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News and Commentary

Doctor Jekyll and Mister Hyde: autophagy can promote both cell survival and cell death

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Via autophagy cells can degrade their own cytoplasmic material in lysosomes. This process has been considered important for survival during starvation conditions, but the genetic evidence for such a role in mammalian cells has been lacking. Recent results with cell lines deficient in autophagy proteins finally demonstrate the essential role of autophagy for survival in mammalian cells deprived of nutrients or growth factors. However, recent results also show that under certain conditions autophagy can promote cell death, particularly if cells are triggered to die but apoptosis is not possible. Importantly, these studies show that autophagy proteins are needed for the induction or execution of cell death. However, the current results suggest that the two modes of autophagy, survival promoting and death promoting, may be activated via different signaling pathways. In addition, further results suggest that under certain conditions autophagy may act as an initiator of cell death that however needs the apoptotic machinery for the execution of the cell demise.

Introduction: Autophagy and Apoptosis

Autophagy is a process where cytoplasmic material, including organelles, is segregated into a double-membrane bound vesicle and then delivered to the lysosomal compartment for degradation.[1,2] Two main functions have been proposed for this process. Firstly, autophagy is a short-term stress response in nutrient-limited conditions or amino-acid deficiency. By degrading their own cytoplasmic components in lysosomes, cells get substrates for both energy metabolism and vital protein synthesis.[3] Secondly, autophagy is suggested to play a role in type II, or autophagic, cell death.[4] Autophagy (*ATG*) genes, which are required for the formation of autophagosomes, were originally discovered in yeast mutants. A uniform nomenclature was recently agreed upon for these genes.[5] Atg5, as a covalent conjugate with Atg12, is essential for autophagosome formation.[6] Another protein essential for autophagosome formation is beclin 1. Interestingly, beclin 1 is homoallelically deleted in many human tumors, and overexpression of the protein induced autophagy and inhibited the tumorigenicity of human breast cancer cells.[7] LC3, the mammalian homologue of the yeast Atg8, is also essential for autophagosome formation. LC3 is lipidated during autophagy activation and this lipidated LC3 serves as a marker protein for autophagic vacuoles.[8] Atg7 and Atg3 are further essential autophagy proteins, which assist in the conjugation reaction between Atg12 and Atg5, and in the lipidation of LC3/Atg8.[9,10] Figure 1 presents a summary of the mammalian autophagic pathway.

Apoptosis, or type I programmed cell death, is the principal mechanism for cell elimination in metazoan organisms. Morphologically, apoptosis is characterized by cell shrinkage, chromatin condensation, and fragmentation of the cell into apoptotic bodies, which are phagocytosed by neighboring cells or professional phagocytes.[4] Biochemically, apoptosis is characterized by DNA fragmentation and caspase activation. Apoptosis can be triggered by external signals or the release of apoptosis mediators from the mitochondria.[11,12] The trigger can be ultraviolet radiation, γ-irradiation, chemoterapeutic drugs, staurosporine, etoposide, growth factor deprivation, the endoplasmic reticulum stress stimuli thapsigargin and tunicamycin, or signaling by death receptors located at the cell surface. Most apoptosis signaling pathways lead to the activation of caspase 8, which then leads to the activation of downstream factors such as Bid or other caspases.[12] Apoptosis is regulated at multiple levels, and Bcl-2 family proteins represent a critical intracellular checkpoint. Bax and Bak are proapoptotic, while Bcl-2, Bcl-X_L, and Mcl-1 are antiapoptotic.[11] Cells deficient in Bax and Bak are unable to initiate apoptosis via the intrinsic mitochondrial pathway.[13]

Autophagy is a Survival Mechanism for Cells Deprived of Growth Factors or Nutrients

Yeast mutants defective in any of the autophagy genes do not survive starvation,[14,15] indicating that in yeast autophagy is essential for survival during nutrient limitation. Similar role has been proposed for autophagy in mammalian cells, since autophagy is induced by nutrient, especially amino acid, deprivation.[3] However, until the mammalian autophagy genes were recently identified, it was not possible to directly test such a role in mammalian cells. Boya *et al.*[16] recently provided genetic evidence showing that autophagy acts as a survival mechanism under nutrient deprivation in mammalian cells. HeLa cells deprived of serum and amino acids activated autophagy as expected. If the autophagy proteins beclin 1, Atg5, Atg12, or Atg10 were downregulated using RNA interference, or the autophagic pathway was inhibited pharmacologically, the cells died via apoptosis during nutrient deprivation. This paper thus provides the long overdue genetic confirmation for the role of autophagy as a survival mechanism in nutrient-deprived mammalian cells. The paper

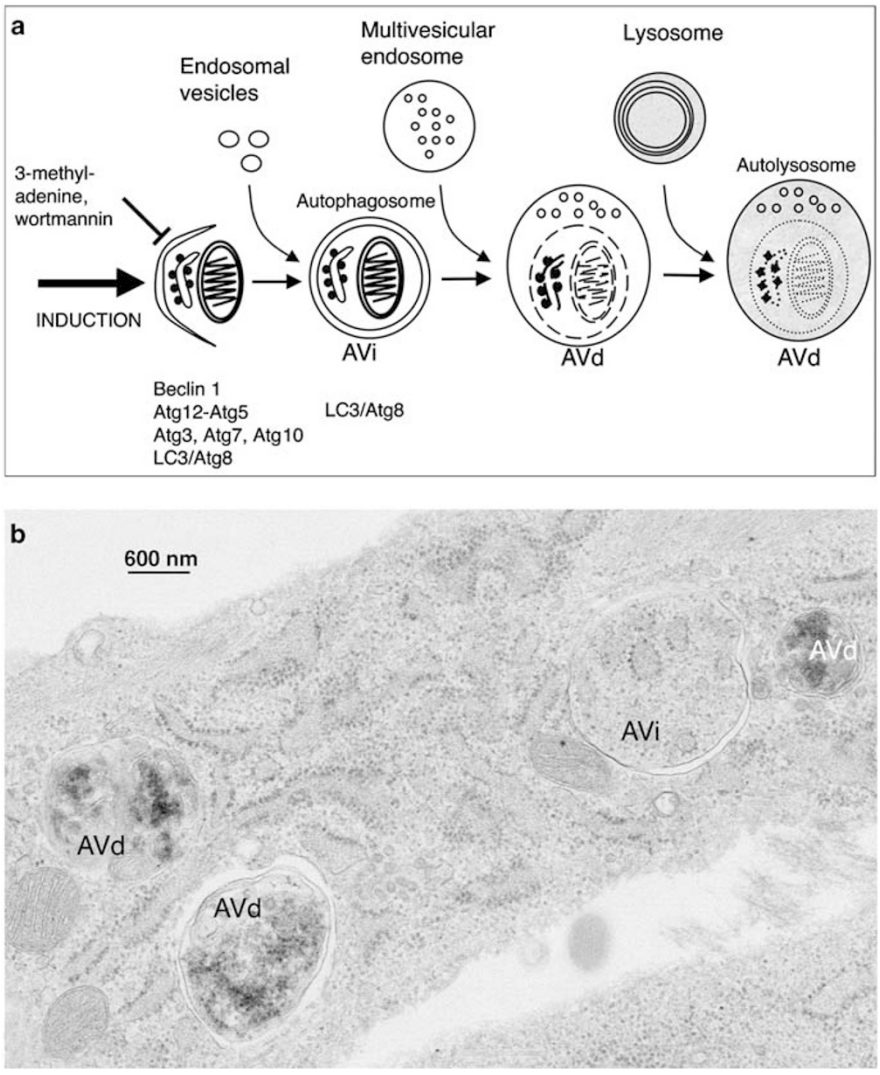

Figure 1 (**a**) Schematic presentation of the formation and maturation of autophagosomes in mammalian cells. The autophagy genes mentioned in the text are indicated in the position where they are likely to function. Panel A is modified from Eskelinen *et al.*[1] (**b**) Electron micrograph from a mouse embryonic fibroblast incubated in serum and amino-acid-free medium for 2 h to induce autophagy. Initial autophagic vacuole (AVi) indicates that the contents of the vacuole look morphologically intact, not yet degraded. Degradative autophagic vacuole (AVd) indicates that the contents look partially degraded, usually more electron dense

also demonstrates that under nutrient limitation, autophagy is needed to prevent apoptosis. When autophagy is prevented, apoptosis activates and the cells rapidly die.

Lum *et al.*[17] recently presented further evidence for the survival-promoting role of autophagy in mammalian cells. Earlier work from this group showed that growth factor limitation lead to a rapid downregulation of cell surface nutrient transporters.[18] Lum *et al.* used immortalized bone marrow cells from Bax- and Bak-deficient mice, which were resistant to apoptotic stimuli. In addition, the bone marrow cells were dependent on the growth factor interleukin 3 (IL3). The authors first showed that following IL3 withdrawal (but still in the presence of nutrients), these cells activated autophagy and underwent progressive atrophy.[17] Despite the presence of extracellular nutrients, the IL3-deprived cells maintained ATP production from catabolism of intracellular substrates via autophagy. The cells survived for weeks by eating their own cytoplasm through autophagy, as evidenced by the continuos presence of autophagosomes and by the progressive disappearance of intracellular organelles including ribosomes, the Golgi complex, the endoplasmic reticulum, and most mitochondria. Surprisingly, the cells fully recovered and even started to proliferate when IL3 was added to the culture medium after 6 weeks of deprivation. Importantly, if autophagy was inhibited during the IL3 deprivation with Atg5 or Atg7 RNA interference, or with 3-methyladenine, the cells rapidly died. This study thus directly demonstrates that under growth-factor deprivation, autophagy is an essential survival mechanism. However, it should be noted that such a survival is a short-term escape from death. The nutrient supply provided via autophagy is eventually depleted when all organelles have been consumed, although this may take several weeks. After this the cells will perish in spite of active autophagy.

Autophagy Genes are Needed for Autophagic Cell Death

Type II, or autophagic, cell death has been extensively described in the literature.[4,19] As the name indicates, this death pathway is associated with the accumulation of autophagic vacuoles in cells, without hallmarks of apoptosis (nuclear condensation, cell fragmentation). The role of autophagy in the death process has been controversial: Autophagy has been suggested both to protect cells from death by apoptosis, and to act as a death mechanism.[20] Thus, the presence of autophagic vacuoles in dying cells could indicate either, that the cells activate autophagy in an attempt to survive, or that autophagy is part of the death process. Compared to the type I, apoptotic cell death, much less is known about the signaling and regulation of the type II, autophagic cell death. Recent studies have, however, indicated some important details, and provide evidence that under certain conditions, autophagy can act as a death initiator and/or executor. Three of these studies are discussed in this commentary.

Yu *et al*.[21] published the first genetic evidence showing that autophagic cell death is dependent on *ATG* genes. The authors observed that inhibiting caspases with zVAD, a caspase inhibitor with broad specificity, surprisingly induced cell death in several cell lines including murine L929 fibrosarcoma cells, mouse RAW264.7 macrophages, murine peritoneal macrophages, and human U937 monocyte cells. Morphologically, the cell death was characterized by accumulation of autophagic vacuoles, without the hallmarks of apoptosis. The cell death was also triggered by downregulation of caspase 8 by RNA interference, but not by downregulation of other caspases, indicating the effect was specific for the inhibition of caspase 8. Importantly, both the autophagy inhibitor 3-methyladenine and downregulation of the autophagy proteins beclin 1 and Atg7, inhibited both the accumulation of autophagic vacuoles and the cell death, indicating the death process was dependent on genes needed for autophagosome formation. This indicates that autophagy was not a failing survival attempt, but really needed for the cell demise. Intriguingly, the authors also showed that the signaling pathway triggering the death process included the activation of receptor-interacting protein (RIP) and Jun amino-terminal kinase (JNK). Both autophagic vacuole accumulation and cell death were inhibited if RIP or JNK were downregulated with RNA interference. RIP and JNK have been described as components of death receptor-induced apoptosis signaling.[12] To date, these signaling components are not known to be involved in the induction of starvation-induced autophagy.[22] It remains to be shown which other signaling components are involved when inactivation of caspase 8, or activation of RIP and JNK, induce autophagic cell death.[23] Taken together, these results suggest that although the same autophagy genes are required for both autophagic cell death and starvation-induced autophagy, there might be differences in the signal transduction pathways activating these two processes. Further, this study suggests that caspase 8 might act as a suppressor of autophagic cell death. It is possible that caspase 8 could act as a hub that regulates which death pathway, apoptotic or autophagic, the cell takes. Thus, apoptosis and autophagy may have complementary roles in cell death: if apoptosis fails, then cells have the option of dying via autophagy. This mechanism might be of importance for instance during viral infection, because many viruses have means to inhibit caspase activation.

Another study showing that autophagy genes are needed for type II cell death was published by Shimizu *et al*.[24] The authors used embryonic fibroblasts from mice deficient in Bax and Bak, which are resistant to apoptotic death stimuli. However, when treated with apoptosis-inducing drugs like etoposide or staurosporine, the double-deficient cells still underwent a cell death. This death was characterized by accumulation of autophagic vacuoles and, importantly, inhibited by downregulation of the autophagy genes Atg5, beclin 1, and by 3-methyladenine. Interestingly, the etoposide-induced autophagic cell death was related to the deficiency of Bax and Bak, and not simply a consequence of the inhibition of apoptosis. The autophagic death was not detected in cells deficient in Apaf-1 or caspase 9 (proteins necessary for apoptosis), or in wild-type fibroblasts treated with the caspase inhibitor zVAD and etoposide. The authors went on to study the role of the antiapoptotic proteins Bcl-2 and Bcl-x_L. Overexpression of one of these proteins in wild-type cells triggered a similar etoposide-induced autophagic death as observed in the Bax/Bak double-deficient cells. Overexpression of Bcl-2 or Bcl-x_L in Atg5$-/-$ cells did not trigger this cell death, indicating the death was dependent on the autophagy protein Atg5. Further, silencing of Bcl-x_L with RNA interference in Bax/Bak$-/-$ cells prevented the etoposide-induced accumulation of autophagic vacuoles and cell death, indicating Bcl-x_L was required for this death pathway. Since Bcl-2 and Bcl-x_L are known to interact with beclin 1,[25] the authors suggested that Bcl-x_L might influence autophagosome formation at least partly via regulation of beclin 1. Interestingly, such a role for Bcl-x_L has not been demonstrated in starvation-induced autophagy. There were also further differences between the death-associated autophagy and starvation-induced autophagy. Most importantly, upregulation of both beclin 1 and the Atg12–Atg5 complex was observed in cells undergoing etoposide-induced autophagic death, but not at all, or much less so, in cells undergoing starvation-induced autophagy. In agreement with the study by Yu *et al*.,[21] Shimizu *et al*. shows that apoptotic stimuli such as etoposide can induce an autophagic cell death in cells where the apoptotic pathway is inhibited. This indicates that under certain conditions, autophagic cell death can act as a substitute for apoptosis.

Pyo *et al*.[26] recently published a further study demonstrating that autophagic cell death is dependent on an autophagy protein, using cells that undergo autophagic cell death after interferon-γ treatment. First the authors showed that downregulation of Atg5 expression suppressed both autophagic vacuole formation and cell death in HeLa cells treated with interferon-γ. Inversely, overexpression of Atg5 induced autophagic cell death. Chronologically, the accumulation of autophagic vacuoles was shown to precede the cell death in both interferon-γ-treated and Atg5 overexpressing cells. Further, 3-methyladenine, or expression of the inactive mutant Atg5, K130R, blocked both autophagic vacuole formation and cell death. Intriguingly, the caspase inhibitor zVAD inhibited cell death, but not the formation of autophagic

vacuoles, suggesting that caspases might be involved in the signaling or execution of the cell death downstream of autophagic vacuole formation. If proven correct with a more specific inhibition of caspases, this would suggest the existence of crosstalk from autophagy to the apoptotic machinery. However, this result seems to be in conflict with the results of Yu *et al*.[21] In their study, zVAD, or downregulation of caspase 8, *induced* autophagic cell death. Clearly, further studies are required to clarify the relationship between caspases and autophagic cell death. In any case, further results in Pyo *et al*. also suggest crosstalk from autophagy to the apoptotic machinery. The authors showed that Atg5 interacted with the Fas-associated protein with death domain (FADD), via the death domain of FADD. FADD is known as a component of the apoptosis-signaling cascade initiated by death receptors.[12] The Atg5-mediated cell death, but not the accumulation of autophagic vacuoles, was blocked in FADD-deficient cells, suggesting that FADD is a downstream effector of Atg5. This result has two implications. Firstly, it shows that the accumulation of autophagic vacuoles can be separated from the cell death. Secondly, it suggests that an autophagy protein (Atg5) can mediate a death signal to a protein involved in apoptotic cell death (FADD). Taken together, the results of Pyo *et al*. suggest that autophagic vacuoles can act as the initiator of the death process, which then mediates the death signal to the apoptotic machinery that finalizes the cell demise. Table 1 presents a summary of the findings presented in these three cell death papers.[21,24,26]

Concluding Remarks

Under nutrient starvation, autophagy has a survival-promoting role. The articles discussed above[16,17] show for the first time in mammalian cells that autophagy proteins are needed for survival under nutrient starvation. On the other hand, the three cell death studies[21,24,26] show for the first time that autophagic cell death is dependent on autophagy proteins (Atg5, Atg7, and beclin 1) that are needed for the formation of new autophagosomes. This demonstrates that the formation of autophagosomes is needed for the cell death, and that under certain conditions autophagy can initiate and/or execute cell death. Two of the studies[21,24] show that autophagic cell death can be an alternative to apoptosis in situations were cells are triggered to die but apoptosis is not possible. However, the exact role of autophagosome formation in these death processes (such as induction of death, degradation of proteins vital for survival, degradation of cell debris) still remains to be shown (see below). These two papers also suggest that the induction of death-associated autophagy is dependent on signaling components (inhibition of caspase-8, activation of RIP and JNK, presence of Bcl-X_L) that are not known to be involved in the induction of starvation-induced autophagy.[3,22] Thus these results suggest that different signaling pathways may trigger the death-associated autophagy and starvation-induced autophagy. On the other hand, the study by Pyo *et al*.[26] suggests that an autophagy protein (Atg5) can interact with an apoptosis protein (FADD). This

Table 1 Summary of the autophagic death pathways described in Yu *et al*., Shimizu *et al*., and Pyo *et al*.

	Yu *et al*. 2004[21]	Shimizu *et al*. 2004[24]	Pyo *et al*. 2005[26]
Cell type	L929, U937, RAW264.7, peritoneal macrophages	Bax/Bak−/− fibroblasts, Bcl-2, or Bcl-x_L overexpressing fibroblasts	HeLa, MCF-7
Induction of cell death	zVAD, downregulation of caspase 8	Etoposide, staurosporine	Interferon-γ, overexpression of Atg5
Morphology	Accumulation of autophagic vacuoles	Accumulation of autophagic vacuoles	Accumulation of autophagic vacuoles
Autophagosome formation and cell death are inhibited by	3-methyladenine, wortmannin	3-methyladenine	3-methyladenine, zVAD inhibits cell death but not autophagosome formation
Dependence of cell death on Atg proteins	Beclin 1, Atg7 (RNA interference)	Beclin 1, Atg5 (RNA interference, Atg5−/− cells)	Atg5 (antisense oligos, overexpression of mutant Atg5)
Specific requirement for autophagosome formation and cell death	Inhibition of caspase 8 (but not caspases 1, 2, 3, 9, or 12)	Deficiency of Bax and Bak (but not Apaf-1 or caspase 9), and etoposide; overexpression of Bcl-2 or Bcl-x_L, and etoposide.	Presence of FADD needed for cell death but not for autophagosome formation
Signaling components needed to induce autophagosome formation and cell death	RIP and JNK signaling	Bcl-x_L	See above
Difference in signaling compared to starvation-induced autophagy	RIP and JNK are not known to be involved in starvation-induced autophagy	Upregulation of Atg12–Atg5 and beclin 1 is not observed during starvation-induced autophagy. Bcl-x_L is not known to be required for starvation-induced autophagy.	

Further details are given in the text

study also suggests that under certain conditions, autophagy might act as an initiator of cell death, and that the apoptosis machinery might be needed for the actual killing of the cell. Clearly, much further work will be necessary to clarify the role and regulation of autophagy in cell death, as well as the relationship between autophagy and the apoptotic machinery.

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