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Review

Regulated necrosis and its implications in toxicology

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ABSTRACT

Recent research developments have revealed that caspase-dependent apoptosis is not the sole form of regulated cell death. Caspase-independent, but genetically regulated, forms of cell death include pyroptosis, necroptosis, parthanatos, and the recently discovered ferroptosis and autosis. Importantly, regulated necrosis can be modulated by small molecule inhibitors/activators, confirming the cell autonomous mechanism of these forms of cell death. The success of small molecule-mediated manipulation of regulated necrosis has produced great changes in the field of cell death research, and has also brought about significant changes in the fields of pharmacology as well as toxicology. In this review, we intend to summarize the modes of regulated cell death other than apoptosis, and discuss their implications in toxicology.

Contents

1. Introduction

Cell death is the ultimate form of cytotoxicity elicited by xenobiotics. There are many modes of cell death. For the first time, cell death modalities have been classified according to their morphological features. Schweichel and Merker examined cell death during the embryonic development of rodents treated with or without embryotoxic substances by electron microscopy, and classified developmental cell death (programmed cell death, PCD) into three types (Schweichel and Merker, 1973). Later, Clarke examined PCD, and proposed a classification of cell death modalities (Clarke, 1990). Type I PCD is characterized by the condensation of nuclei as well as chromatin, blebbing of the plasma membrane, and shrinkage of the cell, often followed by fragmentation into multiple cell bodies and their subsequent engulfment by phagocytes. Degeneration of intracellular

Abbreviations: PCD, programmed cell death; FADD, fas-associated protein with death domain; FLIP, FLICE-inhibitory protein; NLRP3, (NACHT, LRR and PYD domain-containing protein3); cIAP, cellular inhibitor of apotosis protein; Z-YVAD-FMK, benzyloxycarbonyl-Tyr-Val-Ala-Asp(-*O*-methyl)-fluoromethylketone; Z-VAD-FMK, benzyloxycarbonyl-Val-Ala-Asp(-*O*-methyl)-fluoromethylketone; RIPK, receptor-interacting kinase; MLKL, mixed lineage kinase-like; PGAM5, phosphoglycerate mutase family member5; Drp1, dynamin-related protein1; TRPM7, transient receptor potential melastatin related7; PAR, poly(ADP-ribose); PARP, poly(ADP-ribose) polymerase; AIF, apoptosis-inducing factor; RLS, ras lethal synthetics; GPX4, glutathione peroxidase4; MKK4, mitogen-activated protein kinase kinase4.

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organelles other than the nucleus, such as ER or mitochondria, appears to be minimal during Type I PCD. Type II PCD is characterized by massive cytoplasmic vacuolization that includes the intracellular contents, suggesting autophagic vacuolization. Type III PCD is also associated with cytoplasmic vacuolization, but the vacuoles seem to be derived from the dilation of cytoplasmic organelles other than lysosomes, and, therefore, is referred to as "non-lysosomal disintegration". Clarke subdivided type III PCD into type IIIA and type IIIB depending on the occurrence of lysosomal vacuolization (type IIIB PCD). Although Schweichel and Merker used the term "necrosis" to indicate the status of cellular degeneration including cell death (Schweichel and Merker, 1973), Clarke used "necrosis" to indicate certain types of cell death: he referred to type III PCD as necrosis (Clarke, 1990). Necrosis is characterized by the apparent passive destruction of cellular homeostasis under microscopic examination, and has been considered a non-cell autonomous form of death. In addition to developmental cell death, the cell autonomous active form of death has also been implicated in liver atrophy, and was referred to as apoptosis by Kerr (Kerr et al., 1972). After extensive examination by Horvitz of its mechanism by genetic screening of developmental cell death-deficient mutants (ced-mutants) of the nematode *C. elegance* (Ellis and Horvitz, 1986), apoptosis has gained much attention not only in the field of cell death research, but also in toxicology (Vaux, 2002). Cysteine-aspartic proteases, also called caspases, are responsible for the execution of apoptosis (Cryns and Yuan, 1998). Caspase family of proteins is comprised from 12 proteins in human. Two major pathways of apoptosis, one is caspase-8-dependent extrinsic pathway which is initiated through ligation of death ligands to their cognate receptors and the other is caspase-9-dependent intrinsic pathway which is caused by cytochrome c release from mitochondria, are both converged at the activation of caspase-3, main executioner caspase responsible for nuclear as well as DNA fragmentation. Although necrosis has long been underestimated by cell death researchers as well as toxicologists due to a lack of knowledge of its regulatory mechanisms, recent research has suggested that necrosis may also proceed in a defined manner with signaling molecules required for the progression of cell death. It should be noted that distinct modes of cell death often proceed in parallel even in a single cell; this is especially the case with death caused by xenobiotics (Raffray and Cohen, 1997). Thus, taking the possible involvement of various cell death modalities into consideration is important for gaining a better understanding of the mechanisms of cytotoxicity by xenobiotics. However, reports indicating the involvement of regulated necrosis in xenobiotics-induced cytotoxicity are very limited except in the context of anti-cancer therapies. In this review article, we describe each cell death in approximately ascending order by time.

2. Pyroptosis

Pyroptosis (Fig. 1 and Tables 1 and 2) is a form of cell death that depends on caspase-1, but does not have the features of apoptosis (Bergsbaken et al., 2009). Pyroptosis was firstly described in *Shalmonella*-induced macrophage cell death, which was considered to be apoptosis (Hersh et al., 1999) but revealed as non-apoptotic cell death later (Brennan and Cookson, 2000; Cheung et al., 2006; Hersh et al., 1999). Pyroptosis is morphologically characterized by the formation of plasma membrane pores as well as final rupture of the membrane, whilst nuclear and mitochondrial degenerations are minimum during pyroptosis. Caspase-1, also called interluekin-1β-converting enzyme (ICE), and caspase-11 are involved in the production of the proinflammatory cytokines IL-1β and IL-18, and the subsequent inflammatory damage that often results in this type of cell death. Pyroptosis is mostly implicated in inflammation, as it is characterized by the secretion of these pyrogenic cytokines (Bergsbaken et al., 2009; Fink and Cookson, 2006). Pyroptosis is initiated, for example, by ligation of TLRs (toll-like receptors) as well as NLRs (NOD-like receptors), and the subsequent activation of intracellular large signaling complexes called inflammosomes. Four types of inflammosomes containing NLRP1, NLRP3, IPAF/NLRC4, and AIM2, respectively, along with caspase-1 as the common effector molecule, have been reported to date (von Moltke et al., 2013). For example, the NLRP3 inflammosome, which comprises NLRP3, caspase-1, and the adapter molecule ASC, is activated not only by PAMPs (pathogen-associated molecular patterns), such as components of the

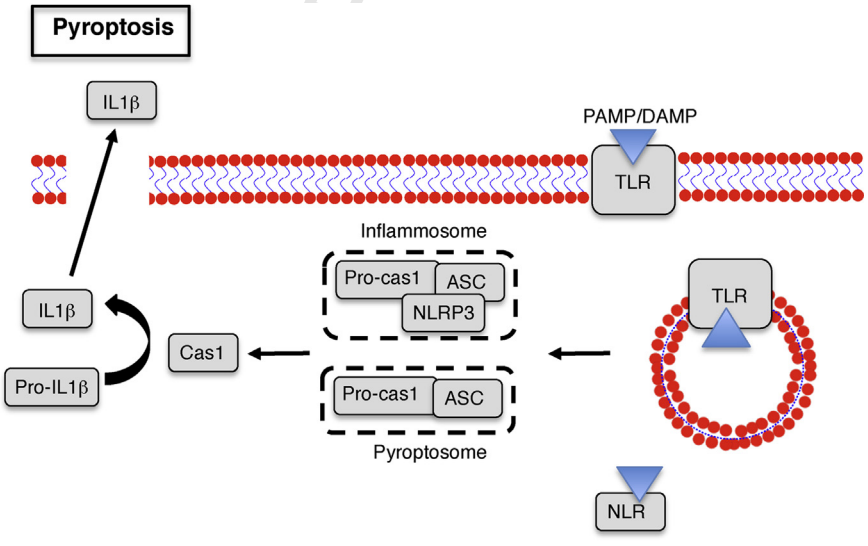

Fig. 1. Typical pathway of pyroptosis. Pyroptosis is typically initiated after the ligation of TLRs (transmembrane proteins reside on plasma membrane and endosome) and NLRs (cytosol proteins) to PAMPs/DAMPs, followed by the formation of the inflammosome comprising, for example, NLRP3, ASC, and pro-caspase-1. The resultant conversion of caspase-1 into its active form ignites a panel of cellular responses including the secretion of pro-inflammatory IL-1β and IL-18. Pyroptosome is another caspase-1-activating complex that contains ASC but not NLRP. Caspase-1 activation leads not only to the maturation of pro-inflammatory cytokines but also to the ultimate cell lysis. Focal plasma membrane pore formation mediates the secretion of cytokines, followed by osmotic cell lysis and release of intracellular pro-inflammatory molecules such as ATP and HMGB1.

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