury (for example, cisplatin or ischaemia–reperfusion) and secondary insults are the mediators released in response to cell stress or cell death, such as TNF superfamily cytokines. **a**, The severity of insult may affect the activation of different cell death pathways. Of note, necrosis is not necessarily associated with a greater severity of insult. **c**, A primary insult might activate several cell death programmes in the same cell that are mutually exclusive so that inhibition of one will result in death from the other. PANoptosis triggered by caspase 6 activation is one example of such an event, and the interaction between extrinsic apoptosis and necroptosis illustrated in Fig. 4a is another example. **c**, Primary or secondary insults may differentially impact the mode of cell death in different cell types. **d**, A primary insult (the cause of the kidney disease) may result in the release of inflammatory mediators by injured kidney cells (secondary insults) and the release of danger-associated molecular signals that trigger an innate immune response that, in turn, amplifies cell death. **e**, Regulated necrosis of innate immune cells might result in the release of inflammatory mediators and the activation of an innate immune response that, in turn, causes a different form of cell death in kidney epithelial cells. **f**, A primary insult might result in immunogenic cell death, and the resultant adaptive immune response might further cause cell death and tissue injury via antibodies or cytotoxic T cells. **g**, Cell death might clear innate immune cells and limit the inflammatory response. In this scenario, inhibition of cell death may result in persistent or amplified inflammation that triggers the death of kidney epithelial cells. **h**, Ferroptosis might induce synchronized renal tubule cell death following the intercellular propagation of cell death in a wave-like pattern, potentially affecting whole nephrons[23]. By contrast, apoptosis and necroptosis occur in isolated cells. Inflammatory cytokines released by cells recruited in response to ferroptosis may, in turn, induce apoptosis and/or necroptosis of individual tubule cells located in nearby tubules.

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macrophages, that in turn, release cytokines that amplify tubular injury, for example, through cytokine-induced necroptosis (Fig. 5d). The release of cytokines might similarly occur following the death of innate immune cells, such as macrophages, by pyroptosis (Fig. 5e). Regulated necrosis can also be immunogenic and stimulate an adaptive immune response to trigger autoimmunity (or alloimmunity in the case of kidney grafts); these immune cells may subsequently induce a different form of regulated death in their target cells (Fig. 5f). The immune response may not necessarily be directed against kidney cell antigens. For example, both necroptosis and ferroptosis may result in NETosis[82,83,101,102], and NETosis is thought to contribute to autoimmunity in anti-neutrophil cytoplasmic autoantibody (ANCA) vasculitis — a disease that is associated with kidney cell death (Fig. 5f). Finally, the death of inflammatory cells might limit inflammation and tissue injury during repair. In this context, inhibition of immune cell death might contribute to persistent inflammation or autoimmunity (Fig. 5g).

Overall, our understanding of the interconnections between different modalities of regulated cell death implies that the impact of targeting regulated necrosis pathways may differ according to the stage of disease or between different diseases according to the primary insult and the presence of secondary mediators of injury. The available evidence also implies that different forms of regulated cell death may coexist simultaneously in the same or different cell types, or be activated sequentially even in the same individual. In this regard, interventions that target secondary or minor forms of cell death rather than the predominant form of cell death or main cellular target of the disease might still provide benefit. For example, therapeutic benefit was achieved by targeting autosis — a form of regulated necrosis that was only observed in pericytes — in a preclinical model of IRI-AKI[20]. Finally, interventions that effectively inhibit a key modality of cell death might become ineffective if cells switch to a different form of death. As an example from the AKI literature, ferroptosis might propagate from cell to cell within the same tubule[23], and secondarily recruit an inflammatory response that may trigger necroptosis or other forms of cell death in adjacent tubules[96,103] (Figs. 5h,6), suggesting that the timing of interventions may be critical[96,103–105]. All of these possibilities must be considered when developing interventions to target cell death.

Regulated necrosis in kidney disease

Although evidence from interventional preclinical studies suggests a role for regulated necrosis in the pathogenesis of both AKI and CKD[17–19,21,23,27–31,70,71,96,101,103,106–152] (Supplementary Tables 2,4), evidence from human interventional studies is lacking. In the future, the clinical relevance of preclinical observations will be informed by the results of clinical trials.

Acute kidney injury

Morphological evidence of tubular cell apoptosis, necrosis and loss of tubule cells from undetermined causes has been observed in human AKI[153]. In general, inhibition of necroptosis and ferroptosis has been demonstrated to be protective in various models of AKI, including preclinical models of IRI-AKI, nephrotoxin-induced AKI, cytokine storm-associated AKI and crystal-induced AKI, whereas the role of pyroptosis in AKI is disputed[127,128,154]. However, only a handful of laboratories have addressed the relative contribution of the diverse forms of cell death under similar experimental conditions.

Ischaemia–reperfusion injury-induced AKI.
IRI-AKI is characterized by morphological evidence of tubule cell apoptosis and caspase

activation[155]. Protection is typically afforded by the inhibition of pro-apoptotic proteins such as BAX and BAK[156], whereas the effect of caspase inhibition is controversial[17,40,157]. In one study, administration of the pan-caspase inhibitor zVAD-fmk at the time of reperfusion prevented the early onset of IRI-AKI (at 24 h)[157]; however, other studies found no benefit of zVAD-fmk at 48 h after reperfusion[17,157], and found it to be ineffective when administered 2 h after reperfusion[157]. Opposing effects of caspase 3 deficiency have also been observed in the acute and chronic stages of IRI-AKI, with increasing tubule injury scores and serum creatinine levels in the early stages AKI and increasing chronic capillary rarefaction and renal fibrosis in longer term follow-up after IRI, illustrating the potential differential impact of cell death pathway interference on different cells types and on short-term versus long-term outcomes[40].

Evidence from interventional studies indicates that necroptosis, MPT-RN and ferroptosis may contribute to the pathogenesis of IRI-AKI[18,23]. In mouse models of IRI-AKI, inhibition of necroptosis through pretreatment with Nec1 or genetic deletion of RIPK3 afforded partial protection from tubule injury and increases in serum urea and creatinine levels[17,18]. Moreover, mice with deletion of both the mitochondrial protein CYPD and RIPK3 demonstrated greater protection from IRI-AKI than mice with deletion of CYPD or RIPK3 alone[18]. However, contradictory results also exist. For example, a randomized, controlled trial in 154 patients undergoing coronary artery bypass grafting surgery with extracorporeal circulation found that prophylactic cyclosporine did not reduce the risk of AKI[158], arguing against a pathophysiological role of MPT-RN in IRI-AKI. However, cyclosporine is nephrotoxic and may not therefore be a suitable agent to administer to patients at risk of AKI. Moreover, some studies have found that MLKL deficiency is not as protective as RIPK3 deficiency or catalytically inactive RIPK1 in preventing IRI-AKI[159], although other studies have found RIPK3 or MLKL deficiency to be equally protective[160]. Surprisingly, mice with deletion of both *Ripk3* and *Mlkl* were not protected from IRI-AKI, illustrating the complexities of cell death pathways[160].

Ferrostatin 1 and the more stable ferrostatin 1 analogue SRS 16-86 protected mice from severe IRI-AKI, reducing tubule injury and improving renal function[23]. Combined inhibition of necroptosis, MPT-RN and ferroptosis offered complete protection from severe IRI-AKI, being more effective than any monotherapy tested[23]. Nec1f, which, as discussed above, targets both RIPK1 and ferroptosis, also improved survival in a mouse model of IRI-AKI[71].

Evidence linking pyroptosis and, more specifically, specific mediators of pyroptosis to IRI-AKI is contradictory. Some studies have found that deletion of caspase 11 or GSDME protects mice from IRI-AKI[127,130], supporting a pathogenic role for pyroptosis in IRI-AKI. By contrast, others have found that *Gsdmd*-knockout mice develop more severe IRI-AKI, which is attenuated by co-deletion of MLKL[128], suggesting that necroptosis might be amplified if pyroptosis is blocked. That study also found that *Gsdme*-knockout mice were hypersensitive to IRI-AKI[128], again contradicting earlier work[130]. The reasons for these discrepant findings are not clear, however, and further work is needed to understand these issues.

Although available evidence suggests that necroptosis, MPT-RN, ferroptosis and potentially pyroptosis may contribute to IRI-AKI, whether individual pathways contribute to the loss of specific cell types and the factors that result in the activation of these pathways, are currently unclear. Moreover, the existence of discrepant results suggests that adverse outcomes may be a consequence of strategies that target specific pathways of regulated necrosis and at this point,
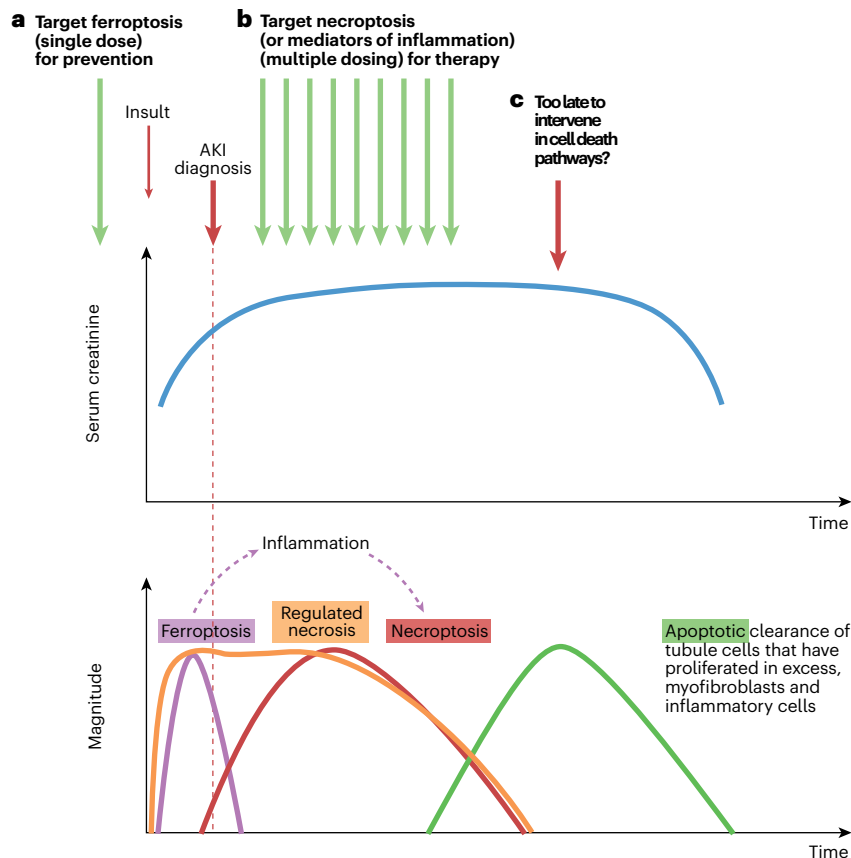
Fig. 6 | Interactions between different forms of cell death and their impact on the design of therapeutic approaches for different contexts of use in AKI. a, In a hypothetical scenario in which an insult causes an initial wave of cell death (for example, an early wave of ferroptosis-induced tubule cell death), the specific form of cell death may be targeted using a preventive approach; for example, with prophylactic administration of an inhibitor. This preventive intervention may avoid the initial wave of cell death and all subsequent consequences, including the development of acute kidney injury (AKI). **b**, If unchecked, the initial wave of regulated necrosis might result in the release of damage-associated molecular patterns (DAMPs) that triggers inflammation and a second wave of inflammatory cytokine-mediated regulated necrosis, such as necroptosis. In this scenario, a single preventive administration of a cytokine or necroptosis inhibitor will not prevent AKI, as it will not prevent the original wave of cell death caused by ferroptosis. However, necroptosis or other forms of inflammation-related cell death may be amenable to treatment, for example, through initiation of therapy after injury has occurred. This scenario represents the usual clinical situation, in which interventions are prescribed after

diagnosis of AKI. Therapy would be expected to be maintained over several days, since DAMPs will be continuously released by ongoing cell death. Successful treatment will not prevent AKI but will accelerate recovery by interfering with the amplification loop of inflammation-related cell death. Biomarkers of regulated necrosis will be needed to assess whether cell death is still ongoing and if so, what is the predominant active mode of kidney cell death. **c**, In advanced stages of AKI, apoptotic cell death acts to clear excess myofibroblasts and leukocytes as well as excess tubule cells; at this stage, interference with apoptosis might be deleterious while targeting other forms of cell death might be futile. This conceptual representation is based on observations in a mouse model of AKI induced by a folic acid overdose[96,103,104] and on descriptions of morphological apoptosis during the recovery phase of human AKI[105]. The role of ferroptosis in the early stages of this preclinical model and of the contribution of necroptosis to longer term outcomes has been validated in several studies[117,143]. However, validation of this conceptual representation for forms of human AKI requires further studies and clinical trials.

the extent to which human studies will reproduce the preclinical data is unclear.

Toxin-induced AKI. Nephrotoxic agents induce tubule cell death, with nephrotoxic AKI being the most common contributor to hospital-acquired AKI. Nephrotoxicity is the dose-limiting effect of the most widely studied nephrotoxic drug, the antineoplastic agent, cisplatin[161,162]. Apoptosis, necroptosis, MPT-RN, ferroptosis and pyroptosis may contribute to cisplatin-induced AKI. However, the literature

is not uniform. In human tubule cells, low cisplatin concentrations induce apoptosis, whereas higher concentrations induce necrosis[163]. In cultured primary proximal tubular cells, combined targeting of necroptosis (with the non-specific inhibitor Nec1) and caspases (with zVAD-fmk) provided better protection against cisplatin-induced death than individual treatments[109,164]. However, in another study that used human proximal tubule HK-2 cells, ferrostatin 1 provided weak protection against cisplatin-induced death, whereas Nec1 was not at all protective[120]. In mouse models of cisplatin-induced AKI,

inhibition of necroptosis through administration of Nec1 or genetic deletion of RIPK3 or MLKL prevented injury but did not reduce cell death as assessed by TUNEL staining[18,109]. Of note, dual inhibition of apoptosis and necroptosis pathways increased survival[18,109]. A role for MPT-RN and ferroptosis in cisplatin nephrotoxicity is supported by the greater survival of *Ppid*-knockout mice compared with that of wild-type mice[18], and the finding that a single prophylactic dose of ferrostatin 1 improved renal function and decreased kidney injury in mice, 72 h after administration of cisplatin[120]. However, similarly to IRI-AKI, the role of pyroptosis in cisplatin-induced AKI is less clear. Some studies have found that deletion of caspase 11, GSDMD or GSDME protects mice from cisplatin AKI[127,130], whereas in at least one study, deletion of GSDMD led to exacerbation of cisplatin-AKI[128].

Of more clinical interest than preclinical cisplatin nephrotoxicity are endogenous toxins, such as haem proteins (for example, haemoglobin and myoglobin, which are released following intravascular haemolysis or rhabdomyolysis, respectively) that may cause AKI, particularly in environments with inadequate access to kidney replacement therapy. Some evidence suggests that ferroptosis contributes to rhabdomyolysis-associated AKI[118]. Despite the activation of apoptosis and necroptosis pathways during rhabdomyolysis, only ferrostatin 1 reduced oxidative stress, cell death and preserved kidney function, both in vivo and in vitro, whereas the pan-caspase inhibitor zVAD and RIPK3 deficiency were not protective[107]. Inhibition of ferroptosis also has protective effects on organs outside the kidneys; for example, by reducing neuron lipid peroxidation and cell death after haemorrhagic stroke[165]. Additionally, inhibition of ferroptosis with three different molecules (ferrostatin 1, liproxstatin 1 and UAMC-3203) reduced both single (including the kidney) and multiple organ dysfunction, and prevented death in a mouse model of multiple organ dysfunction induced by intravascular haemolysis[166].

Thus, a body of evidence suggests the involvement of different specific forms of regulated necrosis in diverse models of nephrotoxicity, but the results are not always concordant. Such divergency may be expected, given different experimental conditions and the possibility that toxin doses may activate different molecular pathways. However, the heterogeneity in preclinical findings limits their clinical translation, given the lack of tools to identity the predominant form(s) of regulated necrosis under clinical conditions. Prevention of haem-induced AKI (that is, with myoglobin or haemoglobin) may offer a better opportunity for clinical development than prevention of cisplatin-induced AKI, unless the interventions can be locally targeted to tubule cells. The observed beneficial effect of targeting ferroptosis fits well with our understanding of the tubule iron overload that occurs with haem nephropathy.

Cytokine storm-associated AKI. A cytokine storm is a life-threatening systemic inflammatory syndrome, characterized by elevated levels of circulating cytokines and immune-cell hyperactivation in response to diverse triggers, including inflammatory disorders, such as endotoxaemia, sepsis and COVID-19, and can lead to the development of AKI[167,168]. Approaches to targeting bacterial sepsis (for example, as induced in animal models by caecal ligation and puncture (CLP)) and endotoxaemia (for example, as induced in animals by administration of lipopolysaccharide (LPS)) differ in that interventions for bacterial sepsis must not interfere with the antibacterial capabilities of the immune system, whereas experimental endotoxaemia is sterile. Targeting of ferroptosis (through administration of Fer1), necroptosis (through pharmacological or genetic inhibition of RIPK3) and

pyroptosis (through deletion of caspase 11, caspase 1 or GSDMD) has demonstrated kidney-protective effects in mouse models of sepsis and endotoxaemia[114,115,148,169]. However, the impact of *Mlkl* deletion is disputed in CLP with at least one study demonstrating a lack of kidney protection in *Mlkl*-knockout mice[114]. Combined deletion of *Ripk3* and *Gsdmd* or of *Mlkl* and *Gsdmd* provided better protection against CLP-induced AKI and death than deletion of either gene alone[169]. Moreover, combined deletion of *Ripk3* and *Gsdmd* also improved survival upon challenge with TNF or LPS than deletion of either gene alone[169]. Disulfiram — a drug that is used to treat alcohol addiction — inhibited GSDMD-induced pore formation, pyroptosis and cytokine release in cultured cells and LPS-induced septic death in mice, while allowing processing of IL-1β and GSDMD[170]. Thus, available evidence in general supports pathogenic roles for ferroptosis, necroptosis and pyroptosis in cytokine storm-induced AKI, suggesting these pathways could represent potential therapeutic targets.

Crystal-induced AKI. Crystal nephropathies can trigger three different pathways of regulated necrosis: necroptosis, ferroptosis and MPT-RN, and cause AKI and the development of CKD. Clear evidence also suggests that the NLRP3 inflammasome and IL-1β contribute to oxalate-induced kidney inflammation and AKI[84], although — as described below — the dependency of this process on pyroptosis has not been demonstrated.

Prophylactic inhibition of necroptosis, MPT-RN or ferroptosis offered protection from calcium oxalate-induced nephropathy 24 h after injury[19,23,108]. In another study, dual blockade of necroptosis and MPT-RN was more effective than monotherapy, but the effect of ferrostatin 1 was not tested[19]. Calcium oxalate may activate the inflammasome in kidney immune cells directly[84] or indirectly through DAMPs, such as ATP, released from tubule cells that undergo necrosis following internalization of calcium oxalate[84,171]. Inhibition of necroptotic tubular cell death prevented inflammasome activation in immune cells[84,108,171]. The dual necroptosis–ferroptosis inhibitor (Nec1f) was also protective[71], whereas the inhibition of apoptosis was not protective[108].

The role of pyroptosis in calcium oxalate-induced AKI has not been well characterized. In one study, neither GSDMD nor MLKL deficiency prevented monosodium urate crystal-induced cell death and IL-1β release in macrophages or in vivo[172]. However, *Gsdmd*-knockout mice had more severe kidney injury at 20 days (that is, during the subacute phase) than wild-type mice[128]. By contrast, other studies have shown that inhibition of the NLRP3 inflammasome protects mice against crystal-induced chronic nephrocalcinosis and fibrosis[173,174].

Folic acid-induced AKI (FA-AKI) is thought to be dependent on crystalluria[104] and to involve a sequence of events characterized by an initial wave of ferroptosis within the first 24 h that triggers TNF-like weak inducer of apoptosis (TWEAK)-mediated inflammation and necroptosis. Targeting of TWEAK or necroptosis shortened the duration of AKI, and led to improved kidney function and histology 96 h after exposure to folic acid in mice. However, in one study, specific targeting of bone marrow-derived RIPK3 induced an early decrease in kidney inflammation independent of effects on tubule cell death and did not improve kidney function 48 h after administration of folic acid[65], suggesting that RIPK3 might have a dual role in FA-AKI by promoting both early inflammation through its function in innate immune cells and later tubule cell necroptosis in response to inflammatory cytokines through its function in tubular cells. FA-AKI has also been used as a model to explore the effect of ferroptosis on the lipidome[103,117,175].

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Chronic kidney disease

Information regarding the role of regulated necrosis in preclinical models of CKD is limited. CKD is a complex entity — it can occur as a consequence of a variety of aetiologies and has variable pathophysiology, which can involve an adaptive response to the loss of functioning nephrons, genetic defects and ongoing toxic, metabolic, mechanical or immune insults. Interfering with cell death pathways may therefore differentially affect processes such as nephron loss, inflammation, autoimmunity, fibrosis and cystogenesis. Although limited, some available data support roles for necroptosis, ferroptosis and pyroptosis on diverse readouts associated with CKD.

For example, inhibition of necroptosis with the non-specific inhibitor, Nec1, preserved renal function in rats with subtotal nephrectomy[111], whereas deletion of *Ripk3* improved kidney function in mice with adenine-induced CKD[116]. Nec1 also decreased kidney inflammation and fibrosis in a model of unilateral ureteral obstruction (UUO)-induced CKD[110] and similarly, *Ripk3* knockout reduced kidney fibrosis in mouse models of UUO, adenine-induced CKD and diabetes[116,144]. However, the effect of *Ripk3* deletion on fibrosis might be independent of necroptosis, since *Mlkl*-knockout mice were not protected from UUO-induced kidney fibrosis[116], and Nec1 has targets other than RIPK1.

Evidence also suggests a pathogenic role for ferroptosis and pyroptosis in the transition of AKI to CKD and in the development of kidney fibrosis. Administration of the antioxidant, liproxstatin 1, or the caspase 1 inhibitor, belnacasan (also known as VX-765), which is used to target pyroptosis, ameliorated the maladaptive kidney response and development of fibrosis 14 days after severe IRI-AKI[176]. Liproxstatin 1 also reduced kidney fibrosis induced by UUO[124]. Deletion of *Gsdme* also decreased inflammation and kidney fibrosis after UUO or subtotal nephrectomy and preserved kidney function in the latter[135,177]. Deletion of *Gsdmd* also provided renal protection against UUO-induced kidney fibrosis through effects on neutrophil function[178]. Disulfiram protected mice from albuminuric nephropathy induced by the presence of *APOL1* risk alleles[152], which are thought to underlie the high incidence of the so-called hypertensive nephropathy in African Americans[179].

TWEAK has pro-proliferative and pro-inflammatory effects as well as an ability to induce cell death[180–182], which probably contributes to the ability of this cytokine to promote cystogenesis[183]. Of interest, administration of Nec1 aggravated disease in a murine autosomal dominant polycystic kidney disease model with selective deletion of *Pkd1* in the kidney, potentially by decreasing the loss of cyst wall cells[184]. Similarly, administration of Fer1 decreased cyst growth and preserved kidney function in a mouse model of polycystic kidney disease with systemic deletion of *Pkd1*, whereas the induction of ferroptosis by erastin further inhibited cystine uptake and aggravated disease[123].

Although it is difficult to dissect the impact of regulated necrosis on autoimmunity, inflammation and kidney cell death in autoimmune disease, regulated necrosis may lead to CKD through the induction of autoimmunity. NETosis is thought to have a key role in the development of autoimmunity against nuclear and neutrophil antigens in systemic lupus erythematosus and ANCA vasculitis[21]. Targeting necroptosis in myeloid cells reduced the formation of NETs and glomerular injury in experimental ANCA vasculitis[101] and administration of the RIPK3 inhibitor, GSK872, inhibited the development of lupus nephritis in MRL/lpr mice[113]. However, MRL/lpr mice demonstrate defects in caspase 8-dependent apoptotic clearance of lymphocytes due to defective FAS signalling and thus, RIPK3 inhibitors might promote caspase 8-dependent apoptosis in this model[72]. The role of pyroptosis in the context of autoimmune kidney disease is unclear. The inhibitor of caspases 1, 4, 5 and 11, Ac-FLTD-CMK, prevented the development of lupus nephritis in MRL/lpr mice[134] — an effect that was attributed to reduced pyroptosis. However, studies that claim an impact of caspase or NLRP3 targeting on disease outcomes through the modulation of pyroptosis should be confirmed by demonstrating that targeting of gasdermins is also beneficial. In this regard, deletion of *Gsdmd* increased mortality, kidney inflammation, albuminuria and autoantibody production and impaired kidney function in a model of imiquimod-induced lupus nephritis[185]. By contrast, the opposite outcome (that is, kidney protection) was achieved following the administration of disulfiram in pristane-induced autoimmune immune complex nephritis[186]. Protection from both autoimmunity and kidney disease was also described in *Gsdme*-knockout mice in the pristane model[187].

Thus, the impact of regulated necrosis in preclinical models of CKD seems to be more complex than in preclinical models of AKI, potentially owing to the presence of differences in cell death dynamics, pathophysiology and disease readouts, and the potential consequences of cell death processes over a prolonged period of time. Of note, preclinical models are not able to mimic the full complexity of human CKD, and better models are likely to be required to better delineate the contribution of cell death pathways to disease pathogenesis.

Towards clinical implementation

The clinical implementation of therapeutic approaches for targeting regulated necrosis rests on the availability of biomarkers that can provide readouts of drug efficacy and the availability of drugs that can be rapidly brought through clinical development with minimal safety concerns.

In search of biomarkers

Contrary to apoptosis, no morphological features that are visible by optical microscopy are sufficiently specific for necroptosis, pyroptosis or ferroptosis to enable their quantification in kidney biopsy samples. This limitation impairs our ability to characterize the occurrence and dynamics of diverse forms of regulated necrosis in clinical kidney disease. Moreover, biomarkers are needed to enable the non-invasive monitoring of specific forms of regulated necrosis and their response to therapy. Although phosphorylation of MLKL (at Ser358) has been proposed as a histological indicator of necroptotic activity[188], it does not indicate that necroptosis has actually occurred, since cells have defence mechanisms that expel phosphorylated MLKL to maintain cell viability[49,62]. Similarly, although the expression of ferroptosis activator ACSL4 correlates negatively with estimated glomerular filtration rate in patients with CKD[56], the presence of ACSL4 does not necessarily indicate that ferroptosis has occurred, since ACSL4 is involved in lipid biosynthesis but not in lipid peroxidation. Moreover, although lipid peroxidation is a hallmark of ferroptosis and can be detected by using BODIPY or probing for malondialdehyde, not all lipid peroxidation results in ferroptosis. In this regard, BODIPY actually detects the lipid oxidation that occurs in non-canonical pyroptosis, which takes place after membrane permeabilization and therefore represents a part of the process that does not contribute to cell killing[189]. Similarly, 4-hydroxynonenal (4-HNE) is a lipid peroxidation end product that forms adducts with proteins and has long been used as a marker of oxidative stress in tissue sections, but may also not represent ferroptotic cell death[190]. Work is ongoing to develop ferroptosis-specific lipidomics panels that can be assessed non-invasively in urine. For example, phospholipid oxidation products (that is, oxygenated polyunsaturated phosphatidylethanolamines) were increased in cell pellets from urine samples obtained from patients with AKI who did not recover[117].

Review article

Drug repurposing

Drug repurposing is the safest way to fast-track a drug for a new clinical indication, although care is needed as many therapeutics are nephrotoxic and may therefore have adverse effects in the context of kidney disease. Of note, a screen of candidate drugs to prevent maladaptive repair in proximal tubule cells identified pyroptosis and ferroptosis as key driver pathways suitable for drug targeting[176].

Multiple studies have found that cilastatin — a DPEP1 inhibitor that is used clinically to prevent DPEP1-mediated degradation of imipenem — protects the kidney against nephrotoxicity induced by drugs such as cisplatin, gentamicin and vancomycin[191–193], or haem proteins[194] and against IRI-AKI[195] and FA-AKI[56]. The mechanism of this kidney-protective effect is unclear and may be multifactorial, since DPEP1 has various functions, including roles in neutrophil sequestration by endothelial cells and is an enzyme and regulator of transport and signalling in tubule epithelial cells. Overexpression of DPEP1 is associated with kidney disease in humans, whereas *Dpep1* knockdown in mice increased total and free GSH levels in kidneys and protected against cisplatin-induced tubule cell apoptosis and ferroptosis with no effect on necroptosis or pyroptosis[56]. Consistent with a role for DPEP1 in decreasing antioxidant defences and in ferroptosis, cilastatin reduced lipid peroxidation in cisplatin-induced AKI[191]. Moreover, the sensitization of cells to ferroptosis in response to dexamethasone through glucocorticoid receptor-induced DPEP1 expression and GSH depletion was prevented by cilastatin or genetic inactivation of *Dpep1* (ref. 196). Additionally, cilastatin has been reported to decrease the uptake of several nephrotoxins by tubule cells, potentially through DPEP1-mediated interference with cholesterol lipid raft dynamics (which by itself may also decrease signalling through death receptors) and interference with, or inhibition of megalin[191,192,194,197]. Thus, although the molecular mechanism of kidney protection is unclear, different laboratories have demonstrated kidney protection by cilastatin in various models of AKI[56,191–193,197–203]. As a consequence of these findings, cilastatin is now undergoing clinical development as a kidney-protective molecule[203].

Vitamin K may also prevent ferroptosis, at least in some clinical scenarios. Vitamin K antagonists, such as the oral anticoagulant warfarin, inhibits the vitamin K epoxide reductase (VKOR), which normally reduces vitamin K epoxide to vitamin K hydroquinone — a radical-trapping antioxidant and inhibitor of (phospho)lipid peroxidation[204]. The ability of vitamin K to reverse warfarin toxicity may be explained by the ability of FSP1 to reduce vitamin K to vitamin K hydroquinone, since VKOR remains inhibited. In 2022, vitamin K_1 and a form of vitamin K_2 — menaquinone 4 (MK-4) — were shown to inhibit ferroptosis in cultured cells and improve outcomes in a mouse model of IRI-AKI[204]. By contrast, the vitamin K antagonist phenprocoumon, which is in clinical use in Germany, exacerbated outcomes[204,205]. This knowledge may drive the reinterpretation of the reported adverse effects of vitamin K antagonists in kidney disease, which include the development of haematuria-associated AKI during periods of warfarin overdosing[206–211], vascular calcification[212] and an increased risk of CKD progression[213,214]. Patients on chronic oral vitamin K antagonist anticoagulants are frequently co-prescribed proton pump inhibitors (PPIs) to decrease the risk of gastrointestinal bleeding whereas haematuria — which may result from anticoagulant overdosing — may lead to an overload of iron in tubule epithelial cells[206–208,210]. Interestingly, PPIs have also been associated with acute tubulointerstitial nephritis, an increased risk of incident and progressive CKD[215] and with AKI in patients treated with immune checkpoint inhibitors, possibly as a consequence of unsuppressed autoimmunity[211]. The induction of an immunogenic form of cell death — such as ferroptosis — could underlie a cause–effect relationship between PPIs, vitamin K antagonists and nephrotoxicity. Such a link is biologically plausible given the ability of vitamin K to prevent ferroptosis and the association between haematuria and iron overload. However, findings from experimental models and systems are contradictory. For example, omeprazole induced tubular cell death in healthy mice in vivo[216] and another PPI, lansoprazole, increased the severity of experimental cisplatin-induced AKI[217]. By contrast, one in vitro study found that omeprazole could scavenge lipid peroxyl radicals, and demonstrated short-term (48 h) anti-ferroptotic effects in cultured cells following cystine deprivation; however, omeprazole was toxic at higher doses[218]. Furthermore, another in vitro study found that the induction of tubule cell death by omeprazole was exacerbated by iron overload, but was not prevented by ferrostatin 1 (ref. 216), questioning the contribution of ferroptosis to this process.

Other compounds that are currently in clinical use or readily available, including indole-3-carbinol, rifampicin, promethazine, carvedilol, oestradiol and T3, also have anti-ferroptotic effects on cultured tubule cells[218]. In vivo, promethazine and rifampicin reduced the severity of cisplatin-induced AKI[218].

The anti-epileptic drug primidone inhibits RIPK1. Administration of primidone 5 days prior to surgery protected mice from IRI-AKI[151]. However, primidone overdose might cause crystalluria and AKI in humans[219], and long-term nephrotoxic effects (including kidney cysts, chronic nephropathy and kidney adenoma) were observed in rats exposed to high doses of primidone in toxicological studies[220]. This pattern of nephrotoxicity (that is, the development of cysts and adenoma) might be compatible with its role in the inhibition of kidney cell death. Another anti-epileptic drug, phenytoin, which shares a hydantoin scaffold with Nec1, attenuated RIPK1 kinase activity and protected mice from IRI-AKI[188].

The potential of agents that modulate iron availability to prevent ferroptosis is also being explored. Specifically, iron binders that decrease iron availability protect cells from ferroptosis[221]. However, this strategy may be risky, especially in the context of proximal tubule cell ferroptosis. For example, the dose-limiting adverse effect of the iron binder deferasirox is proximal tubule cell injury that leads to Fanconi syndrome and/or AKI[222]. Mechanistically, deferasirox induces necrosis in a manner that is dependent on the depletion of iron and is prevented by the anti-apoptotic protein BCL-X$_L$ but is not prevented by necroptosis inhibitors[223]. By contrast, preconditioning with iron sucrose (FeS) protected mice against selected forms of AKI via a mechanism associated with upregulation of hepcidin[224,225]. A FeS formulation ('RBT-3') is under clinical development for AKI[224].

Safety considerations

Safety aspects were usually not addressed in early animal studies; however, they are a key consideration for clinical translation. As discussed above, some agents that target components of regulated necrosis pathways are nephrotoxic and may therefore have limited utility in patients with kidney disease. The existence of preclinical findings showing that targeting cell death can actually exacerbate kidney injury[40,128,184] highlights these safety concerns. Interference with cell death processes has potential to induce devastating adverse effects in organs or systems beyond the kidney, for example, by inducing malignancy or autoimmunity through the impaired clearance of premalignant or autoimmune cells, respectively. In support of this possibility, suppressed regulated necrosis has been observed in malignant cells[33]. A further cause for concern in the context of oncology is the potential

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nephrotoxicity of therapies that aim to promote regulated necrosis in malignancy. For example, whole-body inactivation of *Gpx4* in mice triggered kidney failure and death within 10–15 days[70]; however, agents are being developed to degrade GPX4 and induce ferroptosis for the treatment of malignancy[226].

Conclusions

Available preclinical evidence suggests that regulated cell death may contribute to changes in cell number and to inflammatory and autoimmune responses driving AKI and potentially CKD. However, discrepancies exist in the outcomes of interventions designed to target different pathways of regulated necrosis in experimental studies, and gaps remain in our understanding of the processes involved. An important first step in addressing these knowledge gaps is to identify non-invasive biomarkers of different forms of regulated necrosis that are consistent across animal models and human disease. Such tools will facilitate the development and translation of therapeutics — a process that is complicated and, beyond a requirement for a better understanding of underlying disease mechanisms, requires identification of the optimal intervention, target and patient population. Timing of the intervention is particularly relevant, since the main modality of regulated necrosis may evolve over time and different molecular or cell targets may be relevant for prevention or treatment. The identification of molecular or histological markers of ongoing necroptosis, ferroptosis or pyroptosis in clinical samples will enable real-time monitoring and allow adjustments to the timing and type of intervention as well as and evaluation of the patient response to therapy.

Given the potential for interaction between different forms of cell death (Fig. 5), pan-cell death inhibitors that act on two or three pathways may increase the likelihood of success[71,95,166,227,228]. However, safety concerns may be exacerbated by such an approach. Such interventions may not only modulate the process in the intended cell targets (for example, tubule epithelial cells in AKI) but also modulate cell death in unintended targets, such as inflammatory cells, fibroblasts and malignant cells. Overall, therapeutic modulation of regulated necrosis is promising based on preclinical studies of preventive and treatment approaches in AKI and CKD, but only clinical trials will confirm its clinical relevance.

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A.B.S. and A.O. wrote the article. All the authors researched data for the article, contributed substantially to discussion of the content and reviewed and/or edited the manuscript before submission.

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