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Autophagy- A review

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Abstract
Autophagy is a self-degradation process that is essential for survival, differentiation, development, and homeostasis. There are at least three forms of autophagy – chaperone mediated autophagy, microautophagy, and macroautophagy– that differ with respect to their mechanisms, physiological functions and cargo specificity all of which are involved in the lysosomal degradation of cellular components. The interplay between autophagy and apoptosis is discussed which is a complex phenomenon and not fully understood. The two processes are regulated by common factors and share common components, whereas the activity of one can regulate the activity of the other.

Keywords: Autophagy, Apoptosis, Necrosis, Autophagosomes, ATG genes.

Introduction

Autophagy is a self-degradation process that is essential for survival, differentiation, development, and homeostasis. There are at least three forms of autophagy – chaperone mediated autophagy, microautophagy, and macroautophagy– that differ with respect to their mechanisms, physiological functions and cargo specificity all of which are involved in the lysosomal degradation of cellular components [1].

Macroautophagy (hereafter called 'autophagy') is a mechanism conserved among eukaryotic cells that starts with the formation of a multi-membrane-bound vacuole, known as an autophagosome, which ultimately fuses with the lysosomal compartment and the degradation of the sequestered material [2]. Autophagy was discovered in mammalian cells and has been extensively investigated in yeast [3]. These studies have identified many genes encoding proteins involved in autophagy (ATG proteins) [4]. ATG proteins participate in the induction of autophagy, the formation, expansion and maturation of autophagosomes, and in the retrieval of autophagic proteins from mature autophagosomes [5].

Macroautophagy occurs at a basal rate in most cells, where it acts as a cytoplasmic quality-control mechanism to eliminate protein aggregates and damaged organelles [6]. The physiological importance of basal autophagy in maintaining tissue homeostasis has recently been demonstrated in conditional brain and liver Atg knockout mouse models [7]. These studies have demonstrated the role of autophagy in preventing the cytotoxic deposition of aggregate-prone proteins in the cytoplasm, and the contribution of autophagy to the elimination of ubiquitinated proteins that are efficient substrates for the proteasome. Although initially autophagy was classified as a form of cell death, it is now generally accepted that this is not the case and that it mainly promotes cell health and survival and, only under peculiar conditions, can it result in cellular death [8].

Molecular Mechanism of Autophagy

Longatti and Tooze [7] give a comprehensive view of the proteins regulating the initial events during autophagosome formation in higher eukaryotes. The activation of ULK kinases is an early regulatory step essential for the induction of many downstream autophagic events. However, a full comprehension of function of these kinases still requires a complete identification of their autophagy-specific protein substrates. After the induction of the ULK kinases, the Beclin 1–Class III PI3-Kinase complex is activated to produce PI3-P-enriched membrane domains, which are probably acting as platforms able to recruit the downstream factors, (Atg5–12–16 complex, LC3 and ATG9) which are required for autophagosomes assembly.

Recent advances in the late steps of the autophagic process, from the expansion of the autophagosomes to their fusion with lysosomes, are described in the review by Noda *et al*. [10] In particular, the authors focus on the role of LC3, the mammalian homolog of yeast ATG8. On the basis of recent experimental data, the authors propose that the main function of LC3 is related to the closure of the autophagosomes by a mechanism named 'reverse fusion' [9].

Autophagy-Apoptosis

The interplay between autophagy and apoptosis is complex and not fully understood. The two processes are regulated by common factors and share common components, whereas the activity of one can regulate the activity of the other. Several pro-apoptotic signals (e.g., those transduced by BH3-only proteins) induce autophagy, whereas signals that inhibit apoptosis (e.g., through Bcl-2 family members) also inhibit autophagy. An additional finding supporting a direct molecular link between autophagy and apoptosis is the observation that ATG5 can undergo calpain-mediated cleavage to generate a pro-apoptotic fragment that functions in the intrinsic mitochondrial death pathway [11]. When NF-kβ is

inhibited in cancer cells, TNFα triggers autophagy that contributes to TNFα-induced apoptotic signaling, including caspase-3 activation. Autophagy stimulation is dependent on the production of ROS and on the inhibition of mammalian target of rapamycin (mTOR). Whether autophagy is able to induce cell death when caspase 3 is inhibited remains to be investigated in this context. Interestingly, autophagy has been shown to regulate the activation of NF-kβ by degrading activators of the canonical and non-canonical NF-kβ pathways. Overall, the findings of these studies suggest that NF-kβ plays an important part in regulating autophagy [8].

Autophagy-Necrosis

Several studies have revealed that autophagy is upregulated by necrosis-inducing stimuli. Although in some cases autophagosomes accumulate mostly in neuronal axons, in others they gather in the perinuclear region [12]. Activation of RIP, a component of the Jun N-terminal kinase (JNK) pathway, in mammalian cells treated with caspase inhibitors also causes toxicity characterized by excess autophagosome formation [13]. The JNK pathway has been associated with caspase-independent necrotic-like damage [14, 15]. Although autophagy is upregulated in the above cases, it was not clear whether it protects cells or contributes to their destruction. Recent studies in C. elegans indicate a causative role of autophagy in necrotic cell death. Impairment of autophagy by downregulation of the autophagy genes bec-1, unc-51 and lgg-1 or pharmacological treatment interfering with autophagy partially suppresses necrotic neuronal death induced by hyperactive MEC-4, DEG-1 and DEG-3 ion channels. Autophagy synergizes with lysosomal proteolytic pathways to facilitate necrotic cell death [16].

Triggering Factors

Several signal transduction pathways that are triggered by common cellular stress as well as more specific signalling pathways can elicit both autophagy and apoptosis.

General stress mediators: ROS, ceramide and Ca2+ ROS can favour pro-apoptotic mitochondrial outer membrane permeabilization (MOMP) as well as stimulate the proteolytic activity of ATG4, thereby stimulating autophagy [17]. The sphingolipid ceramide is a prominent apoptosis inducer that acts through the intrinsic pathway, but it can also stimulate autophagy. Interestingly, another sphingolipid, sphingosine-1 phosphate, is a potent inhibitor of ceramide-induced apoptosis but also induces autophagy, which indicates how the intracellular milieu can contribute to 'deciding' between the two processes [18]. Increases in free Ca2+ ion concentrations in the cytosol ([Ca2+]c), Ca2+ efflux from the ER and Ca2+ overload of mitochondria all constitute prominent proapoptotic signals [19]. In addition, [Ca2+]c increases can trigger autophagy, presumably by activating calmodulin dependent kinase-β. This activates AMPK, which then inhibits mTOR, thereby de‑inhibiting autophagy [20]. However, this is not the only pathway that links increases in [Ca2+]c to catabolism. [Ca2+]c is a strong stimulator of calpains, which might contribute to autophagy [21] or apoptosis. [22] These findings underscore the extent to which the apoptotic cell death- and autophagy-inducing pathways can be intermingled by intracellular signals.

The role of p53. The transcription factor p53 is a quintessential tumour suppressor and apoptosis inducer. However, accumulating evidence suggests that p53 has a positive role in cell survival in response to physiological (as opposed to genotoxic) stress; for example, by stimulating antioxidant pathways [23] and autophagy [24].

BH3-only proteins and BH3 mimetics. BCL2 homology-3 (BH3) domains are present in all members of the BCL2 family. They can bind to BH3 receptors (which are present in the multi domain members of the BCL2 family), thereby inhibiting the anti-apoptotic BCL2-family members (such as BCL2 and BCL-XL) or activating the pro-apoptotic BCL2-family members (such as BAX and BAK) [23]. Pharmacological BH3 mimetics such as ABT737 and HA14-1 can induce autophagy in cells that do not undergo apoptosis or before they undergo apoptosis. In this context, inhibition of autophagy by knockdown of *BECN1* promotes apoptosis; conversely, inhibition of caspases promotes autophagy. The autophagy induced by ABT737 specifically concerns mitochondria and not the ER. Endogenous BH3-only proteins also induce autophagy. The BH3-only protein BAD, which is known to be activated by the withdrawal of obligate growth factors (presumably by dephosphorylation), is involved in the induction of autophagy [24, 25].

Death-associated protein kinase (DAPK) family. DAPK (also named DAPK1) is a calcium/calmodulin-regulated Ser/Thr death-promoting kinase [26]. DAPK1 controls either type I apoptosis or type II autophagic cell death in response to various stimuli including interferon‑γ, activation of Fas/CD95 receptors, tumour necrosis factor-α (TNFα), transforming growth factor-β (TGFβ), detachment from the extracellular matrix and oncogenes [27]. DAPK1 is a prominent tumour suppressor and its expression is lost in many tumours, mainly owing to DNA methylation [26]. In addition, a germ-line predisposition to chronic lymphatic leukaemia caused by a mutation in the promoter region of DAPK1 has recently been discovered. DAPK1 can induce apoptosis by several pathways: activation of p19ARF (which inhibits the negative regulator of p53, MDM2). The DAPK1 relatives DAPK-related protein kinase‑1 (DRP1; also known as DAPK2) and ZIP-kinase (or DAPK3) can also participate in controlling both apoptosis and autophagic cell death [27]. Physical interactions and a complex cascade-like signalling connection that amplifies both processes exist between the members of DAPK family. Notably, these kinases differ in their intracellular localization; whereas DAPK1 is mainly associated with the actin cytoskeleton, DRP1 is highly concentrated in the lumen of autophagic vesicles upon its ectopic expression. So, the DAPK family of proteins seems to consist of stress-activated kinases that link different cellular stresses to type I and type II cell death [26].

Conclusion

Autophagy can be a cell death mechanism in mammalian cells either by selectively targeting key cell survival elements or as a result of excessive bulk self-digestion. Identifying what determines selective autophagy in mammalian cells would provide important information about the impact of selective autophagy on cell survival. Moreover, recent studies of the regulation of Atg gene expression provide an important line of research to understanding the consequences of elevated Atg

for cell survival. Regarding autophagic cell death, it is generally assumed that excessive autophagy leads to self-destruction of mammalian cells. One intriguing aspect of autophagy is the increasing number of regulators now known to be shared with apoptosis. Many of these common regulators act by anti-apoptotic and antiautophagic pathways (e.g. Bcl-2 and NF-kB).

Conflict of Interest: None Declared

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