An Overview of Pathways of Regulated Necrosis in Acute Kidney Injury

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Summary: Necrosis is the predominant form of regulated cell death in acute kidney injury (AKI) and represents results in the formation of casts that appear in the urine sedimentation, referred to as *muddy brown casts*, which are part of the diagnosis of AKI. Pathologists referred to this typical feature as acute tubular necrosis. We are only beginning to understand the dynamics and the molecular pathways that underlie such typical necrotic morphology. In this review, we provide an overview of candidate pathways and summarize the emerging evidence for the relative contribution of these pathways of regulated necrosis, such as necroptosis, ferroptosis, mitochondrial permeability transition–mediated regulated necrosis, parthanatos, and pyroptosis. Inhibitors of each of these pathways are available, and clinical trials may be started after the detection of the most promising drug targets, which will be discussed here. With the global burden of AKI in mind, inhibitiors of regulated necrosis represent promising means to prevent this disease.
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Over the past decades, the field of cell death research has become more and more interesting with the discovery of new forms of regulated cell death besides the classic caspase-dependent apoptosis, which is characterized by cell shrinkage, pyknosis, and karyorrhexis. Determination of the form of cell death has shifted from morphologic parameters to biochemical analyses. Necrosis, which is classically characterized by cell swelling (oncosis) and subsequent disintegration of the cellular membranes and release of so-called danger-associated molecular patterns (DAMPs), is tightly regulated by integrated molecular cascades and the formation of intracellular protein complexes. Because necrosis is the main form of cell death observed in acute kidney injury (AKI), ischemia-reperfusion injury, and chemotherapy-induced nephropathies, it is of great interest to dissect these molecular cascades, which provide us with possible novel therapeutic approaches to reduce acute kidney injury–associated mortality and to reduce the immunogenic potential of necrotic cell death (necroinflammation[1,2]) in case of renal transplantation. Therefore, the current review is focused on regulatory pathways leading to necrotic cell death that are relevant in the context of renal diseases.

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FORMS OF REGULATED CELL DEATH WITH A NECROTIC PHENOTYPE

A recent publication by the Nomenclature Committee on Cell Death[3] stressed the use of biochemical definitions of cell death, which currently limits the types of regulated cell death with a necrotic phenotype (RN) to necroptosis, mitochondrial permeability transition (MPT)-mediated regulated necrosis (MPT-RN), parthanatos, ferroptosis, and pyroptosis. The complex role for autophagy in necrotic cell death in the context of renal diseases will be discussed in detail by other authors in this issue of *Seminars in Nephrology*. Besides the aforementioned necrotic pathways, caspase-dependent apoptosis and other currently nonrecognized pathways such as NETosis/ETosis will not be discussed in this review because NETosis was shown to overlap with the necroptosis machinery.[4] In the following parapgraphs, we discuss the current knowledge on the RN pathways and their relative contributions to renal diseases in detail.

NECROPTOSIS

Necroptosis is characterized by the dependency on the receptor-interacting protein kinase (RIPK)-3 kinase domain that mediates the phosphorylation of the necroptosis executing pseudokinase mixed lineage kinase domain-like (MLKL). This process appears to require oligomerization of active RIPK3 molecules,

dependent on their RIP homotypic interacting motif (RHIM) domains.[5,6] Upon ligation of death receptors, a submembranous death-inducing signaling complex (also called complex I) is generated. Intracellular death domains show sequential similarities with death effector domains (found in Fas-associated death domain and procaspases such as procaspase 8), caspase recruitment domains (CARDs), and pyrin domains, which are considered death-fold protein motifs. Death folds (death domains, death effector domains, CARDs, pyrin domains) can be found on a large variety of (receptor) proteins, including various pattern recognition receptors (PRRs) of the innate immune system (Toll-like receptors [TLRs], nod-like receptors) and downstream TLR mediators (such as MYD88), which are known to be expressed in renal parenchymal cells.[7] The prototypic complex I that has been investigated in many different cell types is the death-inducing signaling complex formed by activation of tumor necrosis factor receptor-1 (TNFR1) by its ligand TNF-α. TNFR1 trimerizes and binds to TNFRSF1A-associated via death domain, TNFR-associated factor 2, cellular inhibitors of apoptosis 1 and 2, and RIPK1. Complex I can be interpreted as a primary docking platform where additional modifications to complex I will determine whether the cell will undergo cytoprotection via nuclear factor κB (NF-κB) and mitogen-activated protein kinase activation, cell death by apoptosis, or cell death by necroptosis. One such modification of complex I is ubiquitination. Both K63 linkages and linear ubiquitination of members of some components in compex I by sevaral E3 ligases (in the case of K63 linkages) and the linear ubiquitin chain assembly complex (in the case of linear linkages) results in prosurvival and/or proinflammatory signaling through NF-κB and mitogen-activated protein kinase.[8] Indeed, men and mice with mutations in members of the linear ubiquitin chain assembly complex suffer from decreased NF-κB activity,[9-11] decreased nod-like receptor protein-3 (NLRP3) inflammasome activity,[12] and increased cell death from aberrant formation of complex II. Therefore, it is the loss of the stabilizing polyubiquitin linkages that result in the cell death decision after death receptor ligation. Physiological stimuli that lead to the removal of these chains on RIPK1 remain to be deteimined in the cause of AKI, but some candidates, such as the weak inducer of TNF signaling (TWEAK) may represent likely candidates because they are up-regulated during several models of AKI.[13]

Complex II may be subdivided into complex IIa and complex IIb (necrosome). Progression from complex I to complex IIb requires deubiquitination of RIPK1 and possibly other factors. Cylindromatosis functions as an important deubiquitinating enzyme that allows binding of complex IIb member RIPK3. The RIPK1–RIPK3 interaction is established through the RHIM and oligomerization of RIPK3 was shown to promote its autophosphorylation and subsequent phosphorylation of MLKL. Some reports found MLKL to be a component of the necrosome, and therefore MLKL may represent the most downstream obligatory member of complex IIb. It is clear that phosphorylation of MLKL at Ser345 is required for the execution of necroptosis,[14] but the precise mechanisms beyond its binding to certain phosphoinositides remain unclear. The exact role for RIPK1 in necroptosis signaling remains controversial because it is involved in survival, apoptotic, and necroptotic signaling. Clearly, RIPK1 was shown to be required to prevent spontaneous RIPK3 oligomerization,[15,16] and this function must be independent of the kinase domain of RIPK1.[17-20] Other RHIM-containing proteins such as TIR-domain–containing adaptor-inducing interferon (IFN)-β[21-23] and DNA-dependent activator of IRFs[24] can directly mediate necroptosis by binding to RIPK3 and subsequent downstream signaling. Next, RIPK3-mediated phosphorylation of MLKL provokes a conformational change in the pseudokinase domain of MLKL that allows exposure of the so-called four helical bundle, and subsequently the oligomerization and membrane translocation of multimerized phosphorylated MLKL (pMLKL) proteins.[25] Besides cleaving the necrosome members, the cFLIP-long/caspase-8 heterodimer inhibits necroptosis by cleaving the pronecrotic deubiquitinating enzyme cylindromatosis. To indicate the importance of the interplay between survival, apoptosis, and necroptosis signaling, human herpes simplex virus transcribes caspase-8 inhibitors and RHIM-containing viral proteins that disrupt both apoptosis and necroptosis as the primary and secondary clearance mechanisms in host cells. Along these lines, human cytomegalovirus additionally encodes as yet unknown proteins that block necroptosis downstream of phosphorylation of MLKL.[26,27]

MPT-RN

Necroptosis can occur in the absence of mitochondria because mitochondrial depletion by experimentally induced mitophagy did not affect formation of the necrosome, the downstream executional signaling cascade, and cell death.[28] However, dysfunction of mitochodria is able to execute RN via MPT. MPT-RN is defined as a regulated form of necrotic cell death that results from a sudden increased and sustained permeability of mitochondrial membranes, thereby providing a shortcut between the mitochondrial matrix and the cytosol. This process may be induced by small solutes (such as Ca^{2+}). Sudden opening of the permeability transition pore complex, also referred to as the mitochondrial permeability transition pore, in response to