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Letter to the Editor

Autophagy in human tumors: cell survival or death?

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Dear Editor,

The development of tumors involves multiple genomic changes that result in abnormal neoplastic cells and necessary alterations in the surrounding support tissue. Similar to the dynamics of a developing tissue or organism, tumors can be viewed as amalgamations of multiple cell types of epithelial, stromal, angiogenic and connective tissue origin that are intricately linked by their interactions.[1] Tumor growth involves two essential deviations from the normal state including the induction of proliferative stimuli, such as c-Myc and E2F, and simultaneous suppression of potentially compensatory cell death.[2] It is well recognized that apoptosis is impaired in many cancers by mutations in genes such as p53,[1,3] but it remains to be determined if nonapoptotic cell death mechanisms are also impaired in neoplastic cells. While compelling evidence indicates that aberrations in cell proliferation and death are the critical determinants of neoplastic growth, recent discoveries suggest that less studied mechanisms may contribute to tumor growth control.

Autophagy is an evolutionarily conserved mechanism of protein and organelle degradation that has been observed in organisms that are as different as yeast and humans. Autophagy involves the sequestration of cytoplasmic structures into vacuoles that are transported to lysosomes for degradation.[4] Recent studies of autophagy suggest that this mechanism of proteolysis may function in the regulation of cell survival and death.[5] There are at least three ways in which autophagy might enhance cancer cell survival. Autophagy may serve to optimize nutrient utilization in rapidly growing cancer cells when faced with hypoxic or metabolic stress similar to the starvation response observed in normal cells.[6] Alternatively, autophagy might aid in the degradation of organelles such as depolarized mitochondria that activate death pathways.[7] Autophagy might also prevent cells from accumulating free radical-induced damage to lethal levels by removing organelles that are sources or targets of such damage.

While the role of autophagy in cell survival during nutrient deprivation is well characterized, less is known about the possible role of this form of proteolysis in cell death even though autophagy occurs in dying cells of diverse organisms.[8,9] Therefore, it is important to consider the possibility that autophagy may play an important role in some forms of programmed cell death. While autophagy might commence as an adaptive response that sacrifices mass for homeostasis and enhances survival, cell death may ensue if the process is carried beyond a threshold. Thus, autophagy may suppress tumor growth by causing cell death, limiting cell size, or otherwise maintaining a low mutation rate, and decreasing the likelihood of aberrant growth.

We initiated a morphological survey for autophagic structures in several different primary human tumors because of the paucity of evidence for autophagy in cancer (Table 1). Tissues were obtained from surgical resection specimens, fixed in 3% glutaraldehyde, processed and sectioned, examined by light microscopy to verify the presence and preservation of viable neoplastic tissue, and analyzed by transmission electron microscopy. Autophagic structures were observed in neoplastic cells, and displayed the morphological features of double and multilamellate membrane-bound vacuoles enclosing cytoplasmic content and organelles (Figure 1). These autophagic vacuoles were typically in the vicinity of the nucleus and were frequently adjacent to swirls of endoplasmic reticulum devoid of ribosomes. The nuclei of these cells lacked apoptotic features such as fragmentation and chromatin margination. Of the 12 tumors studied, seven had evidence of autophagy including ganglioneuroma, infiltrating ductal carcinoma of the breast, adenocarcinoma of the lung, pancreatic adenocarcinoma and pancreatic islet cell tumor. Taking into account the small sample size for some of the tumor types examined, it seems reasonable to expect that autophagy occurs in many tumors.

The precise role of autophagy in cancer development, progression and response to therapy is not understood. The recognition of Beclin-1 (Atg6), a gene that functions in autophagy, as a haploinsufficient tumor suppressor raises intriguing possibilities about the importance of autophagy in cancer.[10–12] It is possible that the mechanism of tumor suppression is through promotion of cell death. Autophagy peaks at precancerous stages and diminishes at the malignant stage in some rat tumor models,[13] suggesting a tumor suppressor role. It is interesting that autophagy is regulated by some of the same pathways of cell growth control that are altered in tumor formation such as the PI3K system.[14]

Table 1 Survey of human tumors for autophagic structures.

Tumors with autophagic structures	Tumors lacking autophagic structures
Ganglioneuroma, mediastinum	Medullary carcinoma, thyroid
Mesothelioma, pleura	Lymphoma, lymph node
Invasive duct carcinoma, breast	Adenocarcinoma, ovary
Adenocarcinoma, lung	Meningioma, brain
Adenocarcinoma, pancreas	Malignant glioma, brain
Islet cell tumor, pancreas	
Adenoma, pituitary gland	

Further, analogs of rapamycin, which stimulate autophagy by inhibiting mTOR, have shown promise in models of tumor therapy.[15–17] Rapamycins are thought to stabilize tumor size rather than cause marked regression, possibly indicating control of cell size as the mechanism. In addition, therapeutics such as tamoxifen, an estrogen antagonist in breast tissue, have been shown to potently induce autophagy in MCF7 breast cancer cells,[18] suggesting the possibility that autophagy contributes to their antineoplastic activity.

A greater understanding of the regulation of autophagy in higher animals would provide better targets for cancer therapies. Although many of the genes that regulate autophagy in yeast appear to be conserved in diverse species,[8,20] several autophagy genes are absent in higher animals, suggesting possible differences in the regulation of this form of proteolysis. Autophagy is thought to be present at basal levels in most tissues and is regulated by the pleiotropic mTOR pathway.[19] Normal cells, unlike rapidly growing cancer cells, would be expected to be less sensitive to proautophagic stimuli due to minimal metabolic demands and normal activity of regulators such as PI3K and Akt. Therefore, it seems likely that drugs that specifically enhance autophagy would be of value because of their high therapeutic index. Elucidation of the pathway downstream of mTOR would help avoid the pleiotropic effects of this kinase. In tumors with deficient apoptosis and/or upregulation of the PIK3/Akt pathway, a combinatorial approach that utilizes autophagy modulators in addition to other chemotherapeutic agents might add value to therapy efficacy.[21]

Nonapoptotic mechanisms of cell death have been largely overlooked in studies of cancer causation, progression and therapy. Although the variation in cell complexity has been recognized in tumors,[1] modest progress has been made in understanding this aspect of cancer biology. It is important for cancer researchers to consider the presence and impact of autophagy and similar less studied processes when interpreting clinical trials and developing drugs for modulation of aberrant cellular pathways.

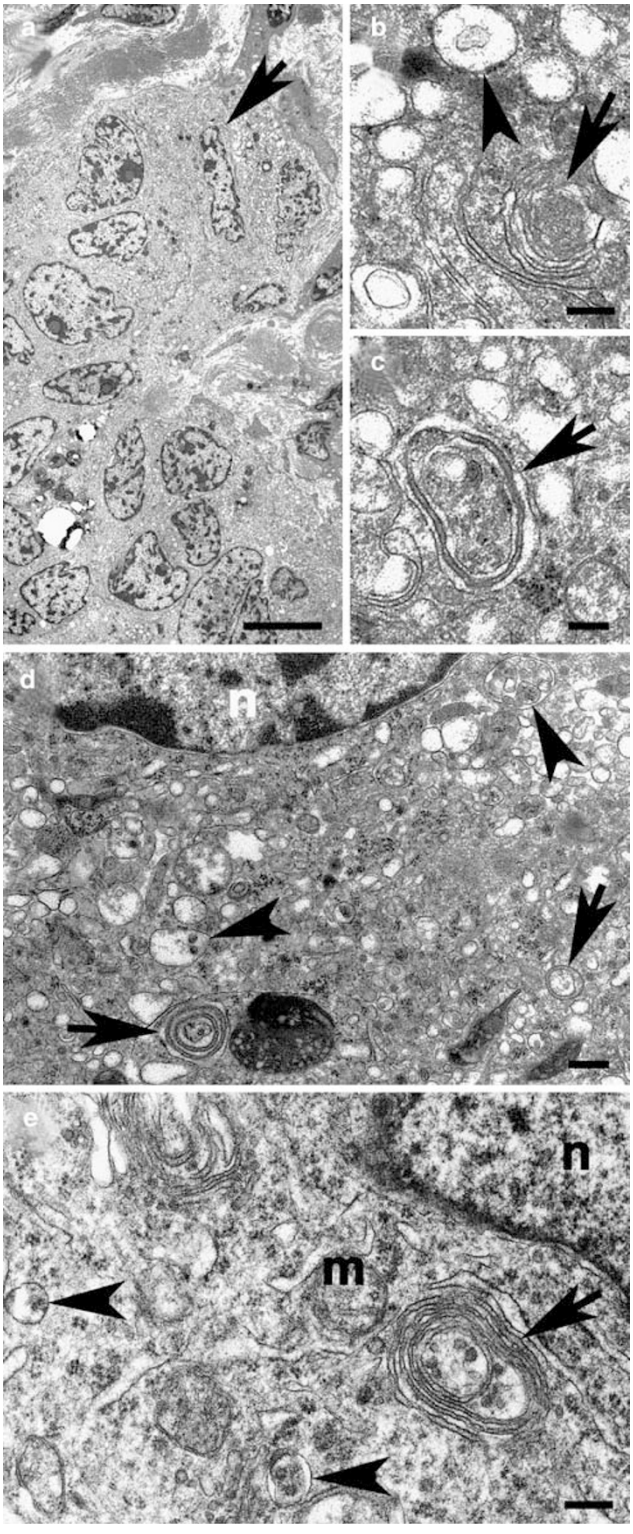

Figure 1 Autophagic structures are present in neoplastic cells of multiple types of tumors. (**a**) Cells of a pancreatic islet cell tumor that display autophagic features and lack the hallmarks of apoptosis (arrow). (**b, c**) Neoplastic pancreatic islet cell tumors cells contain early-stage multilamellate (arrows) and single membrane-bound (arrowhead) autophagic structures. (**d**) Adenocarcinoma of the lung contains several multilamellate (arrows) and single membrane-bound (arrowhead) autophagic structures, while the nucleus (n) appears normal. (**e**) A ganglioneuroma cell with a normal nucleus (n) and mitochondria (m) contains multilamellate (arrows) and single membrane-bound (arrowhead) autophagic structures. Scale bars = 10 μM (**a**), 0.3 μM (**b–e**). All the tissues were obtained from surgical resection specimens, immediately fixed in 3% glutaraldehyde, processed and embedded in Embed 812. Semi-thin sections were cut, stained with toluidine blue, and examined using light microscopy to verify the presence and preservation of viable neoplastic tissue. Thin sections were stained with uranyl acetate–lead citrate and examined using a Zeiss EM 10 transmission electron microscope

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AS Alva[1], SH Gultekin[2] and EH Baehrecke[*,1]*

[1] Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742, USA
[2] Department of Pathology, Mount Sinai School of Medicine, One Gustave L Levy Place, 1468 Madison Avenue, Box 1134, New York, NY 10029-6574, USA
* Corresponding author: EH Baehrecke, Center for Biosystems Research, University of Maryland Biotechnology Institute, College Park, MD 20742, USA. E-mail: baehreck@umbi.umd.edu

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