
Review Article

Programmed Necrosis-mediated Cell Death and Disease: Recent Advances

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Abstract

Cell death classification has been based on morphological characteristics for decades. Modern advances in molecular technology enabled us to explore cell death pathways on a level of unprecedented detail. While necrosis has been considered a negative unpreventable form of cell death, recent findings demonstrate that a subtype of necrosis, namely necroptosis, is a regulated programmed process. Hence, therapeutic intervention to inhibit necroptosis-mediated loss of cells in a disease process is possible. This review highlights recent advances in programmed necrosis (necroptosis) and its potential relationship to human disease.

Keywords: Necroptosis, programmed cell death, necrosis, caspase-independent cell death

Introduction

“There are remedies for all things but death”—Thomas Carlyle. Cell death on the other hand, might be a different story. Can we prevent massive neural cell death following a cerebrovascular stroke, minimize cardiomyocytes loss after myocardial infarction and preserve kidney tissue during acute renal failure or save hepatocytes in hepatitis? Scientists in the cell death research field seek answers for these and similar questions to reduce the toll of disease on human health. Cell stress in the course of a disease often turns-on a survival, stress-induced, response to preserve the cell. Overwhelming or sustained stress could lead to accidental loss of cell viability or the activation of a regulated cell death/suicide pathway. Hence, an in depth

understanding of the different mechanisms of cell death enables us to identify therapeutic intervention targets to stop the cell death process and preserve target tissue viability in the course of a disease. In 1971, John F Kerr described a cell death form he initially called “shrinkage necrosis”⁽¹⁾ but the following year he changed its name in a landmark paper published in the *British Journal of Cancer*⁽²⁾. He described a distinct active programmed form of cell death and named it “apoptosis” (from Greek *apoptōsis*—falling off; *apo*—off or without, *ptosis*—falling). The term programmed cell death was widely used interchangeably with the term apoptosis until recently when other forms of programmed cell death have been uncovered. In 2000, John Kerr received the Paul Ehrlich and Ludwig Darmstaedter Prize for his description of “apoptosis”

shared with Robert Horvitz. The European prize is second only to the Nobel Prize. It is worth mentioning that after Kerr et al. seminal work; many scientists have contributed significantly to the field of programmed cell death research. Most notably, Sydney Brenner, H. Robert Horvitz and John E. Sulston who shared the 2002 Nobel Prize in Physiology or Medicine “for their discoveries concerning genetic regulation of organ development and programmed cell death”. Historically, cell death has been classified based on different morphological characteristics of dying cells into 3 types. Type I cell death characterized by condensation then fragmentation of the nucleus and cytoplasm; associated with “apoptosis”. Type II characterized by large autophagic vacuoles that fuse with lysosomes; associated with “autophagy” (from Greek autophagy – self-eating; auto – self, phagein – to eat). Type III characterized by disintegration of the cells into debris; associated with “necrosis” (from Greek *nékrōsis*; state of death)⁽³⁾. Following the significant advances in biochemical and genetic investigation of cell death, the scientific community recently adopted definitions for various cell death modules based on specific molecular characteristics⁽⁴⁾. Recent developments have uncovered multiple cell death pathways i.e. necroptosis, parthanatos, pyroptosis, entosis, ferroptosis, paraptosis and auto-phagic cell death. This review highlights recent advances in the non-apoptotic cell death mechanism, necroptosis, and sheds the light on its emerging relationship to disease processes.

Programmed necrosis (necroptosis)

Traditionally, necrosis has been long viewed as accidental passive (unregulated) process. Hence, therapeutic prevention of necrosis was never considered. Recent studies however, demonstrated that a

form of regulated necrosis results in programmed cell death, later coined as “necroptosis”. The first evidence for the existence of programmed necrosis came from Jürg Tschopp laboratory in 2000. That original work utilized T-cells and demonstrated that Fas induced a caspase-independent form of cell death that was absent in T-cells lacking Fas-associated death domain (FADD) protein or lacking receptor-interacting protein kinase (RIPK)⁽⁵⁾. [*Fas receptor was well known before this study as a cell surface death receptor that mediates apoptosis. FADD is well characterized as an adaptor protein that bridges death receptor signaling of the tumor necrosis factor receptor superfamily, such as the Fas-receptor, to the caspase cascade (procaspases 8 and 10) to mediate apoptosis*⁽⁶⁾. *The receptor-interacting protein kinases (RIPK) are serine/threonine protein kinases that contain c-terminal caspase activation and recruitment domains. They act in signaling complexes that sense cellular stress, initiate stress response and mediate cell death*⁽⁷⁾].

Molecular highlights of necroptosis

Apoptosis is known to proceed through a molecular cascade that involves cleavage and activation of caspases that in turn cleave other proteins including RIPK1 and RIPK3 in the process of executing apoptotic cell death. When caspases especially caspase-8 are inhibited by gene deletion, RNA interference or by pharmacological inhibitors, RIPK1 and RIPK3 are not degraded. Instead the two proteins execute programmed necrotic cell death⁽⁸⁾. Programmed necrosis or necroptosis is used here to indicate RIPK1 or RIPK3-dependent necrosis. Necroptosis can be activated by tumor necrosis factor alpha (TNF α), TNF-related apoptosis-inducing ligand (TRAIL) and Fas ligand (FasL); the same factors that

initiate apoptosis⁽⁹⁾. Thus, these receptors seem to function as general death receptors. Their activation could lead to cell death via alternative routes. Upon activation of these receptors, the mechanism by which the death route is chosen, apoptosis vs. necroptosis is still under investigation. Recent work suggests that death stimulus-mediated regulation of cellular energetics might dictate the choice between apoptosis and necrosis. In this process, RIPK3 acts as a switch that directs TNF α -induced cell death from apoptosis to necrosis⁽¹⁰⁾. Another elegant recent study utilized RIPK3 point mutant knock-in mice, implicate the kinase activity of RIPK3 as the switch that determines whether a cell dies from necroptosis or apoptosis⁽¹¹⁾. The formation of a multi-protein complex called the “necrosome” constitutes the main molecular signaling event in necroptosis. The necrosome contains RIPK1 and RIPK3, the two main players that mediate necroptosis⁽¹²⁾. As key regulators of programmed necrosis, RIPK1 and RIPK3 are therapeutic targets in multiple conditions. The identification of necrostatins, the RIPK1/necroptosis inhibitors, stimulated basic and translational research in the field of programmed necrosis⁽¹³⁾. Necrostatin, a tryptophan derivative, is a small molecule that inhibits the kinase activity of RIPK1 without affecting other RIPK1-mediated functions⁽¹⁴⁾.

Necroptosis in health and disease

Since FADD and caspase-8 are major proteins involved in apoptotic cell death, one might expect that lack of these proteins confer resistance against cell death. Surprisingly, deletion of the FADD or caspase-8 genes in mice is embryonic lethal due to massive necrotic cell death. Concurrent deletion of RIPK1 or RIPK3 rescues this embryonic lethality indicating an anti-necrotic

role for FADD and caspase-8⁽¹⁵⁻¹⁷⁾. Hence, FADD and caspase-8 inhibit RIPK1/RIPK3 dependent cell death or necroptosis in utero, a function required for normal embryonic development. While the field is in its infancy and we still have a lot to learn about its implications, programmed necrosis is also involved in multiple pathophysiological processes. These include ischemia-reperfusion injury, neurodegeneration, traumatic injury, toxic tissue-injury, infections, chronic inflammation and organ transplantation.

Neurological disorders

Using small molecule inhibitors such as necrostatin, multiple studies have demonstrated that blocking necroptosis in neural ischemia-reperfusion injury models is neuroprotective^(13,18-21). Necrostatin dose-dependently reduced brain infarct volume and improved functional neurological score following transient focal cerebral ischemia in mice⁽¹³⁾. Necrostatin conferred delayed but persistent neuroprotection when administered immediately after unilateral carotid artery ligation followed by 45 minutes of hypoxia in mice neonates⁽¹⁹⁾. In a rat model of high intraocular pressure-induced retinal ischemia, necrostatin pre-treatment preserved retinal thickness, reduced neural cell death and improved retinal function in electroretinography following ischemia⁽²¹⁾.

The motor neuron disease amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive motor neuron loss, paralysis and eventually death. Motor neurons exposed to ALS astrocytes were demonstrated to die by necroptosis. This novel finding identifies the programmed necrosis pathway as a new therapeutic target for ALS⁽²²⁾. Necrostatin delivered intracerebroventricularly delayed disease-signs onset and motor behavior decline in R6/2 mouse model of Huntington's disease⁽²³⁾. Reactive

oxygen species, reactive nitrogen species and energy metabolism regulation, all play important roles in the pathophysiology of neurodegenerative diseases. RIPK1 and RIPK3 also regulate free radicals and cellular energetics. It will be interesting to explore whether necroptosis plays a major role in other neurodegenerative diseases initiation and/or progression.

Necrostatin decreased neuronal death, decreased inflammatory cells infiltration and improved motor and memory functions following controlled cortical impact in mice⁽²⁴⁾. Necrostatin was also found to reduce lesions, inflammatory mediators, improved functional outcome after traumatic spinal cord injury⁽²⁵⁾. These studies suggest that programmed necrosis is involved in the pathogenesis of tissue damage following cerebrovascular stroke, traumatic brain and spinal cord injury. Hence, necrostatin and other specific programmed necrosis inhibitors might improve structural and functional recovery after these nervous system disorders.

Infectious diseases

Programmed necrosis is emerging as an important player in host-pathogen interaction. Several recent studies provide evidence that implicate necroptosis in the innate immune response. For example: 1) necroptosis is stimulated by pathogen recognition receptors (PRRs), such as Toll-like receptors⁽²⁶⁻²⁸⁾, 2) viral infection/ proteins regulates apoptotic and programmed necrotic cell death^(16,29,30), 3) necroptosis is induced by the anti-viral cytokine, interferon (IFN)⁽³¹⁾, 4) bacteria counteract host defenses by sensitizing cells to necroptosis⁽³²⁾. Recent evidence suggests an antiviral role for necroptosis. For example, vaccinia virus infected cells die by necroptosis in an attempt to limit viral replication as an antiviral strategy. Hence, sacrificing infected cells to save the rest in a tradeoff between

tissue pathology and viral titer/dissemination⁽³⁰⁾. Reovirus, important in gastrointestinal and respiratory infections, also activates necroptosis as a cellular defense mechanism against infection⁽³³⁾. On the other hand, influenza A virus induces cellular necroptosis without affecting viral dissemination. This suggests that cell death in this case is contributing to the severity of the disease rather than a defense mechanism⁽³⁴⁾.

Mycobacterium tuberculosis infection induces necroptosis to lyse the cells and release the bacteria, which helps propagating the disease. Blocking necroptosis at a stage that follows the oxygen burst (to fight the bacteria), but before the lysis of the cell, confers resistance to infection⁽³⁵⁾. Salmonella Typhimurium infection induces macrophage cell death via necroptosis. However, the role of necroptosis in the pathogenesis of the disease is not clear yet⁽³⁶⁾. A bacterial protein was identified recently as an inhibitor of necroptosis. Hence, it counteracts host defense and facilitates bacterial colonization⁽³²⁾. Sepsis is one of the major complications of certain infections. Recent evidence suggests that necroptosis is involved in the pathogenesis of sepsis and might serve as a therapeutic target as well. Genetic inhibition of necroptosis by RIPK3 gene deletion or pharmacologic inhibition by necrostatin protected against lethal systemic inflammatory response syndrome (SIRS)⁽³⁷⁾.

Cardiovascular diseases

Necrostatin given with reperfusion after left anterior descending coronary artery ligation decreased myocardial cell death and reduced infarct size in mice⁽³⁸⁾. In another study, necrostatin, administered with reperfusion, not only reduced myocardial infarct size and inhibited necrotic cell death, but also limited adverse remodeling, inhibited inflammation and preserved cardiac

performance following cardiac ischemia-reperfusion in mice⁽³⁹⁾. RIPK3-LDLR double knockout mice showed reduction in advanced atherosclerotic lesions due to lower macrophage necroptotic cell death compared to LDLR knockout mice. This finding suggests that macrophage death by necroptosis is involved in the pathogenesis of advanced atherosclerosis⁽⁴⁰⁾.

Gastrointestinal disorders

RIPK3 knockout mice showed reduced inflammation-induced tissue damage and cell loss in cerulein-induced acute pancreatitis^(10,41). Intestinal epithelial cells-specific deletion of FADD gene leads to spontaneous epithelial cell necrosis, loss of Paneth cells, enteritis and severe erosive colitis. Concurrent deletion of the RIPK3 gene prevented the spontaneous pathology in these mice. These findings implicate necroptosis in inflammatory bowel disease⁽⁴²⁾. Intestinal epithelial cells-specific deletion of caspase-8 gene lead to spontaneous inflammatory lesions in the terminal ileum and mice were more prone to colitis. The mice also lacked Paneth cells due to increased necroptosis, and had decreased numbers of Goblet cells. Moreover, increased RIPK3 was observed in human Paneth cells and elevated necroptosis in the terminal ileum of patients with Crohn's disease. These findings suggest that necroptosis is involved in the pathogenesis of Crohn's disease⁽⁴³⁾. Mice deficient in RIPK3 but not RIPK1 and not treated with necrostatin were protected from chronic ethanol-induced hepatocyte injury. These results give an example where RIPK3 regulates necroptosis independently from RIPK1 and implicates RIPK3-dependent necroptosis in alcoholic liver disease⁽⁴⁴⁾. Genetic deletion of RIPK3 or inhibiting its expression using antisense morpholinos in wild-type mice reduced immediate but not de-

layed acetaminophen induced necrotic liver cell death⁽⁴⁵⁾.

Renal disorders

Necrostatin given after reperfusion decreased tissue injury and renal failure leading to increased survival following lethal kidney ischemia-reperfusion insult in mice⁽⁴⁶⁾. Another study demonstrated that necroptosis and cyclophilin-D-mediated mitochondrial permeability transition are independently involved in tissue damage following renal ischemia-reperfusion injury suggesting the benefits of combined therapy⁽⁴⁷⁾. Genetic deletion of RIPK3 gene almost blocked acute tubular necrosis and lowered injury score following kidney ischemia-reperfusion injury. Moreover, in an experimental kidney transplantation model, donor kidneys from RIPK3 knockout mice were associated with lower serum creatinine levels, reduced inflammation, and histopathological injury. Animals that received RIPK3 knockout kidneys had a greater rejection-free survival to day 100 and overall longer survival compared to those that received wild type kidneys⁽⁴⁸⁾.

Cancer

Cell death is not always an undesired event. Cancer types that resist apoptotic cell death induction might be prime target for induction of necroptosis as a therapeutic strategy. Drug-resistant and drug-sensitive cancer cell lines were proven susceptible to the necroptotic inducer, shikonin⁽⁴⁹⁻⁵¹⁾. Shikonin also induced cell death in primary and metastatic osteosarcoma, likely by inducing necroptosis⁽⁵²⁾. The mitotic kinase Polo-like kinase 1 (Plk1) was found to be upregulated in castration-resistant prostate cancer cells. These cells were sensitive to pharmacologic inhibition of Plk1 likely through activation of necroptosis⁽⁵³⁾.

Summary

“I would rather live than die. I would rather die than survive as a monster”— Robert Fanney

While cell death prevention is a prime objective in multiple diseases, its induction is invaluable in treating cancer. RIPK1/RIPK3-dependent programmed necrosis or necroptosis is a caspase-independent cell death pathway. Inhibition of this pathway is required for normal embryonic development. Since necroptosis mediates cell death in multiple experimental disease models, it might serve as a therapeutic target in these diseases. What we have learned so far is still limited. Comprehensive dissection of the pathway is important to understand its functional relevance in more details and to identify more targets for therapeutic intervention. In many disease processes, cell loss might be due to more than one cell death pathway. It is important to explore these possibilities and consider combinational targeting for these pathways to obtain a better outcome. On the other hand, induction of necroptosis is potentially useful in tumor management especially in drug-resistant cancers.

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