**Here, There Be Dragons: Charting Autophagy-Related Alterations in Human Tumors**

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**Abstract**

Macroautophagy (or autophagy) is a catabolic cellular process that is both homeostatic and stress adaptive. Normal cells rely on basal levels of autophagy to maintain cellular integrity (via turnover of long-lived proteins and damaged organelles) and increased levels of autophagy to buoy cell survival during various metabolic stresses (via nutrient and energy provision through lysosomal degradation of cytoplasmic components). Autophagy can function in both tumor suppression and tumor progression, and is under investigation in clinical trials as a novel target for anticancer therapy. However, its role in cancer pathogenesis has yet to be fully explored. In particular, it remains unknown whether *in vitro* observations will be applicable to human cancer patients. Another outstanding question is whether there exists tumor-specific selection for alterations in autophagy function. In this review, we survey reported mutations in autophagy genes and key autophagy regulators identified in human tumor samples and summarize the literature regarding expression levels of autophagy genes and proteins in various cancer tissues. Although it is too early to draw inferences from this collection of *in vivo* studies of autophagy-related alterations in human cancers, their results highlight the challenges that must be overcome before we can accurately assess the scope of autophagy’s predicted role in tumorigenesis. *Clin Cancer Res; 18*(5); 1214–26. ©2012 AACR.

**Introduction**

When charting the role of autophagy in the process of human tumorogenesis, one quickly finds oneself at the edge of the known world, a place that mythical medieval cartographers would have marked with the warning, "Here, there be dragons!" Macroautophagy (referred to here as autophagy) is a well-studied catabolic cellular process that involves the lysosomal degradation of bulk cytoplasm, long-lived proteins, and damaged organelles. Normal cells rely on this process at both homeostatic levels (to safeguard cellular integrity against damaging cellular debris) and stress-induced, elevated levels (to facilitate cell survival through increased nutrient and energy provision). However, its role in cancer pathogenesis has yet to be fully explored. Although researchers have made many forays into this sparse area of the map, they have drawn many of their conclusions from studies that monitored cancer-associated autophagy changes in cell lines and animal models. Questions remain as to whether these observations are applicable to human cancer patients, and whether these changes will be revealed in alterations in the autophagy genes and proteins themselves. The answers to these questions will require genomic scrutiny of a multitude of tumor samples, and the development of reliable biomarkers and *in vitro* assays applicable to measuring the dynamics of autophagy in human tissues. Such practices and tools are in their infancy but are urgently needed to tame the dragon that is the current paradoxical and context-dependent working model of autophagy’s role in tumorigenesis.

Evidence from preclinical cancer models supports autophagy as both a tumor suppressor and an agent of tumor progression depending on the cancer type, stage, and therapy context (for excellent reviews, see refs. 1–3). *In vitro*, the scope of autophagic involvement with aspects of the tumorigenic state encompasses links between autophagy and many hallmarks of cancer [e.g., evasion of apoptosis, self-sufficiency in growth signal, and DNA damage stress (4–6)] and the corresponding cancer cell stress phenotypes [e.g., hypoxia, genomic instability, and aneuploidy (6)]. Recent studies have revealed associations between modulated autophagy and a variety of tumor-associated physiologic changes, including restructured stroma (7), altered survival signals (8–11), angiogenesis in response to hypoxia (12), altered metabolism (13), altered proliferation (14), genomic instability (15), aneuploidy/copy-number variation (16, 17), and immune-system modulation (18). In an emerging area of investigation, researchers are conducting...
Autophagy-Related Alterations in Human Tumors

Translational Relevance

Autophagy mediates lysosomal degradation and recycling of proteins and organelles. Preclinical studies suggest that autophagy acts as a double-edged sword in cancer pathogenesis, contributing to the integrity of the genome in precancerous cells while conferring a survival advantage to tumor cells in harsh microenvironments (e.g., therapeutic cytotoxicity). Ongoing clinical trials involving hydroxychloroquine (a lysosomal inhibitor that blocks autophagosome-lysosome fusion) are investigating whether this drug can augment the efficacy of existing anticancer agents and overcome therapeutic resistance. However, the tools that are necessary to monitor autophagy status in patients are still under development. To identify diagnostic and prognostic biomarkers, researchers are cataloging autophagy alterations at the DNA, RNA, and protein levels in human tumor specimens. The results of these studies may provide novel biomarkers for patient stratification, identify new targets for the design of specific autophagy inhibitors, and help guide the implementation of therapeutic autophagy modulation. This review highlights recent progress in the examination of human autophagy alterations in cancer.

Sounding the Depths: DNA Sequence Mutations in Autophagy-Related Genes and Regulators

Autophagosome formation requires intricate cellular membrane dynamics that are governed by the autophagy-related (ATG) genes and presided over by an interconnected web of regulatory proteins that permit or restrict autophagy, largely through the outcome of their interactions with mTORC1 and the Beclin-1–PI3K type 3 (phosphoinositide-3-kinase type 3) complex (reviewed in refs. 19 and 20). ATG genes were first identified in yeast genetic screens seeking mutants with defects in protein turnover (21). To date, investigators have identified more than 30 ATG genes in yeast, many of which have mammalian homologs (22). Mammalian autophagy genes can have multiple paralogs, some of which have distinct functions, and recent studies reported mammalian-specific autophagy-associated genes [e.g., RB1CC1/FIP200 (23)]. In a small (but growing) number of studies, investigators have begun to record genomic alterations found in ATG genes and regulators in human cancer tissue (Table 1). Such lesions, including single-nucleotide variants (SNV) and small insertions/deletions (indels), among other types, may prove vital for tumor initiation, expansion, and/or survival.

mTORC1 meets the ULK1 complex: autophagy induction and its oncogenic big brother

mTORC1, a master positive regulator of cell growth and proliferation, forms the integrational hub of an extensive network of regulatory proteins that transmits extrinsic and intrinsic signals regarding cellular nutritional status. On the basis of mTORC1’s influence over cellular metabolism, its regulation by common oncogenic signaling pathways [e.g., PIK3CA–AKT1 and RAS–ERK (10)], and the observation that aberrant mTORC1 signaling is found in 40% to 90% of human cancers (24), researchers have intensively studied mTORC1’s role in tumorigenesis and the therapeutic benefit of inhibiting its overactivation in cancer (25).

mTORC1 inhibits autophagy through its interaction with the serine/threonine-protein kinase ULK1 (ULK1) induction complex. In vitro studies also showed that this interaction can negatively feed back on mTORC1 itself (26, 27), and potentially represents a homeostatic mechanism for restricting mTORC1 signaling to an appropriate range (27). Given the intimate relationship between mTORC1 signaling and autophagy, it is likely that cancer-associated sequence changes in the mechanistic target of rapamycin (serine/threonine kinase) or MTOR (the gene encoding the effector kinase of the mTORC1 complex) and/or aberrant mTOR protein expression would perturb autophagy, making autophagy an important mediator of the effects of this common dysregulation in human cancer.

Two groups (28, 29) recently investigated 10 somatic mutations in MTO1 that are listed in the Catalogue of Somatic Mutations in Cancer (COSMIC) database (Table 1). To test for potential oncogenic mutations in MTO1, these groups expressed 4 of 6 nonsynonymous point mutations in HEK293 cell lines and examined increases in mTORC1 substrate phosphorylation. Both groups determined that 2 of the mutations (S2215Y and R2505P) led to increased mTORC1 activity, maintained kinase activity even under nutrient-starved conditions, and resulted in a higher proportion of cells in the synthesis phase of the cell cycle. In vitro selection of MTO1-activating
### Table 1. Cancer-associated sequence changes in autophagy genes and key regulators, summarized from the literature

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>DNA Δ (frequency)</th>
<th>Protein Δ</th>
<th>Protein expression (frequency)</th>
<th>Cancer</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MTOR</strong></td>
<td>Autophagy inhibition</td>
<td>Somatic c.404T &gt; C&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.M135T</td>
<td>n/d</td>
<td>Melanoma</td>
<td>COSMIC 753546</td>
</tr>
<tr>
<td><strong>MTOR</strong></td>
<td>Somatic c.22G &gt; T&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.A8S</td>
<td>Present (cell lines)</td>
<td>Lung (large cell)</td>
<td>COSMIC 753390</td>
<td></td>
</tr>
<tr>
<td><strong>MTOR</strong></td>
<td>Somatic c.6031A &gt; G&lt;sup&gt;c&lt;/sup&gt;</td>
<td>p.M2011V</td>
<td>n/d</td>
<td>Ovary (mucinous)</td>
<td>COSMIC 753344</td>
<td></td>
</tr>
<tr>
<td><strong>MTOR</strong></td>
<td>Somatic c.6644C &gt; A&lt;sup&gt;c&lt;/sup&gt;</td>
<td>p.S2215Y</td>
<td>Present (cell lines)</td>
<td>Colorectal ADCA</td>
<td>COSMIC 753426</td>
<td></td>
</tr>
<tr>
<td><strong>MTOR</strong></td>
<td>Somatic c.7427C &gt; T&lt;sup&gt;c&lt;/sup&gt;</td>
<td>p.R2505P</td>
<td>Present (cell lines)</td>
<td>Kidney (clear cell)</td>
<td>COSMIC 948155</td>
<td></td>
</tr>
<tr>
<td><strong>MTOR</strong></td>
<td>Somatic c.7514G &gt; C&lt;sup&gt;c&lt;/sup&gt;</td>
<td>p.Q1866&lt;sup&gt;c&lt;/sup&gt;</td>
<td>n/d</td>
<td>CRC</td>
<td>COSMIC 20407</td>
<td></td>
</tr>
<tr>
<td><strong>RB1CC1/ FIP200</strong></td>
<td>Induction complex</td>
<td>Somatic deletions&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Various</td>
<td>Absent</td>
<td>Breast&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Chano et al. (117)</td>
</tr>
<tr>
<td><strong>BECN1</strong></td>
<td>Nucleation</td>
<td>Exon 2: 24 C &gt; A&lt;sup&gt;d&lt;/sup&gt; (1/180)</td>
<td>p.N8K</td>
<td>n/d</td>
<td>Early gastric</td>
<td>Lee et al. (33)</td>
</tr>
<tr>
<td><strong>BECN1</strong></td>
<td>Exon 11: 1165 C &gt; T&lt;sup&gt;d&lt;/sup&gt; (1/180)</td>
<td>p.R389C</td>
<td>n/d</td>
<td>Early gastric</td>
<td>Lee et al. (33)</td>
<td></td>
</tr>
<tr>
<td><strong>BECN1</strong></td>
<td>Exon 11: 1049 C &gt; G&lt;sup&gt;d&lt;/sup&gt; (1/50)</td>
<td>p.P350R</td>
<td>n/d</td>
<td>Colorectal ADCA</td>
<td>Lee et al. (33)</td>
<td></td>
</tr>
<tr>
<td><strong>BECN1</strong></td>
<td>17q21 LOH</td>
<td>n/d</td>
<td>Reduced (15/17)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Breast</td>
<td>Liang et al. (62)</td>
<td></td>
</tr>
<tr>
<td><strong>UVRAG</strong></td>
<td>Nucleation/ fusion</td>
<td>Exon 8: various indels&lt;sup&gt;d&lt;/sup&gt; (32/102)</td>
<td>Truncated protein</td>
<td>Present (cell lines)</td>
<td>CRC</td>
<td>Knævelsrud et al. (40)</td>
</tr>
<tr>
<td><strong>UVRAG</strong></td>
<td>Somatic c.708_709del AA&lt;sup&gt;d&lt;/sup&gt; (2/137)</td>
<td>p.Ser237fsX1</td>
<td>n/d</td>
<td>Gastric ADCA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Kim et al. (39)</td>
<td></td>
</tr>
<tr>
<td><strong>UVRAG</strong></td>
<td>Somatic c.709delA (1/137)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.Ser237ValfsX6</td>
<td>n/d</td>
<td>Gastric ADCA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Kim et al. (39)</td>
<td></td>
</tr>
<tr>
<td><strong>ATG2B</strong></td>
<td>Membrane cycling</td>
<td>Somatic c.3120delA (10/75)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.Lys1040AsnfsX2</td>
<td>n/d</td>
<td>Gastric, CRC</td>
<td>Kang et al. (49)</td>
</tr>
<tr>
<td><strong>ATG5</strong></td>
<td>Elongation</td>
<td>Somatic c.334C &gt; T (1/45)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.Leu334Phe</td>
<td>Present (79/100)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>Gastric&lt;sup&gt;b&lt;/sup&gt;</td>
<td>An et al. (48)</td>
</tr>
<tr>
<td><strong>ATG5</strong></td>
<td>none found</td>
<td>Present (73/95)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>CRC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>An et al. (48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>ATG5</strong></td>
<td>Somatic c.721C &gt; T (1/45)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.His241Tyr</td>
<td>Present (45/50)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>HCC&lt;sup&gt;b&lt;/sup&gt;</td>
<td>An et al. (48)</td>
<td></td>
</tr>
<tr>
<td><strong>ATG5</strong></td>
<td>Somatic c.704delA (2/75)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.Lys235ArgfsX4</td>
<td>n/d</td>
<td>Gastric, CRC</td>
<td>Kang et al. (49)</td>
<td></td>
</tr>
<tr>
<td><strong>ATG9B</strong></td>
<td>Membrane cycling</td>
<td>Somatic c.27delG (10/75)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.Arg10GlufsX138</td>
<td>n/d</td>
<td>Gastric, CRC</td>
<td>Kang et al. (49)</td>
</tr>
<tr>
<td><strong>ATG9B</strong></td>
<td>Elongation</td>
<td>Somatic c.293delC (3/75)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>p.Pro98GlnfsX8</td>
<td>n/d</td>
<td>Gastric</td>
<td>Kang et al. (49)</td>
</tr>
</tbody>
</table>

**Abbreviations:** ADCA, adenocarcinoma; CRC, colorectal cancer; HCC, hepatocellular carcinoma; MSI, microsatellite instability; n/a, not applicable; n/d, not determined.

<sup>a</sup>Frequency of DNA change or indicated protein expression observation (indicated where reported): number of samples with indicated change/observation out of total samples tested for that cancer type.

<sup>b</sup>Primary tumor (no treatment), indicated where reported.

<sup>c</sup>Heterozygous mutation.

<sup>d</sup>Zygosity not reported.

<sup>e</sup>Samples used for expression analysis were independent of samples used to detect DNA changes.
mutations would concurrently inhibit autophagy, suggesting the possibility that autophagy may have a tumor-suppressive role in these types and stages of cancer. However, of the 4 tested nonsynonymous changes listed in COSMIC, only 2 showed hyperactivation of mTOR. The remaining coding changes (Table 1) have yet to be functionally characterized, and it is plausible that nonsense mutation Q1866* would hinder mTOR activity, thereby indirectly increasing autophagy in this particular tumor (a carcinoma of the large intestine). Ultimately, final judgment regarding the frequencies of somatic gain-of-function and loss-of-function mutations of MTOR, and their effect on autophagy function, awaits the results of resequencing studies that will examine multiple samples from single tumor types, preferably controlled for tumor stage, genetic background, and therapeutic context.

Interacting with Beclin-1: mutational targeting of a dynamic regulation complex

Through an ever-expanding list of interactions with positive and negative regulators of autophagy, Beclin-1 (BECN1) enables the lipid kinase signaling of PI3K type 3, which is required to gather the autophagic machinery to the site of autophagosome nucleation. In 1999, Aita and colleagues (30) established a link between defective autophagy and cancer by showing that monoallelic deletion of (mouse) Becn1, a deletion previously observed in 40% to 75% of breast, ovarian, and prostate cancers in humans, leads to spontaneous tumorigenesis (e.g., lymphomas and lung and liver cancers) in aging mice (31, 32). Although the remaining allele of (mouse) Becn1 was neither mutated nor silenced, autophagy was downregulated in these mice, and BECN1 has since been regarded as a haploinsufficient tumor suppressor (31).

Beclin-1 continues to be intensively studied with respect to its role in cancer pathogenesis. Recently, Lee and colleagues (33) screened for SNVs in BECN1 in human cancers and employed targeted sequencing to examine BECN1 coding regions. Out of 548 patient samples [from gastric cancer, colorectal cancer, breast cancer, hepatocellular carcinomas (HCC), leukemias, and non–small cell lung cancers], they found 11 SNVs. Three exonic variants (p.N8K, p.R389C, and p.P350R) were expressed in HT1080 (human fibrosarcoma) cells stably transfected with a marker for autophagosome formation (eGFP-LC3). Although all lines retained the ability to form eGFP-LC3–stained puncta upon autophagy induction, further characterization of these mutations in cells deficient for wild-type Beclin-1 may help to define their effects on autophagy function.

In addition to mutations in BECN1, mutations in Beclin-1 binding partners may also provide an efficient way for tumor cells to hijack autophagy modulation. Dynamic autophagy regulation may be achieved by the coexistence of distinct populations of Beclin-1–PI3K type 3 complexes defined by their various combinations of Beclin-1 interactors (20), and therefore mutually exclusive Beclin-1 binding partners, such as UV radiation resistance-associated gene (UVRAG) and Beclin-1-associated autophagy-related key regulator or Barkor (ATG14), are prime targets for mutation analysis (34). UVRAG is monoallelically deleted in various human cancers [e.g., breast and colon malignancies (35–37)] and is predicted to be a tumor suppressor (38). Heterozygous truncating frameshift mutations, caused by various indels in a polyadenine tract in exon 8 of UVRAG, are found in HCT116 colon cancer cells, and at high frequency in colon and gastric carcinomas with microsatellite instability, where by simple genomic sequence repeats become vulnerable to DNA damage and are abnormally shortened or lengthened (37–40). Whereas expression of wild-type UVRAG was shown to suppress the proliferation and tumorigenicity of HCT116 colon cancer cells [confirming a tumor-suppressor role for UVRAG (38)], autophagy induction and level were not affected in colon cancer cell lines that endogenously express truncating frameshift mutations (40). UVRAG’s alternate role in endocytic trafficking is now hypothesized to be the mediator of its tumor-suppressor functions (40, 41). Similarly, Beclin-1 was recently suggested to participate in endocytosis (42), introducing a potential for autophagy-independent mechanisms of Beclin-1 tumor suppression.

Although UVRAG loss-of-function mutations were not shown to modulate autophagy, mutational profiling of other Beclin-1 binding partners is still a valuable approach for examining autophagy alteration in cancer. The sheer number of proteins that have been shown to regulate PI3K type 3–mediated autophagosome nucleation (20, 43) makes the Beclin-1 interactome a dynamic and important regulator of autophagy, within which lies a world of possibilities for the ever-opportunistic cancer cell.

Mutations in core ATG genes: a potential wrench in the autophagic machinery

The core autophagy machinery in mammals can be broken down into 4 functional groups (Fig. 1): (i) the induction complex [ULK1 and regulators (ATG13 and RB1CC1/FIP200)]; (ii) mediators of autophagosome nucleation [Beclin-1–PI3K type 3 core complex (with PIK3R4/p150)]; (iii) mediators of autophagosome elongation [ubiquitin-like ATG12 and microtubule-associated protein 1 light chain 3 (MAP1LC3) and their E1-, E2-, and E3-like conjugation machinery (ATG3, ATG5, ATG7, ATG10, and ATG16)]; and (iv) the ATG9–ATG2–ATG18 cycling complex, which is thought to deliver lipids from source to growing autophagosome (20). Although some autophagy-independent ATG functions have been described (44–47), these highly conserved proteins are the most specific to the autophagy process, and patterns of expressed, cancer-associated, somatic mutations identified in these genes would be tantalizing evidence of autophagy’s involvement in cancer pathogenesis.

Various somatic mutations have been identified in ATG2B, ATG5, and ATG9B in gastrointestinal cancer (Table 1; refs. 48, 49). Two somatic point mutations in ATG5 (p.Leu334Phe and p.His241Tyr) were identified by...
targeted sequencing of 135 patient samples of gastric cancer, colorectal cancer, and HCC (48). Subsequent quantitation of ATG5 protein levels by immunohistochemistry (IHC) showed loss of expression in 21% (gastric), 23% (colorectal), and 5% (HCC) of the entire sample set (48). In a separate study, Kang and colleagues (49) conducted a targeted examination of frameshift mutations in the coding regions of ATG genes that harbor mononucleotide repeats (at least 7 nucleotides long), which they predicted to be vulnerable to indels, that would result in protein truncation. They identified frameshift mutations in ATG2B, ATG5, and ATG9B (singly or in combination) in 28.1% of gastric cancer samples and 27.9% of colorectal cancer samples (Table 1). Although autophagy function was not evaluated in that study, the existence of truncating sequence changes in core ATG genes suggests that loss of autophagy may benefit primary gastrointestinal neoplasms.

Future comprehensive studies to identify all types of ATG mutations in other cancers, combined with functional studies to measure the impact of these mutations on autophagy level and flux (a measure of the functional integrity of the autophagy process), will be extremely valuable for assessing whether the autophagy pathway is a target for somatic mutation in human cancers.

**On the horizon: high-throughput sequencing and functional validation**

With an onslaught of next-generation sequencing studies of cancer genomes likely around the corner, a glut of autophagy-associated sequence and transcript changes, from an array of disease contexts, is anticipated. In addition, genome-wide analyses of epigenetic changes will add a further dimension to the exploration of autophagy-related alterations in human cancer samples (50, 51). However, lists of genomic changes are just the beginning. We must have a way to functionally characterize these changes in samples of diseased tissue. Unfortunately, the current roster of reliable autophagy assays can only be performed in vitro, and cell lines are notorious for harboring idiosyncratic genetic changes that may confound results. A major hurdle for researchers will be the development of in vitro markers of autophagy level and flux in human tissues.

**Bumpy Seas: Measuring Expression of Autophagy Transcripts and Proteins in Human Cancer Tissues**

With growing numbers of clinical trials testing the effectiveness of hydroxychloroquine (a lysosomal inhibitor that blocks autophagosome-lysosome fission) in various cancers, and in vitro evidence supporting dual roles (tumor-promoting and tumor-suppressing) for autophagy in tumorigenesis (52–54), the search for direct markers of autophagy in cancer patients is of paramount importance for both clinicians and scientists. Currently, the number of autophagy proteins that can be studied in human tissues is limited, and none can be used in cancer patients as independent markers of autophagy induction or inhibition (for summaries of autophagy-related alterations at the expression level assembled from the literature, see Tables 2 and 3; details of the antibodies used in these studies are provided in Supplementary Table S1).

A variety of autophagy assays have been widely applied to measure autophagy in cultured cells (55, 56), and a subset of these assays can be reliably used in mouse models (57). However, most of these methods involve genetic and/or pharmacological manipulations and are not applicable to the assessment of autophagy in human tissues. Although quantitation of autophagosomes by electron microscopy is feasible for human samples, it is not practical for the clinical setting (53), is not suitable for assessment of whole tumor samples (58), and, of most importance, is insufficient without confirmation of autophagic flux. Because patient tumor biopsies represent a snapshot of the processes taking place in tumor tissue (and cannot reflect dynamic fluctuations in autophagy), the development of a reliable flux assay for biopsies remains a challenge.

Currently, a common approach for evaluating autophagy status in human tumor samples is to assess the expression of various autophagy markers [e.g., Beclin-1, MAP1LC3, and sequestosome-1 (also known as p62)]. However, inconsistencies in sample preparation, antibody quality, and the use of arbitrary cutoffs and scoring systems (53) can lead to discordant results. Quantitative PCR arrays are also used to determine mRNA levels of autophagy-related genes and regulators; however, they have limited value for assessing autophagy function in cancer tissues due to the occurrence of post-translational regulation of autophagy proteins (55).

Because an ideal assay for assessing the dynamic process of autophagy in human samples is still lacking, the primary objective of many of the studies outlined below was to evaluate the prognostic value of specific autophagy proteins in various cancers, rather than to directly assess autophagic activity. Therefore, it should be noted that although many of the reported expression changes suggest a reduction of autophagy as a principal alteration type in a variety of cancers (Table 3), one must interpret these data carefully, taking into account the technical limitations of autophagy detection in human tissues. One must also consider the potential for confounding factors in comparison studies, such as variations in general tumor characteristics (e.g., primary vs. recurrent, and drug-sensitive vs. drug-resistant) and therapeutic regimens (e.g., treatment vs. no treatment, and intervention type). It is particularly important to control for disease context when interpreting autophagy status, given the wealth of in vitro data that show the flexibility of autophagy as an agent of both tumor suppression and tumor progression. Nevertheless, should a larger pattern of autophagy reduction continue to emerge as a common cancer-associated alteration, this could suggest an important in vivo tumor-suppressor function for autophagy.
Table 2. Protein and mRNA expression levels of autophagy-associated genes in various human tumor samples, summarized from the literature.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Protein</th>
<th>mRNA</th>
<th>Prognostic value</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BECN1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>IHC</td>
<td>↓</td>
<td>n/d</td>
<td>Liang et al. (62)</td>
</tr>
<tr>
<td>Cervical SCC</td>
<td>IHC</td>
<td>↓</td>
<td>LN metastasis, histologic grade</td>
<td>Wang et al. (61)</td>
</tr>
<tr>
<td>Brain tumors</td>
<td>IHC</td>
<td>↓</td>
<td>High-grade tumors</td>
<td>Miracco et al. (67)</td>
</tr>
<tr>
<td>Ovarian</td>
<td>IHC</td>
<td>↓</td>
<td>Histologic grade, tumor stage</td>
<td>Shen et al. (82)</td>
</tr>
<tr>
<td>HCC</td>
<td>IHC, WB</td>
<td>↓</td>
<td>Recurrent disease, OS</td>
<td>Ding et al. (64)</td>
</tr>
<tr>
<td>Lung</td>
<td>IHC, WB</td>
<td>↓</td>
<td>n/d</td>
<td>Liu et al. (66)</td>
</tr>
<tr>
<td>CRC (st. IIIB)</td>
<td>IHC</td>
<td>(85.2%)</td>
<td>Longer OS with ↑</td>
<td>Li et al. (54)</td>
</tr>
<tr>
<td>CRC</td>
<td>IHC</td>
<td>various</td>
<td>Poor OS with ↓ or ↑</td>
<td>Koundourakis et al. (71)</td>
</tr>
<tr>
<td>NHL</td>
<td>IHC, WB</td>
<td>(20%)–(90%)</td>
<td>Pathologic stage, poor OS with ↓</td>
<td>Nicotra et al. (74)</td>
</tr>
<tr>
<td>EN NKTCN (nasal type)</td>
<td>IHC</td>
<td>(86.2%)</td>
<td>Advanced stage, poor OS with ↓</td>
<td>Huang et al. (73)</td>
</tr>
<tr>
<td>Breast</td>
<td>IHC</td>
<td>↓ (in majority of samples)</td>
<td>mRNA↑ in BRCA1-positive tumors</td>
<td>Li et al. (51)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>IHC, WB</td>
<td>↓</td>
<td>Tumor progression</td>
<td>Miracco et al. (69)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>IHC</td>
<td>↓↑</td>
<td>Locally advanced disease, ulceration, increase vascular density, poor OS</td>
<td>Sivridis et al. (70)</td>
</tr>
<tr>
<td>GBM</td>
<td>IHC, WB</td>
<td>↓</td>
<td>High-grade tumors, poor OS with ↓</td>
<td>Huang et al. (68)</td>
</tr>
<tr>
<td>ICC</td>
<td>IHC</td>
<td>↑</td>
<td>LN metastasis, poor OS with ↓</td>
<td>Dong et al. (72)</td>
</tr>
<tr>
<td><strong>LC3</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>CRC</td>
<td>IHC, WB</td>
<td>↑</td>
<td>n/d</td>
<td>Sato et al. (80)</td>
</tr>
<tr>
<td>Lung</td>
<td>IHC, WB</td>
<td>↓</td>
<td>n/d</td>
<td>Liu et al. (66)</td>
</tr>
<tr>
<td>GI tumors</td>
<td>IHC</td>
<td>↑</td>
<td>n/d</td>
<td>Yoshioka et al. (79)</td>
</tr>
<tr>
<td>Breast</td>
<td>IHC</td>
<td>↑</td>
<td>LC3B (cancer tissues only)</td>
<td>Othman et al. (81)</td>
</tr>
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<td>NHL</td>
<td>IHC</td>
<td>(20–90%)</td>
<td>Pathologic stage</td>
<td>Nicotra et al. (74)</td>
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<tr>
<td>Melanoma</td>
<td>IHC</td>
<td>↓</td>
<td>Poor OS with ↓ or ↑</td>
<td>Miracco et al. (69)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>IHC</td>
<td>↓</td>
<td>Ulceration with ↓</td>
<td>Sivridis et al. (70)</td>
</tr>
<tr>
<td>GBM</td>
<td>WB</td>
<td>↑</td>
<td>High-grade tumors, poor OS</td>
<td>Huang et al. (68)</td>
</tr>
<tr>
<td>Breast, endometrial, CRC</td>
<td>IHC, WB</td>
<td>3 different expression patterns</td>
<td>OS (see text)</td>
<td>Sivridis et al. (86)</td>
</tr>
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<td><strong>GABARAP</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Thyroid</td>
<td>IHC</td>
<td>↑</td>
<td>n/d</td>
<td>Roberts et al. (94)</td>
</tr>
<tr>
<td>CRC</td>
<td>IHC</td>
<td>↑</td>
<td>Shortened OS</td>
<td>Miao et al. (95)</td>
</tr>
<tr>
<td>Breast</td>
<td>IHC</td>
<td>↓</td>
<td>n/d</td>
<td>Klebig et al. (96)</td>
</tr>
<tr>
<td><strong>p62</strong></td>
<td></td>
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<tr>
<td>Breast</td>
<td>WB</td>
<td>↑</td>
<td>n/d</td>
<td>Thompson et al. (104)</td>
</tr>
<tr>
<td>Gastric, esophageal, CRC, HCC</td>
<td>IHC</td>
<td>↑</td>
<td>n/d</td>
<td>Su et al. (102)</td>
</tr>
<tr>
<td>Prostate, high-grade PIN</td>
<td>IHC</td>
<td>↑</td>
<td>n/d</td>
<td>Qian et al. (103)</td>
</tr>
<tr>
<td>Breast</td>
<td>IHC</td>
<td>(20.3%)</td>
<td>Tumor grade; metastasis; OS; EGFR, HER2, HER3, and HER4 expression</td>
<td>Rolland et al. (97)</td>
</tr>
</tbody>
</table>

(Continued on following page)
Dysregulation of Beclin-1 transcript and protein levels in human tumors

Beclin-1 is underexpressed in some cancers and overexpressed in others (Tables 2 and 3). This observation supports the current hypothesis that autophagy-mediated damage mitigation both suppresses tumor initiation in precancerous cells and promotes tumor survival in established cancers (2, 3, 59). Under this model, reduced Beclin-1 levels might point to defective autophagy as a mechanism of early tumorigenesis in certain cancers. By similar logic, increased Beclin-1 expression might be a hallmark of autophagy upregulation in advanced tumors. However, reported correlations of Beclin-1 protein expression with tumor type, stage, and histologic grade, and organ involvement in the malignant process are unpredictable. In addition, recent evidence supports the existence of noncanonical, Beclin-1–independent autophagy (60); therefore, changes of Beclin-1 expression in tumor tissue should be interpreted with caution, and should not be considered a direct representation of fluctuations in autophagic activity.

Decreased Beclin-1 protein expression is observed in various tumors, including cervical squamous cell carcinoma (61), ovarian cancer (61), breast cancer (62), HCC (63, 64), lung cancer (63, 66), and some human brain tumors (67, 68). In ovarian cancer, Beclin-1 levels were shown to correlate with clinicopathologic stage, whereas in cervical cancer this correlation was not found (61).

In other cancers, such as melanoma (69, 70) and colorectal cancer (71), both decreased (69–71) and increased (70, 71) Beclin-1 protein expression was detected. The abnormal Beclin-1 levels correlated with patient prognosis; however, these results cannot be generalized, because the prognostic value of Beclin-1 expression appears to be context and cancer-type dependent. In contrast to studies showing a correlation between lower expression levels of Beclin-1 and aggressive (64, 67), recurrent (64), and metastatic disease (69, 72), there is evidence that in other cancers, such as lung cancer (63), expression of Beclin-1 is not affected by disease stage. In many cases, lower Beclin-1 expression was associated with worse prognosis (54, 64, 72–74), and high expression was associated with better prognosis (74). However, in patients with colorectal cancer, extensive over- and underexpression of Beclin-1 both correlated with poorer overall survival compared with other groups (71).

Abnormal Beclin-1 expression was detected at both the protein level and the mRNA level (51, 65–67, 69, 72). Low Beclin-1 mRNA and protein expression levels correlated (51), and were detected in non–small cell lung cancer (66). HCC
(64), and sporadic breast invasive ductal carcinoma samples (51).

The overall pattern revealed by these studies clearly highlights the relevance of abnormal (low or high) expression levels of Beclin-1 as a tumor marker and prognostic factor in various malignancies. However, the role of Beclin-1 in tumorigenesis requires further evaluation. Studies conducted with large sample sizes and unified scoring systems could systematize such information for application in the clinical setting.

**Tumor-related mRNA and protein alterations of MAP1LC3 and its orthologs**

The expression levels of MAP1LC3 or LC3 have been determined in many cancers. However, only a few recent studies differentiated among the various forms of LC3. The 3 main isoforms of human LC3 (LC3A, LC3B, and LC3C) differ in their post-translational modifications, exhibit distinct expression patterns across human tissues (75), and may serve distinct functions. Each isoform converts between cytosolic (LC3-I) and autophagosome-bound (LC3-II) forms depending on autophagy status (76, 77). Increased levels of LC3-II protein may indicate an accumulation of autophagosomes as a result of autophagy induction or, conversely, a block in downstream lysosomal fusion or turnover events (78). To distinguish between these 2 possibilities, investigators have developed autophagic flux assays for *in vitro* studies (78); however, to the best of our knowledge, none of these techniques have been adapted for use in human patient specimens. In addition, evidence of autophagy-independent LC3-II generation has been reported (78). Therefore, data on LC3-II accumulation in human tissues should be interpreted carefully, and not merely assumed to indicate autophagy activation. Ideally, a combination of IHC (for detection of total LC3 in tissues), Western blot (for evaluation of LC3-I and LC3-II levels in tumor lysates), and autophagosome visualization by electron microscopy should be applied along with markers of autophagic flux (see below).

Elevated expression of LC3, and specifically LC3-II, was observed in a large proportion of gastrointestinal (79, 80) and breast (81) cancers (Table 2). In a series of cutaneous melanocytic lesions (69) and astrocytic tumors (68), the expression level of LC3-II decreased during progression of the disease, and this trend was also seen at the mRNA level (69). Decreased LC3-II expression was related to more-aggressive disease and poor prognosis (68), and was also observed in lung cancer (65, 76) and ovarian tumors (82, 83). In most cases, LC3 expression levels resembled Beclin-1 expression levels; however, a significant correlation between the expression levels of these 2 proteins was found in only a small number of studies (68).

Definitions of the LC3 expression pattern in tumors, which are used to score LC3 expression as associated with autophagosome formation, differ from one IHC analysis to another. The classic punctate pattern of autophagosome accumulation, which mimics the vesicular distribution usually observed *in vitro*, has been described in a variety of tumors, including pancreatic cancer (84) and GI stromal tumors (85). Recently, 3 IHC patterns of expression of the LC3A isoform have been categorized as diffuse cytoplasmic, perinuclear, or stone-like structures (86–89). In various cancers, stone-like structures were associated with high-grade tumors and extremely poor survival rates, whereas perinuclear accumulation of LC3A is associated with favorable prognosis. In several studies, the diffuse cytoplasmic pattern was shown to have no relation to survival status (86–89). The authors hypothesized that stone-like structures represent an excessive autophagic response (86), and that diffuse cytoplasmic LC3A reflects basal autophagy. Although the significance of these LC3A patterns with respect to autophagy status requires further evaluation, these reports represent a promising step toward obtaining autophagy-specific IHC analyses with prognostic relevance.

According to these results, it would appear that in most cases the LC3 expression level is tumor specific and mimics the Beclin-1 expression pattern (Table 3). Further study of

<table>
<thead>
<tr>
<th>Table 3. Abbreviated summary of altered expression of autophagy-associated mRNA and protein levels in various cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beclin-1</td>
</tr>
<tr>
<td>Cancer</td>
</tr>
<tr>
<td>Breast</td>
</tr>
<tr>
<td>Lung</td>
</tr>
<tr>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Brain tumors</td>
</tr>
<tr>
<td>Melanoma</td>
</tr>
</tbody>
</table>

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<sup>a</sup> Details of the LC3 forms assessed are listed in Supplementary Table S1.

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↓ Low expression of protein or lower number of positive tumor samples compared with normal tissues.
↑ High expression of protein or higher number of positive tumor samples compared with normal tissues.
— Not determined.
all LC3 forms in additional cancer types, stages, and histologic grades is required to better assess the value of LC3 in the pathogenesis and prognosis of cancer.

It is important to recognize that MAP1LC3 proteins represent just 1 subfamily of 3 that have been defined for the 8 human orthologs of yeast Atg8 [4 LC3 genes (MAP1LC3A, MAP1LC3B, MAP1LC3B2, and MAP1LC3C), 3 GABARAP genes (GABARAP, GABARAPL1, and GABARAPL3), and 1 GATE-16 gene (GABARAPL2)]. The orthologs share high amino-acid sequence homology, and recent in vitro studies showed that both LC3 and GABARAP/GATE-16 proteins are essential for autophagosome biogenesis. LC3 proteins facilitate autophagosome elongation, and GABARAP/GATE-16 proteins are suggested to enable autophagosome closure (90–92). In addition, GABARAP/GATE-16 members function in intracellular protein trafficking (93). Expression profiling of a GABARAP family member was performed in human tumor specimens (without investigation of a direct link to autophagy) of thyroid cancer (94) and CRC [where GABARAP protein was overexpressed compared with matched, adjacent normal tissue (95)], and breast cancer [where both mRNA and protein levels for GABARAP were reduced (although they were not assessed in the same sample) compared with nonmatched, normal breast tissue (96)]. Future classification of GABARAP/GATE-16 proteins with respect to autophagy-specific function may reveal additional targets for IHC assessment of autophagy status in tumors.
Cancer-associated alterations in p62 protein levels: a measure of autophagic flux?

Expression levels of sequestosome-1 (p62), a multifunctional protein (97) that is preferentially (but not solely) degraded by autophagy and therefore is exploited as a marker of autophagic flux (78, 98), have been examined in a variety of malignant and normal tissues (Tables 2 and 3). Only a few studies have examined both mRNA and protein levels. Such an approach could help distinguish between genuine modulation of autophagic flux (i.e., changes in p62 protein expression level but not mRNA level) and transcriptional regulation of the gene (i.e., corresponding changes in both mRNA and protein levels), but it would likely be limited to situations in which the flux and p62 transcript level are not concurrently altered. For example, recent reports showed that p62 transcript upregulation can occur together with an increase in autophagic flux in vitro (99, 100). The use of the p62 level as a marker of flux on its own is not recommended for in vitro studies (56), and IHC analyses for p62 in tumors should be interpreted with similar caveats in mind.

High p62 expression, which putatively represents defective autophagy (101), has been described in a variety of digestive-system cancers (101–103) and breast cancer (104). The correlation of high expression with reduced 5-year survival in breast cancer (97) suggests that p62 should be considered for further evaluation as a prognostic marker for the disease. In gastrointestinal cancers, accumulation of p62 was associated with DNA damage response activation (101), cell differentiation (102, 103), and tumor metastasis (102). In lung cancer, p62 was overexpressed in adenocarcinomas but not in squamous cell carcinomas (105), and was required for RAS-induced transformation. Given the mounting evidence that autophagy is important in RAS-induced tumorigenesis (11, 84, 106, 107), we anticipate future investigations of the relevance of p62 in RAS-driven tumors.

Core autophagy proteins: uncharted waters

Currently, tumor expression data for additional autophagy-associated proteins are scarce. A high expression of ATG16L1 [oral carcinoma (108)] and ULK1 [esophageal squamous cell carcinoma (109)] was detected in tumor but not in normal tissue, and correlated with positive lymph node status (108) and shorter survival time (109). ATG5, which is involved in early autophagosome formation, was found to be underegulated in gastrointestinal cancers (48). Although the frequency of ATG5 mutation and/or loss of protein expression was low (48), these findings represent some of the first results obtained from IHC evaluation of ATG proteins in gastrointestinal cancers, and as such, make an invaluable contribution to the in vivo examination of autophagy’s predicted roles in human cancers.

Overactive mTOR: mutation and expression-level studies agree

Several recent reports showed elevated tumor-associated expression of mTOR and its pathway markers (e.g., phospho-S6 and phospho-4EBP1) in breast cancer (110, 111), high-stage urothelial carcinoma of the bladder (112), gastric cancer (113), metastatic renal cell carcinoma (114), and malignant glioma (115, 116). As expected for a major inhibitor of autophagy, mTOR expression inversely correlated with Beclin-1 level (115) and was associated with advanced tumor grade (116) and worse patient prognosis (110, 111). The observation of increased protein expression of mTOR and its substrates in various human tumor samples further supports the findings of mutational studies (discussed above) that identified mTOR-activating mutations in large-intestine adenocarcinoma and kidney clear-cell carcinoma at the DNA level (29).

Conclusions

The challenges we face in our attempt to assess autophagy in human cancer tissues are clear. We require comprehensive surveys of autophagy gene sequence and transcript changes for large sample sizes of single tumor types (controlled for stage and treatment), reliable tissue biomarkers that can be used as readouts for autophagic status, in vivo assays that can test flux, and markers with diagnostic, prognostic, and/or predictive value for clinicians and patients. Ideally, along with more-sophisticated sequencing technologies and new IHC biomarkers for tissue profiling, a noninvasive strategy to evaluate autophagy flux in human patients will be developed that can stratify patients without the necessity of a biopsy. With these tools in hand, we must examine more cancers and more stages, bearing in mind the contexts of treatment status and tumor genetic background, so that we may faithfully interpret the role of human autophagy in tumorigenesis and address the following questions: When are autophagy and tumorigenesis partners? When are they rivals? What is the best way for us to intercept therapeutically? And, of equal consideration, will therapeutic autophagy modulation adversely affect healthy tissues that rely on the cytoprotective roles of basal and stress-induced autophagy? Although complete answers to these questions await key technologic advancements before our explorations can reach full sail, researchers are poised to discover the hitherto uncharted contributions of autophagy to human cancer pathogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Autophagy-Related Alterations in Human Tumors


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