Autophagy in Health and Disease: A Double-Edged Sword

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Autophagy, the process by which cells recycle cytoplasm and dispose of excess or defective organelles, has entered the research spotlight largely owing to the discovery of the protein components that drive this process. Identifying the autophagy genes in yeast and finding orthologs in other organisms reveals the conservation of the mechanism of autophagy in eukaryotes and allows the use of molecular genetics and biology in different model systems to study this process. By mostly morphological studies, autophagy has been linked to disease processes. Whether autophagy protects from or causes disease is unclear. Here, we summarize current knowledge about the role of autophagy in disease and health.
Table 1. Possible roles of autophagy in health and disease.

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<th>Disease state</th>
<th>Beneficial effects of autophagy</th>
<th>Negative effects of autophagy</th>
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<td>Cancer</td>
<td>Acts as a tumor suppressor; may be involved in type II PCD in cancer cells, could limit cell size or may remove damaged organelles that could generate free radicals and increase mutations.</td>
<td>May allow survival of cancer cells within the nutrient-poor environment of a tumor, could prevent cell death, and may protect against some cancer treatments.</td>
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| Liver disease | Allows removal of nonfunctional endoplasmic reticulum resulting from accumulation of aggregated α1-antitrypsin 2 protein. | Increased mortality due to excessive mitochon- 
drial autophagy. |
| Muscular disorder | Increased autophagy may compensate for defects in lysosomal function. | Increased autophagy or defects in completing autophagy result in the accumulation of autophagosomes that may impair cell function. |
| Neurodegeneration | Allows the removal of protein aggregates before they become toxic. | May induce cell death in neurons that accumulate aggregated proteins. |
| Pathogen infection | Cellular defense against invasion by bacteria and viruses. | Subversion of the autophagic pathway allows pathogens to establish a replicative niche and supplies nutrients for growth. |

Fig. 1. Conceptual model of macroautophagy.
Fig. 3. Action and subversion of autophagy during bacterial infection. Bacteria may be taken up by phagocytosis and the resulting phagosome can fuse with endosomes and then the lysosome; the bacteria are then degraded within the phagolysosome (not shown). Some pathogens such as L. monocytogenes, escape this pathway by lysing the phagosome membrane. The bacteria may subsequently become targets for autophagy. In addition, degradation of host cell proteins within the late autophagosome or autophagolysosome may supply the nutrients needed for growth of the pathogen.

Fig. 2. Schematic model of autophagic regulation.

Stimulation of the class I PtdIns 3-kinase at the plasma membrane through the insulin receptor results in the generation of PtdIns(3,4)P2 and PtdIns(3,4,5)P3 (dark pink circles). These phosphoinositides allow binding and activation of Akt/PKB and its activator PDK-1. Along with amino acids, Akt/PKB activates mTor (additional components in this pathway are not depicted). Subsequent phosphorylation of a downstream effector, possibly analogous to Alg1 or other ATG gene products as demonstrated in yeast, inhibits autophagy. PTEN dephosphorylates 3-phosphoinositides and antagonizes the action of class I PtdIns 3-kinase. A class III PtdIns 3-kinase complex, which includes Beclin 1/Alg6, generates PtdIns(3)P (purple circles) to control the membrane dynamics that are associated with autophagosome formation. Rapamycin inhibits mTor, while wortmannin and 3-methyladenine inhibit the class III PtdIns 3-kinase; the effect is to induce or inhibit autophagy, respectively. Autophagy is also regulated through heterotrimeric G proteins and other kinases and phosphatases that are not depicted.
Following growth factor withdrawal, Bax\(^{-/-}\) Bak\(^{-/-}\) cells activate autophagy, undergo progressive atrophy, and ultimately succumb to cell death.

An additional consequence of growth factor limitation is a rapid decline in the surface expression of nutrient transporters including the major glucose transporter GLUT1, the LDL receptor, amino acid transporters and receptors for iron uptake.

This decrease in nutrient transporter expression has been proposed to perturb mitochondrial physiology resulting in the induction of apoptotic cell death.

An alternative explanation is that the decline in surface expression of nutrient transporters simply reflects a secondary response to the decreased metabolic demand on the cell following the cessation of growth and the withdrawal from the cell cycle.
Cells from Bax\textsuperscript{–/–} Bak\textsuperscript{–/–} animals fail to undergo apoptosis in response to serum deprivation, loss of attachment, and growth factor withdrawal. Thus, Bax and Bak are essential and redundant regulators of apoptosis and extracellular signals.

(A) Two independent clones of Bax\textsuperscript{–/–} Bak\textsuperscript{–/–} IL-3-dependent cells (parental) stably transfected with either Bax, Bak, or empty vector (vec) were generated, and expression levels were assessed by Western blot. The IL-3-dependent Bax / Bak / cell line FL5.12 is shown for comparison.

(B) Kinetics of cell death in Bax- or Bak-reconstituted cells following IL-3 withdrawal. Viability was measured by propidium iodide exclusion. Data are averages of three experiments standard deviation (SD).
(C) **Cell viability** of Bax⁻/⁻ Bak⁻/⁻ cells in the presence or absence of IL-3. Cells were washed and cultured in the presence (open squares) or absence (closed diamonds) of IL-3. At the indicated time points, cells were collected and viability was assessed. Cells grown in the presence of IL-3 were passaged every 2–3 days to restore a cell concentration of 7.5 × 10⁵ cells/ml. The medium in IL-3-deprived cultures was replaced with an identical volume of fresh complete medium without IL-3 every 10 days. Data are averages of three independent experiments ± SD.

(D) **Cell numbers** of cultures that were grown in the presence or absence of IL-3 and were cultured as in (C). Data are averages of three independent experiments ± SD.

(E) **Cell size** of cultures that were grown in the presence or absence of IL-3 and were cultured as in (C). Data are averages of three independent experiments ± SD.

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**Figure 2. Metabolic Effects of IL-3 Withdrawal on Bax⁻/⁻ Bak⁻/⁻ Cells**

(A) Glycolytic rate of cells grown in the absence of IL-3 as measured by the conversion of 5-³H-glucose to ³H₂O at the indicated time points. The data presented at week 0 represent values of control cells growing in IL-3 throughout the time course of the experiment. Data are averages of three experiments ± SD.

The time-dependent loss of GLUT1, the major glucose transporter expressed on these cells

(B) Western blot analysis of GLUT1 expression in cells cultured in the absence of IL-3. The GLUT1 expression at week 0 is representative of GLUT1 expression of cells grown in IL-3.
Figure 2. Metabolic Effects of IL-3 Withdrawal on Bax--/- Bak -/- Cells

Coincident with the decline in glycolysis, there was a decline in mitochondrial membrane potential

(C) Mitochondrial membrane potential as measured by TMRE staining in cells grown without IL-3 (solid histogram) at the indicated time point. Baseline TMRE was determined by using cells treated with the uncoupler CCCP (dotted histogram). The numbers in the top right corner indicate the average mean fluorescence intensity of three independent experiments. The week 0 time point indicates the mean fluorescence intensity of cells growing in IL-3 and is representative of the values obtained for such cells over the time course of the experiment.

(D) ATP levels in cells grown without IL-3 and expressed as arbitrary units (AU). ATP levels for IL-3-grown cells did not decline significantly over the time course of the experiment (data not shown). Data are averages of three independent experiments ± SD.

Cellular ATP levels also fell, but the decline in glucose transporter expression was greater than that expected based on the ATP decline, suggesting that cells were utilizing alternative substrates to maintain their bioenergetics.
The continued decline in cell size of the G0/G1 arrested cells following growth factor withdrawal suggested the possibility that cells were utilizing macroautophagy to catabolize intracellular substrates to maintain their survival.

Figure 3. Growth Factor Withdrawal Induced Autophagosome Formation Is Required for Survival

(A) Electron microscopy of cells grown in the absence of IL-3 for 48 hr (a–c) showing the presence of autophagosomes. Arrowheads depict representative autophagosomes quantitated in (d). Scale bar, 100 nm. (d) Quantitation of the number of autophagosomes per cross-sectioned cell cultured in the presence or absence of IL-3 for 48 hr. Error bar represents SD. Statistical significance determined by Student’s t test.
**Autophagocytosis**

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(B) Immunofluorescence with anti-LC3* antibody on cells grown in the presence (a) or absence (b) of IL-3 for 48 hr.

(*) antibody specific for the mammalian homolog of the yeast Atg8 protein, microtubule-associated protein-1 light chain-3 (LC3).

(C) Immunoblot analysis of LC3-I processing into LC3-II in cells transfected with control or two independent shRNA constructs against ATG5 (hp-2 and hp-7) followed by culture in the presence or absence of IL-3 for 48 hr. Actin was used as a loading control.

Inhibition of Autophagy Leads to Cell Death

(D) Time course of cell viability following IL-3 withdrawal in cells with inactivation of ATG5. Data are averages of three experiments ± SD. Western blot analysis of ATG5 protein expression in cells transfected with vector control, hp-2, or hp-7 shRNA is shown as a representative experiment. Actin was used as loading control.

(E) Time course of cell viability following IL-3 withdrawal in cells transfected with FITC tagged-siRNA for ATG7 (Yu et al., 2004) or a control siRNA. Cells which had incorporated the siRNA for ATG7 or control were purified by FACS sorting based on FITC-positive cells, and viability was assessed at the indicated time points. Data are averages of three experiments ± SD.
Figure 4. Persistent Autophagy in Long-Term Growth Factor-Withdrawn Cells

(A) Electron microscopy of cells grown in the presence (a) or absence (b) of IL-3 for 6 weeks. Scale bar, 8.5 μm. Magnification image of a cell grown in the presence (c) or absence (d) of IL-3 showing autophagosomes (arrows). Scale bar, 2.3 μm.

Higher magnification of cells grown in the absence of IL-3 (e and f). Arrowheads depict autophagosomes in cells containing recognizable cellular material (e) or a late autophagosome fusing with a lysosome (f). Arrowheads depict representative autophagosomes quantitated in (B).

Long term deprivation (6 weeks)
Figure 5. Cell Death Following Inhibition of Autophagy Is Reversed by Methylpyruvate

(A) Viability of cells grown in the presence (top panel) or absence (bottom panel) of IL-3 for 6 weeks treated with 5 mM 3-MA (closed squares) or 10 M CQ (open triangles). PBS was used as a vehicle control (closed diamonds).

3MA (3-Methyladenine) and CQ (chloroquine) = inhibitors of automacrophagy

(B) Immunofluorescence staining of LC3 in cells grown in the presence (a) or absence (b) of IL-3 for 6 weeks. Cells grown in the presence or absence of IL-3 were treated for 18 hr with 5 mM 3-MA (c and d) or 10 M CQ (e and f) followed by LC3 staining. PBS was used as a vehicle control.

(C) DNA fragmentation assay was performed on Bax -/- Bak -/- cells grown in the presence or absence of IL-3 for 6 weeks and treated for 36 hr with 5 mM 3-MA, 10 M CQ, or PBS as a vehicle control. IL-3-dependent Bax -/- Bak -/- FL5.12 cells grown in the absence of IL-3 for 36 hr were used as a positive control for DNA laddering.
A cell-permeable form of pyruvate, methylpyruvate (MP), was added to the cultures at the time 3-MA or CQ treatment. Once internalized, this substrate can be oxidized in the tricarboxylic acid cycle to produce NADH to fuel electron transport and ATP production.

Figure 7. IL-3 Restimulates Glycolysis and Growth/Proliferation in Growth Factor-Deprived Cells

Despite the loss of cell surface nutrient transporters, the absence of an observable Golgi/ER, and a profound decline in total protein content, the cells cultured in the absence of IL-3 had higher levels of surface IL-3 receptor than cells grown in the presence of IL-3.
Cell size recovery following IL-3 readdition is dependent on the duration of deprivation. Histogram of mean cell size (fL) in cells restimulated with IL-3 for the indicated number of days following 2 (left panel) or 6 (right panel) weeks of growth factor withdrawal.
Macroautophagy Is a Conserved but Self-Limited Survival Mechanism

Based on the results, macroautophagy appears to be an evolutionarily conserved survival strategy. Macroautophagy can support growth factor-independent cell survival of hematopoietic cells for several weeks.

Thus, it appears eukaryotic cells share a common survival pathway that promotes cell-autonomous survival in the face of starvation and/or neglect. Animal cells may have evolved an apoptotic response in part to limit this form of cell-autonomous survival. Nevertheless, as previously demonstrated in unicellular organisms, macroautophagy is a self-limited survival strategy and ultimately will result in cell death if not reversed.