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Macroautophagy: a mechanism for mediating cell death or for promoting cell survival?

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Macroautophagy is a ubiquitous mechanism for the bulk removal of macromolecules and cell organelles from the cell. Periyasamy-Thandavan and colleagues report that cisplatin activates autophagy in renal tubular cells and that autophagy plays a role in decreasing apoptosis of tubular cells induced by cisplatin. This finding provides novel evidence that autophagy may play a role in ameliorating the effects of acute injury on the kidney.

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There are three types of autophagy. Microautophagy is a process that has been well characterized only in yeast; during this process cytosolic components are sequestered by lysosomes at the lysosomal membrane by septation and/or invagination. Chaperone-mediated autophagy is the direct translocation across the lysosomal membrane of cytosolic proteins that have been 'tagged' with a specific peptide sequence. This Commentary will focus entirely on macroautophagy (hereafter referred to as autophagy), which is a ubiquitous, genetically programmed and evolutionarily conserved process in which long-lived cytoplasmic components, including macromolecular aggregates and cellular organelles (such as mitochondria, peroxisomes, and endoplasmic reticulum), are sequestered into vesicles for bulk degradation by a lysosomal degradative pathway.[1–3]

The process of autophagy begins with the formation of crescent-shaped 'initiation' membranes that envelop and sequester cytosolic components before forming double-membraned vesicles called autophagosomes (Figure 1). The origin of initiation membranes remains uncertain. Autophagosomes fuse with lysosomes to form autolysosomes, which degrade the sequestered contents into their basic components (amino acids, fatty acids, and so on). These molecules are returned to the cytosol for recycling (Figure 1).[1–3] Although the proteasome provides another mechanism for mass degradation of proteins, this process requires proteins to be tagged by ubiquitination and to be unfolded so they can enter the degradative ubiquitination channel. In contrast, autophagy has the ability to degrade all forms of peptide and lipid macromolecules as well as entire cellular organelles.

Autophagy is a genetically programmed process. Autophagy-related genes (ATGs) were first isolated in yeast. Subsequently, a number of mammalian orthologues of yeast ATGs have been identified. These genes encode the proteins necessary for the complex series of events that constitute the autophagic cycle (Figure 1).[1–3] The signaling mechanisms responsible for the regulation of autophagy remain uncertain. However, it is clear that autophagy is activated by the target of rapamycin complex 1 (TORC1), as factors that activate TORC1 (insulin and growth factors) stimulate autophagy, and rapamycin-induced inhibition of TORC1 reduces autophagy.[2,4,5]

The biologic processes controlled by autophagy in mammals have not yet been clearly elucidated. Paradoxically, current evidence appears to suggest that autophagy can cause cell death in some situations while acting as a prosurvival mechanism in others.[5] 'Autophagic cell death' is presumed to result from an excessive level of cellular autophagy and is a form of programmed cell death (PCD type II) that is morphologically distinct from apoptosis (PCD type I).[3] Apoptosis is characterized by cell shrinkage, DNA fragmentation, and the rapid phagocytic removal and degradation of apoptotic cell fragments. In contrast, autophagic cell death is associated morphologically with the accumulation of autophagic vesicles (autophagosomes). A fundamental feature that distinguishes apoptosis from autophagic cell death is the source of the lysosomal enzymes used for degrading the dying cells. Apoptotic cells are degraded by the lysosomes of phagocytic cells, whereas in autophagy the endogenous lysosomal machinery of the dying cell serves this purpose.[5]

What data support the view that autophagy can cause cell death? There are morphologic studies that have demonstrated the accumulation of increased numbers of dying cells containing many autophagosomes in some disease states, such as degenerative diseases of brain and muscle.[3,4] However, these findings are only correlative in nature and cannot be considered direct

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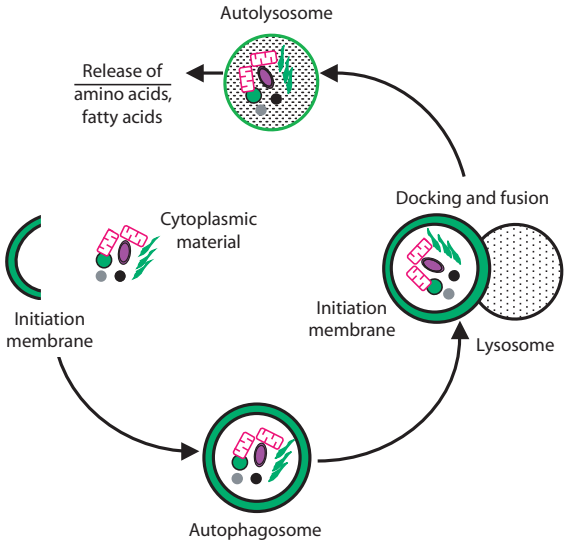

Figure 1 | The autophagic cycle. Autophagy is a lysosomal pathway that is responsible for the bulk degradation of macromolecules and cytoplasmic organelles. Autophagy is initiated by the formation of small, crescent-shaped membrane structures that elongate and enlarge into double-membraned vesicles called autophagosomes. Autophagosomes sequester macromolecules and organelles within the cytosol. Then the outer membrane of the autophagosome fuses with a lysosome to form an autolysosome. The contents of the autolysosome are degraded by acidic lysosomal hydrolases, and the resultant products of this process (amino acids, lipids, and other molecules) are returned to the cytosol for reuse.

proof of autophagic cell death. Studies using pharmacologic agents have demonstrated that inhibition of autophagy can prevent cell death.[3,6,7] However, in many of these studies, autophagy appears to have triggered apoptosis, rather than acting as the direct cause of cell death. Other *in vitro* studies, in which the expression of genes essential for autophagy were 'silenced' with RNA interference (RNAi), have provided the most convincing evidence to date that the autophagy is capable of killing cells, at least under some circumstances. In cells treated with caspase inhibitors[8] and in murine embryonic fibroblasts lacking the proapoptotic protein Bax or Bak,[9] inhibition of the expression of autophagy genes with RNA-mediated interference induced autophagic cell death. However, in both reports, the apoptotic pathway was defective in the cells studied. Although these studies clearly demonstrate that autophagy can induce cell death by pathways independent of apoptosis, they still leave unresolved the question of whether autophagy can ever cause death in cells in which the apoptotic machinery is intact. Thus, although many different lines of evidence suggest that autophagic cell death can occur, the importance of this form of cell death in physiologic or pathologic states remains to be determined by further scientific scrutiny.[3,5]

Although the role of autophagic cell death seems as yet uncertain, there is more substantial evidence that autophagy can promote cell survival.[10] In fact, autophagy represents an ancient and evolutionarily conserved mechanism that is essential for supporting cell survival in the absence of nutrients or growth factors.[10] The re-addition of nutrients or growth factors after periods of deficiency generally leads to continued cell viability.[10] However, if growth factor or nutrient depletion continues for too long, cells will ultimately die by apoptosis. Thus, autophagy appears to represent a highly effective, if self-limited, mechanism for prolonging cell survival in the absence of nutrients and growth factors.[10]

More recent data suggest that autophagy can protect cells against acute injury induced by hypoxia and oxidative injury.[1–3,11,12] Periyasamy-Thandavan *et al.*[13] (this issue) provide highly novel evidence that cisplatin activates autophagy within renal tubular cells, and that inhibition of autophagy promotes apoptosis of cultured renal tubular cells treated with cisplatin. This study is the first to demonstrate a role for autophagy in ameliorating the functional and structural effects of acute renal injury induced by cisplatin.[13] The extent to which autophagy can ameliorate acute kidney injury caused by other types of renal insults (such as ischemia) remains to be determined.

The mechanisms responsible for the prosurvival effect of autophagy are uncertain. It seems likely that some benefits of autophagy are metabolic in nature.[10] Autophagy degrades unnecessary macromolecules and organelles into nutrients that can be reused for generating energy and for rebuilding essential cell structures. This mechanism most likely explains the survival effect of autophagy when it is activated by periods of bioenergetic stress imposed by decreased extracellular supply of nutrients or cell injury. The amino acids generated by autophagy can be processed to support the tricarboxylic acid (TCA) cycle, which carries out two important prosurvival functions. The TCA cycle is an important source of $FADH_2$ and NADH (which are necessary for ATP generation by the electron transport chain) and can also convert intermediates in the cycle into various metabolites, including cytosolic acetyl-coenzyme A (acetyl-CoA), sterols, and nucleotides. Loss of TCA cycle activity would compromise the ability of the cell to generate these substances. The importance of constitutive TCA activity in autophagic cells is supported by studies showing that providing pyruvate, a TCA substrate, during nutrient deprivation suppresses autophagy while maintaining cell viability. In addition, the turnover of lipids during autophagy might also be important in maintaining cell viability. Acetyl-CoA, an end product of β-oxidation and glycolysis, combines with oxaloacetate in the entry step of the TCA cycle. A deficiency of acetyl-CoA would result in decreased TCA activity, even in the presence of sufficient oxaloacetate. In cells confronting a deficiency of nutrients, β-oxidation might play an increasingly important part in supplying acetyl-CoA.

In addition to its role in optimizing metabolic conditions in stressed cells, autophagy may also function as a 'waste disposal' system, clearing fully formed cytosolic organelles that accumulate from the cytoplasm and that are injurious to the cell. It is not yet known whether the disposal system of autophagy occurs randomly or whether it involves the selective targeting of particular organelles. However, it is possible that autophagy preferentially removes aging or oxidatively damaged organelles

and protein aggregates that have the potential to cause or exacerbate cell injury. This could be particularly important for damaged mitochondria, which have a decreased ability to produce ATP and an increased potential for producing toxic reactive oxygen species. Another important organelle that may be targeted for turnover through autophagy is the peroxisome. Peroxisomes are organelles that function in lipid metabolism and breakdown of hydrogen peroxide and are 'marked' for turnover when they are in excess or damaged. The role played by autophagy in promoting cell survival by preventing the accumulation of aged or damaged cytosolic organelles and macromolecules still needs to be determined by additional research.

In summary, autophagy has entered the research spotlight during the past few years because of the discovery of mammalian orthologues of yeast autophagy genes and the elucidation of the mechanisms involved in the autophagic cycle (Figure 1). These studies have revealed the highly conserved nature of autophagy and have implicated autophagy pathogenesis of a wide array of disease states. The study by Periyasamy-Thandavan *et al.*[13] highlights the potential role of autophagy as a survival mechanism following cytotoxic injury to the kidney and is also relevant to the potential protective role of autophagy in acute injury to other organs, such as brain, heart, and liver.[6,11]

As our understanding of the mechanisms involved in autophagy continues to evolve, it is likely that specific pharmacologic agents will become available that can enhance or inhibit autophagic activity.[14] These agents could prove useful for the treatment of diseases in which autophagy plays an important role.[14] However, a great deal of additional research is necessary in order to define the role of autophagy in the pathogenesis of different disease states before we are able to determine the therapeutic utility of pharmacologic agents that modulate the autophagic cycle.

DISCLOSURE

The author declared no competing interests.

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Once-yearly intravenous zoledronate does not impair renal function in postmenopausal women

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Zoledronate, a potent third-generation amino-bisphosphonate previously used for the treatment of bone metastasis, was recently shown to significantly reduce the risk of vertebral and hip fractures in osteoporotic postmenopausal women when infused at a dose of 5 mg per year for 3 years. The renal follow-up of this pivotal study that included more than 5,000 patients (estimated creatinine clearance >30 ml per min) is reported by Boonen *et al.* and shows the long-term renal safety of zoledronate in this osteoporotic population.

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Bisphosphonates, potent inhibitors of osteoclastic resorption, are bone-seeking drugs with very high affinity for mineralized collagen matrix. Therefore, they are cleared rapidly from the circulation, with about half the administered dose taken up by the skeleton while the other half is excreted, unmetabolized, by the kidneys.[1] After being embedded in bone at the remodeling sites,[2] they are slowly released from bone into the circulation,[3] when a remodeling cycle is again initiated at the bone surface. Therefore, because bisphosphonates remain in bone for years when administered daily, the idea of exploiting the considerable skeletal persistence of these drugs was raised, and bisphosphonates were then given weekly, and after that monthly, and are now proposed to

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