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Pathophysiological role of different tubular epithelial cell death modes in acute kidney injury

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Abstract

The histological substrate of many forms of intrinsic acute kidney injury (AKI) has been classically attributed to tubular necrosis. However, more recent studies indicate that necrosis is not the main form of cell death in AKI and that other forms such as apoptosis, regulated necrosis (i.e. necroptosis and parthanatos), autophagic cell death and mitotic catastrophe, also participate in AKI and that their contribution depends on the cause and stage of AKI. Herein, we briefly summarize the main characteristics of the major types of cell death and we also critically review the existing evidence on the occurrence of different types of cell death reported in the most common experimental models of AKI and human specimens. We also discuss the pathophysiological mechanisms linking tubule epithelial cell death with reduced glomerular filtration, azotaemia and hydroelectrolytic imbalance. For instance, special relevance is given to the analysis of the inflammatory component of some forms of cell death over that of others, as an important and differential pathophysiological determinant. Finally, known molecular mechanisms and signalling pathways involved in each cell death type pose appropriate targets to specifically prevent or reverse AKI, provided that further knowledge of their participation and repercussion in each AKI syndrome is progressively increased in the near future.

Key words: apoptosis, autophagy, ferroptosis, necroptosis, pathophysiology

Acute kidney injury: an overview

Acute kidney injury (AKI) refers to a number of aetiologically different conditions suddenly resulting in decreased glomerular filtration, increased plasma creatinine and variably diminished urinary output [1]. AKI is a very serious condition from the sanitary and economic points of view. It is especially relevant in determined clinical circumstances, such as those related to patients in intensive care units, critically ill patients and patients with multiorgan failure. In these circumstances, mortality remains at 50–80% of cases [2]. Overall incidence of AKI is estimated at 1–2% of hospital admissions and 2–7% during hospital stay [2, 3]. Beyond acute consequences, AKI increases medium and long-term cardiovascular morbimortality, favours progression to chronic kidney disease, and is a cause of permanent

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dialysis dependence. AKI-associated cost poses 5% of hospital expenditure [4] and 1% of overall health expenditure [3] in developed countries. From the pathogenic point of view, AKI is often classified in three types: (i) pre-renal AKI; (ii) renal, parenchymal or intrinsic AKI; and (iii) post-renal or obstructive AKI. Insults may produce one of these three AKI types, although most frequently combined pathophysiological patterns result, which depend on the intensity, dose or exposure level of the insult and exposure time. Often, though, the situation is even more complex, as several insults may act simultaneously on the patient.

Pre-renal AKI accounts for 60–70% of all AKI cases [5]. Pre-renal syndromes develop with a transient increase of plasma creatinine that resolves by withdrawal of the cause and hydration. In pre-renal AKI, glomerular filtration decreases as a consequence of deregulated renal haemodynamics. This involves strong reduction in whole renal blood flow, reduction of intraglomerular pressure by altered (or overwhelmed) regulation of afferent and efferent arteriole synchronized contractility, or both. At least theoretically, pure pre-renal AKI courses with no injury to renal tissues, as it results merely from deregulated haemodynamics. Common causes of pre-renal AKI are severe hypotension (caused e.g. by surgical or traumatic blood loss, burns and mild sepsis), dehydration (as from vomiting, diarrhoea, bleeding or hypovolaemia), heart failure, liver failure, narrowing of renal arteries, renal microangiopathy, exposure to vasoactive drugs and toxins, and others [6]. It is uncertain whether purely pre-renal AKI is rife in the overall clinical AKI casuistry, the initial cause or simply a part of the aetiology of more complex syndromes also involving intrinsic damage, first because prolonged or severe pre-renal AKI may give rise to ischaemic scenarios leading to parenchymal damage, and second, because certain pre-renal AKI causes, such as drugs and toxins, may also cause parenchymal damage directly. Animal models of pre-renal AKI (Table 1) include (i) severe hypovolaemia (by exsanguination); (ii) drug-induced renal haemodynamic deregulation; (iii) hepatorenal syndrome (liver cirrhosis of any origin).

Unlike in pre-renal AKI, in renal and post-renal AKI, deadly or subdeadly alterations in renal structures, mostly involving renal tubuli, are the key pathophysiological element [5, 7]. The commonest form of renal AKI is acute tubular necrosis (ATN), characterized by tubular epithelial cell death and dysfunction in one or several tubular segments [8]. Tubular cell dysfunction, resulting from death or sublethal alterations, is the initiating event in ATN leading to renal dysfunction (i.e. reduced glomerular filtration rate, GFR) and renal failure. Different mechanisms link tubular damage with reduced glomerular filtration [9]: (i) tubular cell injury impedes appropriate tubular reuptake, which activates the tubuloglomerular feedback mechanism to reduce filtration and minimize hydroelectrolytic loss; (ii) tissue debris, dead cells and cell residues occlude renal tubules, which hampers filtration in obstructed nephrons and reduces overall glomerular filtration rate; (iii) tubular damage and cell death lead to activation of extant cells, which produce pro-inflammatory mediators and vasoactive cytokines. These, in turn, contribute to keep glomerular filtration low and to further amplify damage to different renal structures. Several rodent models of renal AKI exist (Table 1) that variably recreate the homologous human disease.

Post-renal AKI occurs upon occlusion of the ureters mostly from stones, cancer, trauma and congenital alterations. Ureteral occlusion leads to rapid degeneration and fibrosis of the occluded kidney. Unilateral occlusion develops with maintained renal function, whereas bilateral occlusion leads to dysfunction. In occluded kidneys, tubular cell death, tissue derangement and inflammation are the most important pathophysiological events. The popular animal model of post-renal AKI is recreated by temporary or irreversible, unilateral ureteral ligation [10].

The degree to which renal models reproduce human diseases is heterogeneous and variable. Ischaemic and toxic models often show strong parenchymal damage, which is not consistently observed to the same extent in the limited histopathological information obtained from humans, even for a similar degree of renal dysfunction [11]. It has been suggested that milder damage resulting from accumulation of sublethal comorbidities may more accurately model human AKI in laboratory animals [11]. Yet, knowledge from animal models has, with limitations, been useful for progressively increasing our understanding of simple and complex human conditions. Because the stronger the parenchymal damage the worse the prognosis, better knowledge of tubule cell death is required. In the last four decades, different forms of cell death have been identified with individual phenotypic and biochemical characteristics, and with yet uncertain or not fully unveiled biological and pathophysiological meaning. It is also uncertain whether some or many of these cell modes are epiphenomenons of the same process yielding to different appearances depending on the circumstances and type of insult or intracellular injury site, or whether they constitute truly differentiated processes. In this article we critically review the evidence existing on the occurrence of different cell modes in different experimental models and clinical studies of homologous or similar circumstances, and their pathophysiological importance and mechanisms.

Summary of cell death modes: differential mechanisms, signalling and characteristics

Since the first descriptions of programmed cell death mechanisms in the mid-1960s, many attempts have been made to classify cell death forms and their physiological and pathological consequences. The first classifications of cell death were based on the morphological characteristics of the dying cells. When biochemical pathways and genes involved in cell death started to be described, the classification of cell death types was based also on biochemical and molecular criteria (Table 2 and Figure 1). Because there are many classifications of cell death relying on different criteria, we have chosen to use in the present review the last published recommendations of The Nomenclature Committee on Cell Death (NCCD) [12]. This classification applies to both *in vitro* and *in vivo* settings and includes apoptosis, regulated necrosis, autophagic cell death and mitotic catastrophe, as well as some other types of cell death such as anoikis, entosis, NETosis, parthanatos, ferroptosis, and pyroptosis (Table 2 and Figure 1).

Apoptosis

Apoptosis is the collapse of a cell through an active, highly regulated process requiring metabolic activity by the dying cell, and characterized by membrane blebbing, cell shrinkage, chromatin condensation and DNA fragmentation, followed by rapid engulfment of the corpse by neighbouring cells, without rupture of the cell membrane [13]. Activation of executioner caspases, a family of cysteine proteases, is necessary to complete this process. The term apoptosis is often used interchangeably with programmed cell death. However, in the strictest sense, programmed cell death may be applied to other forms of cell death that require gene expression without fulfilling some of the morphological criteria of apoptosis. [24]. The signalling mechanisms leading to cell death by apoptosis have been extensively reviewed recently [25, 26]. Apoptosis can be divided into intrinsic or extrinsic

Table 1. Major types of acute kidney injury in humans, including their major characteristics

Human syndrome	Pre-renal/ renal	Causes	Characteristics	Animal model
Pre-renal azotaemia	Pre-renal	• Hypotension • Fluid loss • Drugs	• Primary glomerular haemodynamic alterations • No parenchymal injury	• Exsanguination [14] • Drugs [15]
Drug nephrotoxicity	Renal	• Drug administration	• ATN • Secondary glomerular haemodynamic alterations • Secondary inflammation	• Drug administration [16]
Metal toxicity	Renal	• Environmental, accidental or professional exposure to metals	• ATN • Secondary glomerular haemodynamic alterations • Secondary inflammation	• Metal administration [17]
CIN	Renal	• ICM administration	• ATN • Secondary glomerular haemodynamic alterations • Secondary inflammation	• ICM administration (in predisposed animals) [18]
Ischaemic AKI	Renal	• Surgery • Transplant • Renal artery occlusion	• ATN • Primary glomerular haemodynamic alterations • Secondary inflammation	• Renal artery clamping [19]
Septic AKI	Renal	• Sepsis • Septic shock	• ATN • Primary glomerular haemodynamic alterations • Primary inflammation	• Cecal ligation/puncture [20] • LPS [21]
Rhabdomyolytic AKI	Renal	• Rhabdomyolysis	• ATN • Secondary glomerular haemodynamic alterations	• i.m. glycerol injection [22]
Nephritis • GMN • TIN • PN	Renal	• Systemic infections • Genitourinary infections • Autoimmunity	• ATN • Primary Infiltration • Primary Inflammation	• Folic acid administration? [23]

ATN, acute tubular necrosis; CIN, contrast-induced nephropathy; GMN, glomerulonephritis; ICN, iodinated contrast media; PN, pyelonephritis; TIN, tubulo-interstitial nephritis.

apoptosis, depending on the main origin of the first signal inducing the cell death.

Extrinsic apoptosis

Typically, extrinsic apoptosis is initiated when ligands such as FAS/CD95 ligand (FASL/CD95L), tumour necrosis factor α (TNFα) or TNF-related apoptosis-inducing ligand (TRAIL) bind to various transmembrane death receptors, namely FAS/CD95, TNFα receptor 1 (TNFR1) and TRAIL receptor (TRAILR)1–2, respectively [27]. When death receptors are activated, apoptosis is induced by a complex cascade of signalling pathways (recently reviewed in [25]) leading to the activation of initiator caspases (mainly caspases 8 and 10), and subsequently effector or executioner caspases (caspase 3,6,7) [28]. Extrinsic apoptosis can also be initiated in the absence of ligands, by oligomerization of death receptors.

Intrinsic apoptosis

Many intracellular stress circumstances, including DNA damage, oxidative stress, cytosolic Ca^{2+} overload, accumulation of unfolded proteins in the endoplasmic reticulum and many others may also activate apoptosis. Different signalling cascades distinctly initiated at specific cell locations, converge on mitochondria to activate a common mechanism of intrinsic apoptosis [29]. When lethal signals prevail, mitochondrial outer membrane permeabilization (MOMP) occurs which leads to mitochondrial transmembrane potential ($\Delta\Psi_m$) dissipation and inhibition of mitochondrial ATP synthesis and Dcm-dependent transport activities. The respiratory chain becomes uncoupled, leading to toxic overproduction of reactive oxygen species (ROS). Also, proteins that are normally confined within the mitochondrial intermembrane space (IMS) are released into the cytosol [25]. Most significantly, cytochrome c, once in the cytosol, binds to apoptosis protease-activating factor-1 (apaf-1) to recruit and activate initiator caspase 9. Once activated, caspase 9 activates executioner caspases and unleashes apoptosis.

Anoikis

Anoikis describes the apoptotic cell death of adherent cells in response to loss of cell-to-matrix interactions [30]. In most cases, the cell death programme triggered by anoikis is the same as described for intrinsic apoptosis [31].

Table 2. Types of cell death and criteria to define these types

Criteria	Types of cell death
Morphological	• Apoptosis • Necrosis • Autophagy • Mitotic catastrophe
Digestion	• Type I: Heterophagy • Type II: Autophagy • Type III: No digestion
Enzymatic dependency	• Caspases-dependent • Calpains-dependent • Cathepsins-dependent • Transglutaminases-dependent • Serine proteases-dependent • Nucleases-dependent
Functional meaning	• Physiological (necessary function) • Pathological (cause of or secondary to disease)
Immunological	• Immunogenic (causes inflammation and immune response) • Non-immunogenic (do not cause inflammation) • Heterophagy • Cell swelling, bleb formation, condensation of chromatin, fragmentation DNA • Caspase (3 or 7)-dependent • Physiological or pathological • Non-immunogenic
Caspase-independent intrinsic apoptosis	• Similar to apoptosis but – Caspase-independent – Serine proteases-dependent
Anoikis	• Similar to apoptosis but – Initiated by cell-ECM loss of contact – Overexpression of the Bcl-2 family member BIM
Pyroptosis	• Heterophagy • Apoptosis-like chromatin condensation, rupture of the plasma membrane • Caspase 1 and caspase 7-dependent • Pathological • Immunogenic
Regulated necrosis	• Heterophagy • Cytoplasm and organelle swelling, the loss of cell membrane integrity • Caspase-independent

Pyroptosis

Pyroptosis is a form of programmed cell death associated with antimicrobial responses during inflammation. In this process, immune cells that recognize several intracellular danger signals produce cytokines, particularly interleukin-1β (IL-1β) and IL-18, swell, burst and finally die. Pyroptotic cells can exhibit apoptotic and/or necrotic morphological features [32]. The most distinctive biochemical feature of pyroptosis is the early activation of caspase-1, which mediates the proteolytic activation of caspase-7 (rather than that of caspase-3) [33]. It is not clear whether pyroptosis is a specific form of cell death or whether it represents a particular case of caspase-dependent intrinsic apoptosis.

Regulated necrosis

Necrotic cell death is characterized by cytoplasmic and organelle swelling, followed by the loss of cell membrane integrity and release of the cellular contents into the surrounding extracellular space, that produces an inflammatory response within the tissue. Necrosis has been considered for a long time as an accidental, uncontrolled form of cell death lacking underlying signalling events. However, there is now a general agreement that necrosis can occur in a regulated manner, and that necrotic cell death has a prominent role in multiple physiological situations [34]. Several triggers can induce regulated necrosis, including alkylating DNA damage, excitotoxins and the activation of death receptors, at least in some circumstances [35]. Two special forms of regulated necrosis are necroptosis and parthanatos.

Necroptosis

Necroptosis is sometimes used as a synonym of regulated necrosis, but it was originally introduced to indicate a specific case or regulated necrosis, which is started by TNFR1 ligation and can be inhibited by the RIP1-targeting chemical necrostatin-1. Since then, an assortment of necroptosis triggers have been identified, such as FAS/CD95, TRAILR (TNF-related apoptosis-inducing

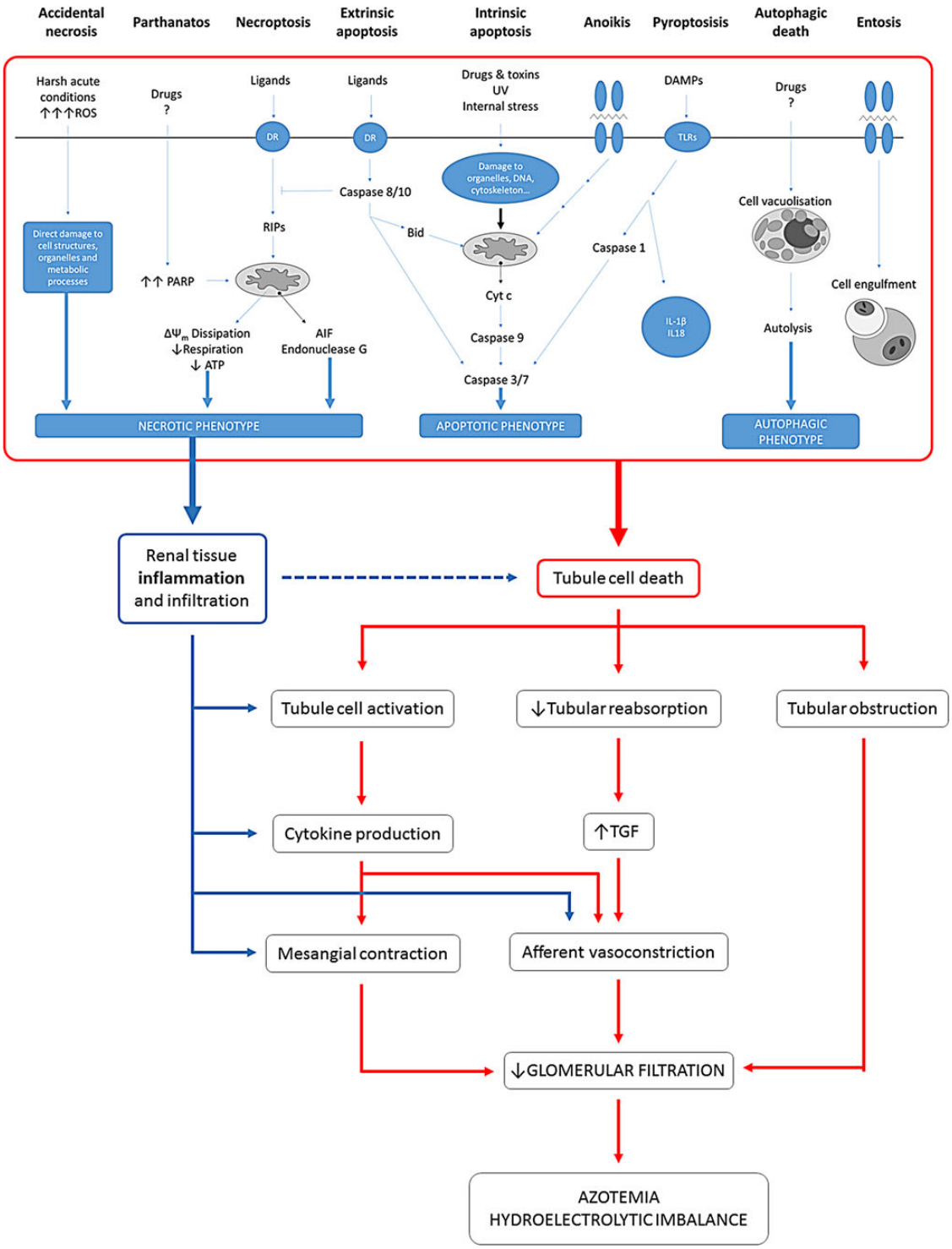

Fig. 1. Schematic representation of the main signalling pathways of different cell death modes, and the pathophysiological mechanisms activated by cell death modes in acute kidney injury.

ligand receptor), TLR3/4 (Toll-like receptor), etoposide and IRI (ischaemia-reperfusion injury) [102]. Necroptosis occurs as a consequence of death receptor signalling upon formation of the RIPK1/RIPK3/mixed lineage kinase domain-like protein (MLKL) containing necroptosome [34, 103]. RIPK3 is activated by phosphorylation and in turn, phosphorylates the pseudokinase MLKL, which has been suggested to be involved in the plasma-membrane rupture [102, 104]. A second necroptotic pathway involves the opening of the mitochondria permeability transition (MPT) pore. Upon MPT pore opening, along with apoptosome-forming proteins, other proteins are released from mitochondria that exert specific events contributing to the apoptotic phenotype in a caspase-independent manner. They include endonucleases such as apoptosis-inducing factor (AIF) and endonuclease G, which degrade

nuclear DNA [12]. Under apoptosis inhibition conditions, the MPT pore pathway leads to a necroptotic cell death mode.

Parthanatos

Parthanatos is a caspase-independent cell death mode involving the DNA damage-responsive enzymes poly(ADP-ribose) polymerases (PARPs), and in particular PARP1 [105]. In physiological conditions, PARP1 cooperates with the DNA repair machinery to ensure genomic homeostasis upon mild DNA damage but PARP1 overactivation has toxic consequences, including NAD+ and ATP depletion, as well as the accumulation of mitochondrial-toxic PAR, which favours $\Delta\Psi_m$ dissipation and AIF release [106, 107].

Ferroptosis

Ferroptosis is a type of cell death characterized by iron dependence, as this type of cell death is also inhibited by the iron chelator, deferoxamine [108], and iron-dependent accumulation of lipid peroxides [104, 109]. Although the aetiology of the iron-dependency of ferroptosis is not yet known, cellular iron may be the most important factor in lipid peroxide generation during ferroptosis. Lipid peroxidation and ferroptosis are inhibited physiologically by antioxidant mechanisms including glutathione peroxidase 4 (GPX4), an enzyme, whose function depends on the glu/cys antiporter in the plasma membrane known as system Xc- [108, 110]. Ferroptosis can be inhibited '*in vitro*' by ferrostatin-1 (Fer-1), a synthetic, potent antioxidant molecule [108, 109, 111]. Morphologically, ferroptosis is characterized by the presence of small mitochondria with condensed membrane densities, and is not associated with chromatin condensation, plasma membrane rupture, swelling of cytoplasmic organelles, or the formation of cytoplasmic vesicles/vacuoles [108].

Autophagic cell death

Autophagic cell death is characterized by massive cytoplasmic vacuolization suggesting that autophagy would actually execute cell death [112]. However, in most cases autophagy is a cytoprotective response activated by dying cells in the attempt to manage cell stress, and its inhibition accelerates, rather than prevents, cell death [113]. Thus, the term autophagic cell death should be used when death can be suppressed by the inhibition of autophagia [114, 115].

Mitotic catastrophe

Mitotic catastrophe is a mechanism of cell death initiated by perturbations of the mitotic apparatus during the M phase of the cell cycle and that is paralleled by some degree of mitotic arrest and ultimately causes cell death or senescence [116].

Entosis

Entosis is a cell death mode that occurs in epithelial cells linked to the invasion of one living cell into another homotypic or heterotypic cell [117], a phenomenon also called cell cannibalism [118]. In most cases, internalized cells appear virtually normal and later disappear, as they are degraded by lysosomal hydrolases. Entosis would be provoked by the loss of ECM interaction, but, in contrast to anoikis, it does not involve the activation of executioner caspases.

NETosis

NETosis is a regulated form of necrosis that is restricted to immune cells like neutrophils (NETosis) and other granulocytes or macrophages (then called ETosis). Pro-inflammatory cytokines such as IL-8 or TNF-α activate neutrophils to undergo a regulated cell death that spreads all chromatin outside the cells in a net-like structure (NETs = neutrophil extracellular traps). TLR2, TLR4, complement, and platelet activation all trigger NETosis whereas increased reactive oxygen species production facilitates NETosis [119]. NETosis spreads histones out of the cell at sites of infection as well as in sterile inflammation. In a similar process, dying renal tubular epithelial cells release histones locally, which promote microvascular and parenchymal injury [119].

Evidence of different cell death modes in animal models and human types of acute kidney injury

Table 3 shows selected references reporting the best evidence found in the literature on the occurrence of distinct modes of cell death in different types of AKI types. Many other studies report evidence for different modes of cell death in many models of AKI. In general, *in vivo* evidence for specific death phenotypes in animal models and human biopsy material is scarce, weak and superficial. Very few studies convincingly determine the mode or modes of cell death occurring in the kidneys. The two most reported and best documented cell death modes *in vivo* are necrosis and apoptosis followed distantly by autophagic death. A handicap for *in vivo* determination of the cell mode is that it is difficult to obtain a body of manifold evidence provided by morphological, biochemical and molecular details. This manifold body of evidence is necessary to unambiguously determine the mode of cell death, because single pieces of evidence are not exclusive to one mode of cell death. Studies are very heterogeneous in their way of addressing the study of cell death mode. They rarely combine morphological, with biochemical and signalling information, but mostly concentrate on one or two of these aspects, typically with superficial probatory depth.

When epithelial cell necrosis is reported, no proof of true 'cell necrosis' is given in most studies, but deduced from simple histological observations. The situation with necrosis is complicated for several reasons. First, because of the initial association between cell death and necrosis, when (before the 1970s) only one form of cell death was known, and termed necrosis. Second, because at that time the pathological pattern characterized by tubule epithelial cell death in acute cases of renal damage, was logically termed 'acute tubular necrosis' in the 1940–50s [120]. The first new mode of cell death identified different from necrosis was apoptosis [13]. Many articles produced in that intermediate period consolidated the association between ATN and cell necrosis, and both were mistakenly used somewhat indistinctly in many publications, even to the present. Third, the term necrosis has been widened in the last decade, when it was progressively realized that specific forms of necrotic-seeming phenotypes were in fact the result of specific cellular death programmes [12]. The first consequence was the distinction between passive and programmed necrosis (or necroptosis). *In vivo*, this is complicated, as necroptosis shares signalling pathways with other forms of death [121]. *In vivo*, phenotypical evaluation of cell morphology within tissue architecture is less explicit than that in cell cultures, with isolated cells.

Apoptosis is also poorly documented in numerous *in vivo* studies. In many papers, there is a mixture of a few *in vivo* data and many *in vitro* measurements, and conclusions of what occurs

Table 3: Types of cell death involved in the most frequently used experimental models of Acute Kidney Injury

		Apoptosis		Passive necrosis		Active necrosis		Autophagy	
		SP	Reference	SP	Reference	SP	Reference	SP	Reference
Toxic	Gentamicin	R	36					R	80
		R	37					R	81
		R	38						
		R	39						
	Cisplatin	M	40	M	47	M	82	M	83
		M	41					M	84
		R	42					M	85
		M	43					M	86
		M	44					M	87
		R,M	45						
		R	46						
	CIN	R	48	R	52				
		R	49						
		R	50						
		R	51						
I/R	Renal artery clamp	R	53			M	82	R	89
		R	54			M	88	M,H	90
		R	55					R	91
		R	56					M	92
								M	93
								M	86
Sepsis	Cecal ligation	R	57					M	94
								R	95
	LPS	M	58					M	96
		P	59						
	Human	H	60						
Metals	Uranium	M	61	R	63				
		M	62	R	64				
	Mercury	R	65	R	66				
				R	67				
	Cadmium	R	68	R	70			R	97
		R,M	69						
	Arsenic			R	71			R	98
Ambient	Malathion								
	Paraquat	R	72	R	73				
Rhabdomyolysis	Glycerol	M	74	R	76	R	99	R	100
		R	75						
Others	Folic acid	M	77	M	79				
		M	78						

R, rat; M, mice; P, pig; H, human.
In addition to the death cell modes described in the table, pyroptosis has been described in ischaemia/reperfusion [133] and NETosis has been reported to play a role in endotoxin and ischaemia/reperfusion-induced kidney injury [101]. The level of documentation of cell type death is very variable in the several papers. Papers in which the type of cell death is poorly documented (usually only basic histology) are marked in red. Papers in blue are those in which the type of cell death is reasonably documented (e.g. paper includes some specific biochemical pathway or TUNEL staining). Papers in black are those in which the death cell type is well documented, including demonstrations by several biochemical pathways or histological techniques.

in vivo are derived from this mixture of results. However, this probatory argument is weak. Cellular models, both primary cultures of renal cells and renal cell lines, are to an undetermined extent phenotypically adrift from their physiological and pathophysiological condition. In addition, they are devoid of undetermined conditionings and determinants only found *in vivo*. Both differences make *in vitro* findings ambiguously predictive of *in vivo* events. A rich body of experimental tools exists to study and manipulate cell models in order to evidence apoptosis. However, *in vivo*, most of these techniques are limited by the need to study whole kidneys for specific molecular events (as from tissue extracts), or to study events on histological samples that provide localization evidence, but more ambiguous molecular specificity. Detection of DNA nicks by terminal deoxynucleotidyl transferase

dUTP nick end labelling (TUNEL) is one of the most used tools to study apoptosis *in vivo*, as DNA fragmentation is considered a hallmark of apoptosis. However, even this signature mark of apoptosis has proved relatively unspecific, since TUNEL-positive cells can also be observed in cells undergoing necroptosis [122]. This exemplifies the need for multifactorial evidence and stresses the difficulty of assessing cell death mode *in vivo*.

Autophagic cell death has even been put into question [123]. A debate exists over whether it constitutes a distinct cell death programme, or merely a defence mechanism of protection from stress that not only does not contribute to cell demise, but rather its inhibition accelerates cell death [124]. Similarly to necrosis and apoptosis, its demonstration *in vivo* is a difficult task. To this difficulty, the uncertainty on its pathophysiological role must be added, when marks of autophagia (i.e. cell vacuolation, activation of specific signalling pathways, etc.) are detected *in vivo*. Other forms of cell death are even less well described or more doubts are cast on their existence as independent forms of cell death or as epiphenomenons of necrosis or apoptosis.

As a conclusion, sound evidence of most if not all cell death modes is available in renal cellular models, but very weak and low level evidence is available from *in vivo* studies and human tissue. Clearly, more focused investigation is necessary to prove the occurrence of specific modes of cell death in different AKI scenarios, their extent in each pathological circumstance and, thus, their repercussion in the overall pathological process. Finally, beyond understanding the pathophysiological role of cell death modes in AKI, markers of these processes in easy-to-obtain biological samples will contribute to perform a progressively more accurate etiopathogenic diagnosis of individual AKI episodes.

Pathophysiological role, significance and repercussion of different cell death modes in acute kidney injury

Many forms of AKI are characterized by tubular epithelial cell death [125], which is a cause of tubular dysfunction and tubular activation. Tubular dysfunction activates the tubuloglomerular feedback mechanism, which reduces glomerular filtration to prevent water and electrolyte loss. Tubular cell death also activates extant cells to proliferate and replace dead cells and to attract immune system cells to aid in repair. Tubule cell activation involves the production of pro-inflammatory and vasoactive mediators (cytokines, reactive oxygen species, etc.) which act in a paracrine and autocrine manner to contract mesangial cells and arterioles, to maintain filtration low. In addition, cell debris also produces tubular obstruction in more distal segments of the nephron, thus reducing glomerular filtration rate and the excretory capacity of the kidney [9].

In addition, tubule cell death is also a cause of further damage, which closes a vicious degenerative circle of injury amplification [9, 126]. Various forms of cell death have been reported to occur simultaneously to different relative and absolute extent depending on AKI type (ischaemic, toxic, septic, etc.), the stage of injury and the level of repair. Each form of cell death produces specific functional and histological consequences. An important reason for these differences is that various cell death modes distinctly stimulate the innate immune and inflammatory responses [127]. Different cell death modes result in a different degree of release of the so-called damage-associated molecular patterns (DAMPs), which stimulate and amplify inflammation and tissue damage [128].

The most studied and best described forms of cell death in AKI are apoptosis and necrosis, but several forms of regulated necrosis and autophagic death have been also described recently. As a general concept, apoptosis results in less aggravation of renal function than necrosis, because apoptosis is far less immunogenic than necrosis [127]. This is because, during apoptosis, release of the cellular content to the medium is limited. *In vivo*, apoptotic cells and their membrane-bound remains (apoptotic bodies) are rapidly removed by phagocytic and neighbouring cells [129]. In contrast, in necrosis and necroptosis, the cell membrane is broken and the cell content is released to the medium [130]. Necrotic debris and poured intracellular content attract immune system cells with the result of increased release of inflammatory cytokines and increased production of reactive oxygen species, all in turn resulting in subsequent or further renal damage [9, 126, 131] (Figure 1). Thus, any manoeuvre that prevents cell death by necrosis, even by transforming it into apoptosis, should have beneficial results for the severity of AKI. However, it should also be noted that the apoptotic signalling pathway, when intercepted or in absence of enough energy supply, may be diverted to necrosis [132].

Pyroptosis is a necrotic-like cell death mode that was thought to occur exclusively in macrophages and leucocytes, but that it has been also described in tubular epithelial cell [133–135]. The major characteristic of pyroptosis compared with other pathways of regulated necrosis is the maturation of pro-inflammatory cytokines such as IL-1 β and IL-18 during the cell death process. Cytokine maturation is cleavage-dependent, and it is mediated by non-apoptotic caspases such as caspase-1 [136]. Membrane rupture leads to the release of these cytokines to the interstitium, inflammation and worsened AKI. Then, pyroptosis shows maximal immunogenic effect among the different types of necrosis. Its role in AKI and its underlying signalling pathways should be further investigated with therapeutic aims.

Therapeutic perspectives and conclusions

There is reasonably solid evidence that apoptosis and necrosis (i.e. necrotic-looking) is involved in the pathophysiology of AKI. Although some evidence that other modes of cell death may take place in the diseased kidneys of AKI, more focused research is necessary to both clearly define distinct cell death modes *in vivo* (including necrosis and apoptosis), and their implication in AKI pathophysiology. Unravelling the occurrence of different cell death modes present in each AKI case, their pathological consequences and the underlying mechanisms and signalling pathways is crucial for a specific and personalized therapy and handling, and a research endeavour for the immediate future [137]. Furthermore, new diagnostic tools in the form of more sensitive imaging techniques and cell death mode-specific biomarkers are needed for an effective theranostic approach to AKI.

Despite the participation of apoptosis in AKI, apoptosis inhibition as a therapy for AKI has been questioned because caspase inhibitors are not successful in improving AKI development and outcome [103]. Despite being the core enzymes responsible for the apoptotic phenotype, caspase activation is a downstream level of apoptosis (in the execution phase). More upstream, initiating apoptotic events, such as mitochondria targeting, result in inevitable cell death [138].

After the discovery of necroptosis and necrostatin-1 (Nec-1) as an inhibitor of necroptosis [139], new strategies were designed for AKI treatment. It was therefore disappointing to realize that Nec-1 could only partially protect from ischaemic AKI [140]. Furthermore, in necroptosis, plasma membrane rupture occurs as

early as 20 min after RIPK3 dimerization [141], and administration of Nec-1, 30 min after the beginning of reperfusion, has no detectable protective effect [140]. Therefore, targeting regulated necrosis may be limited to such disorders in which AKI may be anticipated, like heart surgery-associated AKI, contrast-induced AKI, or kidney transplantation.

Regarding other cell death modes, pyroptosis may be targeted by caspase inhibitors or cytokine response modifier A [142]. In ferroptosis, ferrostatin-1 has been demonstrated to be useful in reducing AKI [111]. The utility of inhibiting these events is yet uncertain. In addition, targeting autophagy results in impairment of renal function in several models of AKI [100].

Thus, therapy that combines apoptotic, necroptotic and ferroptotic inhibitors might be potentially useful, but more research is necessary. Finally, translation of such results into clinical trials is highly problematic. Control groups are required for any single- and double-therapeutic strategy, and support of such studies might become long-winded in the absence of strong, convincing preclinical evidence.

Conflict of interest statement

None declared.

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