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Review

Autophagy and the invisible line between life and death

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ABSTRACT

For a considerable time cell death has been considered to represent mutually exclusive states with cell death modalities that are governed by their inherent and unique mode of action involving specific molecular entities and have therefore been studied primarily in isolation. It is now, however, becoming increasingly clear that these modalities are regulated by similar pathways and share a number of initiator and effector molecules that control both cell death as well as cell survival mechanisms, demanding a newly aligned and integrative approach of cell death assessment. Frequently cell death is triggered through a dual action that incorporates signaling events associated with more than one death modality. Apoptosis and necrosis regularly co-operate in a tightly balanced interplay that involves autophagy to serve context dependently either as a pro-survival or a pro-death mechanism. In this review we will assess current cell death modalities and their molecular overlap with the goal of clarifying the controversial role of autophagy in the cell death response. By dissecting the key molecular pathways and their positioning within a network of regulatory signalling hubs and checkpoints we discuss a distinct approach that integrates autophagy with a resultant cell death manifestation. In doing so, former classifications of cell death modalities fade and reveal the intricate molecular proportions and complexities of the cell death response that may contribute towards an enhanced means of cell death control.

1. Introduction

Juan Ponce de León's 16th century quest for the fountain of youth signified humanity's longing for immortality. Today this quest continues, but the approach has changed from searching for a magical spring to deciphering cell death. This demands to fully comprehend the cells constant battle to stay alive, to elucidate the molecular underpinnings of cellular viability and longevity, and delaying the onset of cell death. One of the key molecular mechanisms that enhance cellular viability is autophagy, which, paradoxically, connects longevity and cell death. Although advances in microscopic applications allowed us to study the physiological mechanisms underlying cell death and contributed substantially to the first 'formal' definitions of cell death modalities, many new fundamental questions on cell death onset control emerge and is reflected by the increasing notion towards a more integrated and complexity-driven approach (Galluzzi et al., 2015, 2012; Kroemer et al., 2009, 2005). This does not only impact the cell death nomenclature, but also asks for a re-evaluation of formerly accepted cell death modalities.

Cell death was originally categorized as type I, II or III (Clarke, 1990; Kerr et al., 1972; Schweichel and Merker, 1973). Type I cell death, apoptosis, displayed as condensation of the nucleus and cytoplasm, fragmentation of the cell, and its subsequent engulfment by a phagocyte leading to its complete removal without a concomitant systemic immune response. Type II cell death, defined as autophagic cell death (ACD), describes lysosomal degradation with little or no association with phagocytes. Lastly, type III cell death, also known as non-lysosomal cell death such as programmed necrosis, represents cell death lacking vacuolar digestion. Given the substantial progress that has been made in the biochemical and genetic dissection of cell death, this characterization has increasingly shifted from a predominantly morphological to a primarily molecular identification of systems (Galluzzi et al., 2012). However, this approach has brought about increasing ambiguity with often-inherent contradictions. Most of the controversy surrounds the role of ACD, to an extend that its existence as a death modality has been questioned (Shen et al., 2012). In this review we aim to provide clarity by assessing current cell death modalities and their molecular overlap with a specific focus on ACD with the aim to better define its role in cell death. Concomitantly, we will re-evaluate the classification of cell death as a specific, predetermined, programmed

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response. We aim to strongly contextualize the role of autophagic flux, molecular overlap between cell death modalities, as well as the relationship between autosis and autophagy. In doing so, we will provide a distinct approach to integrate cell death classifications, which allows to better define the intricate proportions and complexity of the cell death response, and may contribute towards developing enhanced means to control cell death.

2. Cell death modalities and their molecular crosstalk

For the last four decades modes of cell death have been thought to represent mutually exclusive states with each modality consisting of its own inherent unique processes involving specific molecular entities and have therefore been studied in isolation. However, it is now becoming more clear that they are regulated by similar pathways and often even share similar effector and initiator molecules (Nikoletopoulou et al., 2013). In some cases the molecular overlap may complicate the assessment of cell death modalities, leading to inconclusive outcomes. The fact remains that more often than not, cell death is triggered through a dual action involving more than one modality. Depending on the molecular setting, apoptosis and necrosis often co-operate in a tightly balanced interplay that involves autophagy to serve either as a pro-survival or a pro-death mechanism. In this section we will provide a comprehensive characterization of each modality and discuss their molecular overlap.

2.1. Cell death modalities

Of the three cell death modalities, apoptosis has been most substantially defined. Apoptosis (Kerr et al., 1972) can be triggered upon cell damage, during normal development and morphogenesis, or in response to stress (Bibel and Barde, 2000) and is activated by either extrinsic or intrinsic stimuli. The extrinsic stimuli involve death receptors such as tumor necrosis factor-α (TNFα), Fas (CD95/APO1) and TNF related apoptosis inducing ligand (TRIAL) receptors while the intrinsic stimuli incorporate mitochondrial signaling (Adams, 2003; Kroemer et al., 2007). Caspase activation results in mitochondrial membrane permeabilization (MMP), which leads to the release of apoptotic proteins such as cytochrome c, Smac and Omi (Salvesen and Dixit, 1999; Green, 2005). The caspase cascade to execute cell death by apoptosis is dependent on activation by cytochrome c. Smac and Omi, as well as enhanced caspase activation. The B cell lymphoma 2 (Bcl-2) family controls apoptosis-mediated MMP and includes anti-apoptotic proteins (Bcl-2, Bcl-xL, and Mcl-1), multi-domain pro-apoptotic proteins (Bak and Bax), and proteins with the BH3 domain (Bid, Bim, Bik, Noxa, and Puma).

Necrosis involves organellar swelling, rupture of the plasma membrane and complete lysis of the cell (Leist and Jäättelä, 2001; Schweichel and Merker, 1973). Cells undergoing necrosis release factors that are sensed by core inflammasome proteins that result in the release of proinflammatory cytokine interleukin-1 beta (IL1β) and activation of inflammatory responses (Los et al., 2002; Zong and Thompson, 2006). Mitochondrial ATP, released from damaged cells, serve as the first trigger to inflammasome activation (Iyer et al., 2009). Typically, necrosis is not associated with caspase activation or normal development, but a "programmed" form of necrosis, necroptosis, has been defined (reviewed in Proskuryakov et al., 2003). Necroptosis has been found to be associated with diverse forms of neurodegeneration and cell death inflicted by infection or ischemia (reviewed in Nikoletopoulou et al., 2013). Necroptosis, especially, shares several key processes with both apoptosis and autophagy.

Autophagic cells display extensive internal membrane remodelling where engulfment of portions of the cytoplasm into large double-membrane vacuoles enable docking and fusing with hydrolase-containing lysosomes (Kuma et al., 2004). Autophagy, first described in yeast, is driven by over 30 well-reserved autophagic genes or proteins (Atg) (Mizushima et al., 2011). The initial stages of autophagy, termed nucleation, involves the activation of class III phosphoinositide 3-kinase PI3K; a component of a multi-protein complex that includes (Atg6/Beclin) by the ULK/Atg1 complex which enables the recruitment of Atg proteins to the isolation membrane (also called the phagophore) (Simonsen and Tooze, 2009). Vesicle elongation and completion (i.e. autophagosome formation) is governed by two ubiquitin-like conjugation systems (Atg12- and microtubule-associated protein 1A/1B light chain 3 (LC3)- conjugation), which enable the covalent conjugation of Atg5 to Atg12, and conversion of LC3-I to its phosphatidylethanolamine (PE)-conjugated form, LC3-II (Ohsumi, 2001). The formation of autolysosomes results from the fusion of mature autophagosomes with lysosomes allowing the degradation of targeted entities through lysosomal hydrolase. Through Akt kinase/TOR (target for rapamycin) kinase signaling, P13K is also able to regulate cell growth, which in turns inhibits autophagy (Blommaart et al., 1997). Alternative pathways for autophagy have been suggested, including Atg5-independent (Nishida et al., 2009) pathways, as well as a Beclin 1-independent autophagy (Chu et al., 2007). The most important pro-survival function of autophagy is likely its ability to delay apoptosis or necrosis (Loos et al., 2011) or to suppress necrotic cell death.

2.2. Molecular overlap between cell death pathways

Molecular overlap between cell death pathways is manifold. For example, TNFα treatment is able to induce classical apoptosis in one, but a necrotic form of cell death in another cell line (Laster et al., 1988). Autophagy, on the one hand, can be activated thereby blocking necroptosis in several cell lines in response to TNFα antigen stimulation and starvation (Bell et al., 2008; Farkas et al., 2011). zVAD, a short peptide that acts as a general caspase inhibitor, is able to prevent apoptosis and triggers necroptosis under TNFα treatment (Wu et al., 2011). Through its inhibitory effect on lysosomal cathepsins, zVAD is also able to prevent autophagy, highlighting autophagy's pro-survival capacity. Autophagic inhibition via mammalian TOR (mTOR) kinase signaling, on the other hand, enhances necroptosis, while starvation protects against zVAD-induced necroptosis (Wu et al., 2008, 2009, 2011). A number of mTOR activators have also been implicated in apoptotic regulation (Thedieck et al., 2007). Energy sensing is central to cell death activation, indicating mitochondria as a nexus of cell death control. Intracellular ATP levels play a determining role in the cross talk between cell death programs under conditions where ATP levels are low or when apoptosis is blocked (Los et al., 2002). High levels of ATP typically induce energy requiring apoptosis, but low ATP levels have been shown to favor necrosis instead (Eguchi et al., 1997; Los et al., 2002). An ATP dependent shift between apoptosis and necrosis underpins the dynamic relationship (Lemasters et al., 1999). TNFα is directly involved in both mitochondrial ATP production, as well as reactive oxygen species (ROS) generation (Skulachev, 2006). It also induces the activation of poly [ADP-ribose] polymerase1 (PARP1) via mitochondrial ROS leading to ATP depletion and subsequent necrosis (Los et al., 2002). Overactivation of PARP1 consumes large amounts of NAD^+ that result in ATP depletion (Sims et al., 1983) and therefore function as a molecular switch between apoptosis and necroptosis through the regulation of intracellular ATP levels (Fig. 1). PARP1 seems to interface directly with pathways that promote autophagy and has been found to link autophagy and necrosis. Furthermore,