A Matter of Life or Death (or Both): Understanding Autophagy in Cancer

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Background

Definition

Autophagy is a conserved response to nutrient deprivation found in yeast, plants, worms, flies, mice, and man (1). In yeast, nutrient deprivation activates a genetic program that results in (a) self-digestion of cytoplasm and organelles and the recycling of amino acids and fatty acids for energy utilization and (b) budding of an immortal spore. The process of autophagy involves formation of a double-membrane vesicle (“autophagosome”) in the cytosol that engulfs organelles and cytoplasm and then fuses with the lysosome to form the “autolysosome,” where the contents are degraded and recycled for protein and ATP synthesis (reviewed in ref. 2). The formation of the autophagosome is mediated by a series of autophagy-specific genes (ATG), whose products have been classified into four groups based on function: (a) a autophagy regulatory complex that responds to upstream events, such as availability of nutrients; (b) a lipid kinase group that controls vesicle nucleation; (c) an ubiquitin-like protein conjugation system required for assembly of the autophagosome; and (d) a group that is required for disassembly of ATG complexes (1).

Function

This form of self-digestion in unicellular organisms leads to self-preservation in times of nutrient deprivation. However, if left unchecked, autophagy has the potential of producing terminal self-consumption. Not surprisingly, autophagy finds its counterpart in multicellular organisms as high up the animal kingdom as Homo sapiens. At the cellular level, nutrient deprivation prompts cells to exit the cell cycle, shrink, autodigest long-lived proteins and damaged organelles, and recycle fatty acids and amino acids for synthesis of macromolecules or oxidation in mitochondria to maintain cellular ATP. Autophagy may thus result in cellular destruction and/or cellular "hibernation" until the supply of nutrients is restored. Whereas autophagic cell death rests largely on indirect evidence, autophagic cell survival is supported by direct evolutionary, genetic, and biochemical studies (reviewed in ref. 3).

Recent articles highlight aspects of autophagy that are relevant to survival. For example, Kuma et al. (4) asked, “How do newborns survive after being cut off from the maternal blood supply and before adequate nutrients are available through suckling?” In this remarkable set of experiments, it was shown that at birth there is up-regulation of autophagy in several organs, most notably heart and diaphragm, two muscles with abrupt increases in energy requirements at birth. Mice lacking Arg5, a gene whose product is critical for the formation of autophagosomes, appeared normal at birth but died within 24 hours of delivery. The Arg5−/− animals have reduced circulating amino acids and decreased cardiac ATP and die presumably due to energy depletion. Electrocardiograms done on the newborns pups revealed cardiac damage. Thompson’s laboratory (5) examined the role of autophagy in the long-term survival of immortalized hematopoietic precursors and primary bone marrow cultures deprived of an obligate growth factor (interleukin-3). In the absence of growth factors, there was decreased expression of membrane nutrient transporters leading to nutrient deficiency. They found that cells in which apoptosis was inactivated by deleting bax and bak survived for >6 weeks in the absence of interleukin-3 by using autophagy to supply precursors for ATP. On readdition of interleukin-3, cells increased nutrient transport and recovered size and the ability to proliferate. Finally, Boya et al. (6) found that nutrient-deprived HeLa cells underwent autophagy rather than apoptosis (believed to occur through sequestration of damaged mitochondria). Consistent with its prosurvival role, blocking the autophagic pathway with either small interfering RNAs against key autophagy genes (beclin1, Atg10, or Atg12) or with drugs (3-methyladenine, hydroxychloroquine, baflomycin A1, or monensin) triggered apoptosis.

Recent Advances

Regulation

Levine’s group provided additional insights into the role of autophagy in what may be a delicate balance between cell death and survival. Beclin-1 (BECN1) is the mammalian homologue of the yeast Atg6 autophagy gene, which is involved in early autophagosome formation (7, 8). Monallelic deletion of BECN1 in mice enhanced tumorigenesis (9, 10), initially suggesting that autophagy was a cell death pathway. However, it was later shown that embryonic stem cells that are null for BECN1 have no changes in sensitivity to death stimuli and that tumors from BECN1 haploinsufficient mice did not show depletion of beclin-1 protein (3). Levine’s group showed that bcl-2 binds to beclin-1 protein and dampens autophagic cell death (11). Using beclin-1 mutants that fail to bind bcl-2, they observed excessive...
autophagy and cell death. These data suggest that bcl-2 can regulate both autophagy and apoptosis and that bcl-2 may calibrate autophagy to levels that are compatible with survival. Beclin-1 can also bind to class III phosphatidylinositol 3-kinase (PI3K) and form a complex that is required for the activation of autophagy (12).

Autophagy is controlled by pathways that impinge on the mammalian target of rapamycin (mTOR). The mTOR pathway integrates the cellular response to growth factors and nutrients through regulation of protein synthesis (Fig. 1). In yeast, TOR provides a link between cell growth and the availability of extracellular nutrients. In mammals, mTOR is also regulated by growth factors through the class I PI3K/Akt pathway (13) and by the down-regulation of surface nutrient transporters during growth factor withdrawal (5). Activation of TOR in yeast inhibits autophagy via phosphorylation of the APG1-APG13 complex (14), a process inhibited by rapamycin in both yeast and mammalian cells (14). Substantial cross-talk exists between the PI3K/Akt pathway and the Ras/Raf/extracellular signal-regulated kinase 1/2 pathways (Fig. 1). For example, epidermal growth factor activation of both extracellular signal-regulated kinase 1/2 and Akt blocked the induction of autophagy, whereas PTEN, a negative regulator of Akt, stimulated autophagy in HT-29 colon cancer (15).

**Protein synthesis**

Tight regulation of protein synthesis is essential for cell survival during nutrient and growth factor deprivation because...
protein synthesis accounts for the consumption of up to 50% of cellular energy (16). The majority of energy is used during peptide elongation either through the hydrolysis of 2 mol GTP for each added amino acid or during the charging of aminoacyl-tRNAs where 1 mol ATP is hydrolyzed per each charged amino acid. Therefore, for cells to survive under conditions of nutrient deprivation, protein synthesis must be limited to conserve energy; if not, depletion of ATP will impede the function of membrane transporters, thereby destroying electrochemical gradients and thus leading to necrotic cell death (3).

In the presence of adequate nutrients, protein synthesis is stimulated and autophagy is inhibited as depicted in Fig. 1. This is mediated through activation of mTOR via PI3K and Akt and inactivation of the tuberous sclerosis complex TSC1 and TSC2. mTOR phosphorylates S6 kinase and increases the translation of mRNAs that encode ribosomal and other proteins involved in translation (17). Protein translation is then initiated by phosphorylating 4EBP1, an inhibitor of initiation, causing its disassociation from eukaryotic initiation factor 4E (eIF4E). Active eIF4 promotes cell proliferation by increasing translation of cyclin D1, c-Myc, and vascular endothelial growth factor (18). In the presence of adequate nutrients/growth factors, three enzymes, AMP kinase, mTOR, and S6 kinase, promote peptide elongation by regulating eukaryotic elongation factor-2 (eEF-2) kinase (Fig. 1).

In the absence of nutrients, protein synthesis is inhibited and autophagy is activated. Nutrient/growth factor deprivation and subsequent ATP depletion induce autophagy by inhibiting mTOR (via activation of TSC2 by AMP kinase) and by decreasing phosphorylation of S6 kinase and 4EBP1 (19, 20). Under these conditions, initiation of translation is repressed by the reformation of the 4EBP4/eIF4E inhibitory complex, and protein elongation is inhibited through activation of eEF-2 kinase by the increased activity of AMP kinase and decreased activity of mTOR and S6 kinase. Thus, eukaryotic cells have evolved a mechanism to withstand nutrient deprivation by decreasing energy utilization through inhibiting protein synthesis and producing ATP through recycling of amino acids produced from autophagic digestion of cellular organelles and proteins.

eEF-2 kinase is a structurally unique enzyme (21) whose activity is increased in cancer (22). An early clue to the importance of eEF-2 kinase in cell survival came from an unlikely source (hibernation experiments in squirrels). When captured and placed in a hibernaculum (low temperature, dim light, and no nourishment), these creatures survive by reducing body temperature, respiratory rate, heart rate, blood pressure, cardiac output, cerebral blood flow, and metabolism. Hibernating squirrels increase the phosphorylation of eEF-2 in brain and liver through activation of eEF-2 kinase and inhibition of PP2A, the cellular phosphatase that dephosphorylates eEF-2 (23). Accordingly, during hibernation, decreasing protein synthesis through inhibition of elongation conserves energy. eEF-2 kinase is tightly regulated by the availability of growth factors and nutrients via insulin-dependent and non-insulin-dependent regulation of mTOR through the class I PI3K/Akt pathway. Proud’s group showed that when nutrients are available the activity of eEF-2 kinase is inhibited by phosphorylation at Ser78 and Ser146 by mTOR and S6 kinase, respectively (16, 24). In times of “famine,” the activity of eEF-2 kinase is increased by phosphorylation at Ser239 by AMP kinase, which is activated at high AMP/ATP ratios (25). Phosphorylation of eEF-2 at Thr56 by eEF-2 kinase decreases the affinity of the elongation factor for ribosomes and terminates elongation. During times of nutrient/growth factor deprivation when mTOR and S6 kinase activities are decreased and AMP kinase is increased, eEF-2 kinase is activated and transiently inhibits protein elongation, thereby conserving energy (Fig. 1).

Oncogenesis

As mentioned above, BECN1 is the mammalian homologue of the yeast Atg6 autophagy gene and was initially identified through its interactions with bcl-2 (7, 8). BECN1 promotes autophagy in breast cancer cells and maps to a cancer susceptibility locus (17q21) that is monoallelically deleted in 40% to 75% of breast and ovarian cancer (26–28). Monoallelic deletion of beclin1 in mice enhanced tumorigenesis leading to the development of carcinomas of the lung and liver as well as lymphomas (9, 10). Overexpression of beclin-1 in MCF-7 cells increased autophagy on amino acid starvation (8) and inhibited proliferation without increasing cell death. Inhibition of PI3K by 3-methyladenine decreased beclin-1-induced autophagy in MCF-7 cells (8), which may be regulated by DAP kinase and DRP-1 (29). BECN1 transfectants were reportedly “less tumorigenic”; however, the authors did not rule out the possibility that this was due to lowered proliferation. Although the BECN1 gene is haploinsufficient in MCF-7 breast cancer, treatment with tamoxifen induced autophagy (30).

Accumulating evidence points to the importance of autophagy in cancers (reviewed in ref. 31). One of the most carefully studied has been glioblastoma multiforme due in good measure to the work of Kondo’s laboratory (31). For example, radiation (32), platelet-derived growth factor receptor antagonists (33), rapamycin (34), and temozolomide (35) all induce autophagy in a variety of glioma cell lines. Our recent studies indicate that eEF-2 kinase may play an important role in autophagic survival of glioma cells and that targeting this enzyme may accelerate cell death (36). As shown by us (36) and others, the activity of mTOR and S6 kinase are decreased by nutrient/growth factor deprivation and this relieves the inhibition of eEF-2 kinase activity produced by these two enzymes (16, 24). Because protein synthesis consumes more ATP than any other cellular process, the activation of eEF-2 kinase conserves energy through terminating protein elongation and thereby supports survival during times of nutrient stress. To test this model in glioma cells, we blocked starvation-induced activation of eEF-2 kinase with small interfering RNA and measured its effects. Stable glioma cell populations depleted of eEF-2 kinase by short hairpin RNA [T98GeEF-2K(−)] showed decreased phosphorylation of eEF-2 at Thr56 and autophosphorylation of eEF-2 kinase compared with isogenic transfectants were reportedly “less tumor-igenic”; however, the authors did not rule out the possibility that this was due to lowered proliferation. Although the BECN1 gene is haploinsufficient in MCF-7 breast cancer, treatment with tamoxifen induced autophagy (30).

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to nutrient withdrawal as manifested by accelerated loss of viability when grown in serum-free medium. In contrast, overexpression of eEF-2 kinase in T98G glioma cells increased autophagy (36). This intolerance to deprivation is consistent with the depletion of ATP due to ongoing protein synthesis in the face of inadequate nutrients.

**Therapeutic Implications**

**Targeting autophagy for cancer treatment: pros and cons**

If autophagy serves as a dominant survival pathway for cancer cells living on the edge of an adequate blood supply, nutrients, and growth factors, then blocking this survival pathway should provide a means to kill the cancer cells that are often resistant to many forms of treatment, including radiation, chemotherapy, and growth factor antagonists. Similarly, if autophagy protects against apoptotic cell death by sequestering damaged mitochondria, then blocking autophagy should accelerate killing by shutting cells into more reliable death pathways. Alternatively, if autophagy is a dominant death pathway, then inhibition could promote survival. Chemotherapeutic drugs (34, 35), growth factor antagonists (33), and radiation (32) can activate autophagy pathways in models of glioblastoma and other tumors, but whether autophagy is a protective cellular response to cell damage or growth factor withdrawal or inhibition or a death-promoting activity remains to be fully elucidated. Inhibitors of autophagy have been reported to both increase and decrease cell death following treatment with anticancer drugs (38–42).

To illustrate this point, consider the withdrawal of a growth factor (e.g., estradiol) in the treatment of breast cancer. If the increase in autophagy observed by several labs was a mechanism of cell death elicited by antiestrogen treatment, then inhibition of autophagy should decrease cell kill. Conversely, if the onset of autophagy represented a survival mechanism, then inhibition of autophagy should ultimately increase cell death perhaps by shutting cells into more permanent death pathways. At this point, few studies have rigorously explored these possibilities in patients, which will be crucial in determining how to deal with this highly conserved cellular response.

There are several potential ways to envision targeting the autophagy pathway. For example, one could disrupt autophagy by the following approaches: (a) block the signaling pathways that initiate autophagy; target key components involved in the formation of the autophagosome (autophagy gene products); (b) inhibit the fusion of the autophagosome with lysosomes to block autolysosome formation (bafilomycin A and antimitotic drugs); (c) disrupt the recycling of autodigested substrates used for resynthesis of ATP; or (d) block the cell’s ability to conserve energy by preventing the termination of protein synthesis. A target that is activated rather than inhibited (e.g., mTOR, Akt, and PI3K) to initiate our sustain autophagy would be preferable, because it is far easier to block a target than to activate one. In that regard, eEF-2 kinase becomes increasingly attractive because it is overexpressed in many forms of cancer (22, 43) and it is activated during autophagy (36); its activity terminates protein elongation and conserves energy (44); and its unique structure makes this kinase amenable to selective inhibition (21, 43). Conditional inactivation of eEF-2 kinase by genetic (conditional knockout) or pharmacologic approaches in cancer models would therefore help address the proper role of autophagy in cancer treatment.

**Summary**

Autophagy is a highly conserved pathway that can be used for survival during nutrient and growth factor deprivation. Under certain circumstances, it seems that autophagy may serve as a pathway to cell death. Already, it is becoming increasingly clear that autophagy is involved in cancer formation, survival, and response to several forms of cancer treatments. A detailed understanding of how this pathway is regulated is now emerging and will allow translation of this information into new approaches to cancer treatment.

**References**

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