Autophagy: A primer for the gastroenterologist/hepatologist

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Autophagy is a conserved cellular pathway that maintains intracellular homeostasis by degrading proteins and cytosolic contents of eukaryotic cells. Autophagy clears misfolded and long-lived proteins, damaged organelles and invading microorganisms from cells, and provides nutrients and energy in response to exposure to cell stressors such as starvation. Defective autophagy has recently been linked to a diverse range of disease processes of relevance to gastroenterologists and hepatologists including Crohn's disease, pancreatitis, hepatitis and cancer. The present article provides an overview of the autophagy pathway and discusses gastrointestinal disease processes in which alterations in autophagy have been implicated. The clinical significance of autophagy as a potential therapeutic option is also discussed.

Key Words: Acute pancreatitis; Alpha-1-antitrypsin deficiency; Autophagy; Cancer; Crohn's disease; Cystic fibrosis; Ischemia-reperfusion injury

The terms 'autophagy' and 'autophagosome' were originally coined by De Duve and Wattiaux (1) in the 1960s to describe an intracellular digestive system closely related to lysosomes. Autophagy is a basal cellular pathway that maintains intracellular homeostasis by degrading proteins and cytosolic contents of eukaryotic cells. Autophagy clears misfolded and long-lived proteins, damaged organelles and invading microorganisms from cells, and provides nutrients and energy in response to exposure to cell stressors such as starvation. On initiation of autophagy, a cup-like isolation membrane that elongates to surround its cytoplasmic target is formed, and eventually seals to form a double-membrane vesicle known as an autophagosome. The autophagosome then fuses with lysosomes resulting in degradation of its contents (2) (Figure 1).

The three subtypes of autophagy – macroautophagy, microautophagy and chaperone-mediated autophagy – are distinguished by their delivery of material to the lysosome for degradation. Recently, the importance of the macroautophagy (hereafter referred to as autophagy) pathway in a variety of human diseases has been recognized. The present review focuses on the role of autophagy in gastrointestinal and liver diseases.

THE AUTOPHAGY PATHWAY

Autophagy-related (ATG) genes and proteins were first identified in yeast (3) and, because the autophagy machinery is an evolutionarily conserved process, their human counterparts were determined shortly thereafter (4). The formation of the autophagosome requires two ubiquitin-like conjugation systems (Figure 1). Complexes of ATG proteins, including ATG5, ATG12 and ATG16L1, are conjugated to the membrane of autophagosomes and are required for the 'matura-
tion' of this compartment. The microtubule-associated protein 1 light chain 3 (LC3), the homologue of yeast ATG8, was identified as a mammalian autophagosome protein, and is also a major tool to monitor autophagy in vitro and in vivo (5). LC3, which is located in the cytoplasm, is recruited to the forming autophagosome membrane by interaction with the ATG12-ATG5-ATG16L complex. This complex is localized to the outer membrane of the autophagosome and supports the elongation of the isolation membrane and lipidation of LC3 (6-8). LC3 is linked to phosphatidyethanolamine (yielding a product known as LC3II), which is located on the inner and outer membranes of the autophagosome (9). LC3II remains associated with autophagosomes until its destruction at the autolysosomal stage. Thus, the amount of membrane-bound LC3II provides a good index of autophagy induction. LC3II located on the outer membrane can be deconjugated by ATG4, releasing LC3I for further use, whereas the LC3II on the inner membrane is degraded after fusion of the autophagosome with the lysosome.

Figure 1) Autophagosome formation. On induction of autophagy, the isolation membrane or phagophore elongates and engulfs the cellular contents. Sealing of the tips leads to completion of the double-membrane autophago-
some. The membrane then fuses with the lysosome resulting in degra-
dation of its contents. Key elements of this process are the autophagy-related (ATG)12-ATG5-ATG16L complex, which recruits light chain (LC) 3 to the membrane, and ATG4, which controls the lipidation and recycling of LC3.
In general, autophagy is regarded as a cell survival mechanism; however, autophagy-related cell death may also occur (20). A link between the autophagic and apoptotic machinery is found at the autophagosome initiation level where Beclin-1, an autophagy inducer, interacts with the anti-apoptotic protein Bcl-2 or the Bcl-2 homologue Bcl-Xl, preventing Beclin-1-dependent autophagy (21). For example, herpes viruses produce a Bcl-2 homologue that promotes survival by preventing apoptotic cell death of virus-infected cells and suppressing autophagy (22). During starvation, the opposite takes place, i.e., dissociation of Bcl-2 from Beclin-1 induces autophagy (23). Additional connections between autophagy and apoptosis include common stimuli such as reactive oxygen species and the tumour suppressor protein p53 (24) (Figure 2). However, the decisive factors distinguishing 'self-eating' autophagy from 'self-killing' apoptosis have yet to be entirely elucidated.

Autophagy was originally considered to be a nonselective process; however, studies performed in the past few years have demonstrated that autophagic adaptor proteins can target substrates for autophagic degradation. One such autophagy receptor is p62 (also known as SQSTM1/sequestome-1), which delivers ubiquitinated protein aggregates to the autophagosome (25) and also targets invading microorganisms for autophagy (26). There is evidence that p62-marked substrates are incorporated into the autophagosome via binding to LC3 (27). However, p62 can also localize to the endoplasmic reticulum (ER)-associated autophagosome formation site independently of LC3 (28).

With the identification of key components and the tools to study the pathway, researchers have begun to investigate the significance of autophagy in various disorders. In the following sections, we highlight the recent knowledge regarding the role of autophagy in human gastrointestinal disorders including infections, inflammatory conditions and cancer.

**GASTROINTESTINAL AND HEPATIC INFECTIONS**

Some microorganisms invade cells in an attempt to generate a niche – either in specialized compartments or free within the cytosol – to escape elimination by host defenses. However, within the cell, autophagy can target pathogens and restrict their growth. Autophagy of microbes is also known as xenophagy. Salmonella enterica serovar Typhi, which causes acute bloody diarrhea, and can also lead to severe invasive infections and sepsis in immunocompromised patients, replicates within Salmonella-containing vacuoles in host cells. Bacterial colonization of the cytosol is prevented by targeting Salmonella via the adapter protein p62 for autophagic degradation (26). In fact, increased cytosolic bacterial growth has been detected in autophagy-deficient cells (29). Autophagosomes can also target bacterial toxins to mitigate their toxic effects on host cells. During infection with Vibrio cholerae, which causes life-threatening diarrhea, the autophagic pathway protects cells against the pore-forming exotoxin V cholerae toxin (VCC). VCC-intoxicated cells induce autophagy to degrade VCC and promote survival. In contrast, cells with defective autophagy are more sensitive to the cytotoxicity of VCC (30).

The gastric pathogen Helicobacter pylori, which causes peptic ulcer disease and gastric cancer, expresses various virulence factors to mediate disease. One of these factors, the vacuolating cytotoxin VacA, is produced by some strains, and its presence is associated with more severe disease. VacA is internalized within the host cell, inducing the formation of vacuoles and altering host cell signalling (31). In addition, H pylori can survive intracellularly in VacA-dependent vacuoles in vitro (32). Short-term exposure to VacA also induces the formation of autophagosomes in gastric epithelial cells in vitro (33). A portion of the toxin is found within these autophagosomes indicating that autophagy targets the toxin. When autophagy is disrupted in host cells, degradation of the toxin is prevented and toxin-mediated cellular vacuolization is increased, indicating that autophagy can eliminate the toxin and mitigate its detrimental effects in host cells.

These studies indicate that autophagy acts as an innate host defense against infectious agents. However, some microorganisms have
developed mechanisms to manipulate the pathway, thereby avoiding clearance. For example, microbes may prevent the maturation of autophagy, block autophagosome maturation or even usurp the autophagosome to create an intracellular niche (34). Shigella, which causes dysentery and whose toxin-producing strains are associated with the development of hemolytic uremic syndrome, can prevent detection by the autophagic machinery. Shigella moves within the cell and promotes cell-to-cell spreading by actin-based motility. The surface component VirG, which is necessary for Shigella’s actin-dependent motility, can be recognized and targeted for autophagy by the ATG5 protein. However, Shigella also secretes the bacterial protein IcsB, which camouflages VirG, thereby enabling Shigella to escape ATG5 detection and subsequent autophagic clearance (35).

Viruses use host cells and their resources for their own lifecycle. The hepatitis B virus replicates by inducing autophagy and the development of autophagic vacuoles, whereas it simultaneously prevents maturation to autolysosomes, thus avoiding lysosomal degradation (36). Similarly, hepatitis C virus (HCV) replication is dependent on autophagy, illustrated by knockout of Beclin-1, which reduces HCV production, enhances cell death and prevents persistent infection (37). Interestingly, autophagic proteins are needed to initiate HCV RNA translation, but not to maintain it, suggesting a contribution of autophagic proteins in the delivery of HCV RNA to the translational apparatus (38).

These examples demonstrate the potential complexity of host-pathogen interplay in the autophagy pathway during gastrointestinal or hepatic infections. Therefore, careful consideration of this complexity is important for the development of novel therapies that, depending on the specific context, could either promote or inhibit the pathway to ensure beneficial effects for the host.

CROHN’S DISEASE

In 2007, a genome-wide association study (39) identified a nonsynonymous single-nucleotide polymorphism (rs2241880) causing a threonine to alanine substitution at position 300 (T300A) of the ATG16L1 gene as a susceptibility variant for Crohn’s disease (CD). The variant is associated with the WD repeat region of ATG16L1, the function of which is unknown. As described above, ATG16L1 forms a complex with ATG5 and ATG12, which is necessary for autophagosome formation. Several groups have attempted to delineate the potential functional relevance of this allele in mediating CD. In one study, decreased efficiency of antibacterial autophagy was observed in human cell cultures overexpressing the ATG16L1 CD variant (40). Another study determined that fetal hepatic macrophages obtained from ATG16L1 knockout mice produced higher levels of the inflammatory cytokines interleukin-1-beta and interleukin-18 in response to bacterial lipopolysaccharide exposure compared with control macrophages. In addition, mice transplanted with the ATG16L1 knockout macrophages were more susceptible to dextran sulphate sodium (DSS)-induced acute colitis (41). Cadwell et al (42) generated mice with reduced ATG16L1 protein (hypomorphs) to address the role of ATG16L1. These mouse models developed changes in the Paneth cell granule exocytosis pathway, which lacked lysozymes in the mucus layer of the ileum (Figure 3). Paneth cells are located in the crypts of Lieberkühn of the small intestine, and secrete lysozymes and antimicrobial peptides into the lumen of the crypt to protect against pathogenic invasion. These Paneth cell abnormalities were also identified in human tissues from CD patients with the risk allele of ATG16L1 (42). Although these studies did not specifically investigate the endogenously expressed CD variant, the findings suggest that the CD variant may alter autophagy and host inflammatory pathways in response to microbes or their products.

The etiology of CD is multimodal and involves genetic, immunological and environmental factors. The multihit hypothesis – that genetically susceptible individuals primed by an unknown event develop CD after another incident occurred – may explain the fact that only a small percentage of individuals with the common disease variant ATG16L1 are actually affected by CD. In a follow-up to their previous study, Cadwell et al (43) found that mice with reduced levels of ATG16L1 raised in a germ-free facility lacked the Paneth cell abnormalities until they were infected with a specific murine norovirus. Subsequent treatment with DSS induced severe colitis with some of the typical features of the transmural colitis seen in CD patients, whereas uninfected ATG16L1 mutant mice and wild-type mice showed only focal ulcerating DSS colitis. In addition, broad-spectrum antibiotic treatment prevented DSS-induced colitis, indicating a requirement for a bacterial component after the viral trigger (43). Another feature highlighted by this study is the possible contribution of viral infections to the pathogenesis of CD.

ATG16L1 T300A is not a disease-causing polymorphism; therefore, the functional relevance of this variant also needs to be evaluated in the context of other risk alleles. Nucleotide-binding oligomerization-domain-containing 2 (NOD2), an intracellular pattern recognition receptor for bacterial muramyl-dipeptide (MDP), was the first gene associated with CD (44). The most prevalent NOD2 polymorphism

![Figure 3](image-url)
L increases the intra-acinar trypsin leading to cell necrosis and inflammation than Cat B. The imbalance between the lysosomal hydrolases Cat B and Cat L increases the intra-acinar trypsin leading to cell necrosis and inflammation (Figure 4).

Autophagy in pancreatitis. Autophagy is induced during pancreatitis, but the degradation of autophagosomes is impaired with accumulation of large vacuoles. Reduced activity of the lysosomal cathepsins (Cat L and B) was detected in autophagosomes, with Cat L more greatly affected than Cat B. The imbalance between the lysosomal hydrolases Cat B and Cat L increases the intra-acinar trypsin leading to cell necrosis and inflammation.

miRNA-196 regulation decreased the host cell response to infection with adherent-invasive Escherichia coli (Figure 3). All three susceptibility variants for CD – ATG16L T300A, NOD2L1007insC and IRGM C313T – in some way link autophagy with microorganisms and distorted host cell responses. Despite the potential involvement of a multitude of environmental and genetic risk factors, the pathogenesis of CD may ultimately be the result of a few pathways that are simply affected at different stages and in different combinations.

**ACUTE PANCREATITIS**

Pancreatic proenzymes are stored in zymogen granules of acinar cells and enzyme inhibitors, such as the serine protease inhibitor Kazal-type 1, bind prematurely activated trypsin to prevent autodigestion of the pancreas. In acute pancreatitis, these protective mechanisms are exhausted, and activated trypsin within acinar cells leads to cell necrosis with further release of additional activated enzymes. Recently, several groups investigated the potential role of autophagy in the pathogenesis of acute pancreatitis. Hashimoto et al (52) showed that induction of acute pancreatitis with supraphysiological doses of cerulain, a cholecystokinin analogue, induces autophagy in acinar cells and that acinar cell-specific ATG5 knockout mice with disrupted autophagy had less severe pancreatitis and less conversion of trypsinogen to trypsin in their acinar cells (52). The authors suggested that autophagosomes engulf zymogen granules and deliver them to low pH and cathepsin B-containing lysosomes where the activation of trypsinogen occurs. However, increased autophagy also occurs in acinar cells upon starvation without a corresponding increase in activation of trypsinogen (53). In another study, Mareninova et al (53) demonstrated that defective autophagy was induced during acute pancreatitis. In this study, impaired activity of the lysosomal cathepsins L and B was detected in autophagosomes, with cathepsin L more greatly affected than cathepsin B. The authors hypothesized that because cathepsin B converts trypsinogen to trypsin, and cathepsin L degrades trypsin, this imbalance would promote increased active trypsin within cells (Figure 4). However, the underlying mechanisms responsible for these observations remain unknown.

In contrast to these studies, Grasso et al (54) provided evidence that selective zymogen autophagy is a protective mechanism in acute pancreatitis because knockdown of vacuole membrane protein-1 (VMP1), an autophagy-related protein that induces autophagy, prevented pancreatic acinar cell death in experimental models (54). In addition, VMP1 was undetectable in normal human acini but present in tissue affected by pancreatitis in association with autophagosome formation.

These apparently contradictory studies suggest a major role for autophagy in acute pancreatitis. However, additional studies are needed to determine whether there is variation in specific types of autophagy during pancreatitis, some of which may be protective and others harmful, thereby providing a potential explanation for these divergent results as well as directing therapeutic options.

**CANCER**

Autophagy can have diametrically opposing effects in tumorigenesis, playing a role in both tumour suppression and promotion (55). Autophagosomes can engulf and eliminate damaged organelles that produce reactive oxygen species and cause DNA damage. Similarly, autophagosomes can target protein aggregates that may modulate tumourigenic signal transduction pathways. Both of these mechanisms can prevent tumour development. In contrast, a shortage of nutrients within the tumour environment can trigger autophagy to provide nutrients and energy, thereby promoting survival of tumour cells and supporting their progression.
Initial evidence supporting a role for autophagy in tumour suppression came from studies in Beclin-1 deficient mice. Aging Beclin-1+/− mutant mice with impaired autophagy developed spontaneous tumours including hepatocellular carcinoma (HCC) (56). Consistent with these findings, human HCC tissue samples showed lower Beclin-1 expression than non-tumour tissue (57), indicating that autophagy has tumour suppressor functions and that Beclin-1 is a haploinsufficient tumour suppressor. Similarly, monoallelic mutations in the ultraviolet radiation-resistance-associated gene (UVRAG), an inducer of autophagy through binding to Beclin-1, are found in human colon cancer (58) and in gastric carcinomas with microsatellite instability (59). Liang et al (60) demonstrated that overexpression of UVRAG induces autophagy and suppresses the proliferation and tumorigenicity of human colon cancer cells. Additionally, mice inoculated with UVRAG mutant tumour cells developed larger tumour masses than mice inoculated with tumour cells in which UVRAG was replaced (60).

In both human gastric adenocarcinomas and HCC tissue samples, mTOR expression is increased, and tumour growth and angiogenesis is constricted in experimental models following treatment with rapamycin, which inhibits mTOR and induces autophagy (61,62). These findings also suggest that rapamycin, which blocks mTOR activation, may have chemotherapeutic potential in gastrointestinal and liver tumours. In support of this contention, a recent study reported improved survival after liver transplantation for HCC in patients treated with a rapamycin-based immunosuppression protocol (63).

In contrast to its tumour-suppressing effects, autophagy may also help tumour cells survive when oxygen and nutrients are diminished. Tumorigenic cells with defective apoptosis but functioning autophagy display a survival advantage under ischemic conditions compared with cells with blocked autophagy (64). Thus, at first, autophagy may protect against tumour initiation, whereas later in established tumours, it promotes survival.

Due to the opposing effects of autophagy in tumorigenesis, targeting this pathway in anticancer therapy may be particularly difficult. For example, inhibition of autophagy increased sensitivity to radiation in radioreistant human cancer cell lines in one study (65), whereas another study showed that induction of autophagy radiosensitized prostate cancer cells (66). The tumour suppressor p53 induces autophagy and apoptosis (67). Treatment of lymphoma cells with the p53 stimulator tamoxifen led to tumour apoptosis and increased autophagy in surviving cells. Cotreatment with chloroquine, a pharmacological inhibitor of autophagy, enhanced p53-dependent apoptosis and tumour regression by blocking autophagy (68). In this instance, it can be reasoned that a combination therapy of apoptosis-activating and autophagy-blocking agents is a very potent treatment strategy. Therefore, autophagy modulation as an adjuvant to standard chemotherapy may improve efficacy by shifting the balance from autophagy to apoptosis, but needs to be tumour and tumour-stage specific (69).

PROTEIN FOLDING DISORDERS

Alpha-1-antitrypsin deficiency

The acute-phase reactant alpha-1-antitrypsin (A1AT) is a protease inhibitor produced in hepatocytes, which protects tissue from neutrophil elastase in particular. A1AT deficiency is primarily associated with respiratory complications, but it is also the most common genetic cause of liver disease in children. The classical Z mutation leads to a misfolded protein, which accumulates in the endoplasmic reticulum (ER), which causes ER stress and inflammation. Monomers can be degraded in proteasomes whereas polymer degradation takes place in autophagolysosomes. Enhancing autophagy with carbamazepine increases the disposal of mutant A1AT and subsequently ameliorates its hepatotoxicity properties, promoted the degradation of mutant A1AT and even decreased hepatic fibrosis (72) (Figure 5). This intriguing study suggests that enhancing autophagy may be a promising pharmacological intervention in A1AT. However, rapamycin failed to show enhanced disposal of mutant A1AT, which may suggest a role for an mTOR-independent pathway. Proteasomal clearance may also be part of the carbamazepine effect because proteasomal inhibitors lessened the degree of carbamazepine-induced degradation of A1AT. More studies are needed to clarify the role of autophagy in A1AT deficiency because carbamazepine seems to influence other intracellular mechanisms. In addition, despite the favourable safety profile of carbamazepine, its feasibility needs to be evaluated if the high doses required in mice are also required in humans.

Cystic fibrosis (CF) is caused most commonly by a ΔF508 mutation in the CF transmembrane conductance regulator (CFTR) gene, which results in a misfolded protein that does not reach the cell membrane and is degraded rapidly. Decreased LC3II and Beclin-1 protein levels were observed in nasal polyps from severely affected CF patients, in lung tissue from CF mice and in human CF bronchial epithelial cells. These levels could not be increased by autophagy induction via starvation or rapamycin, suggesting defective autophagy (73). Previously, it was shown that tissue transglutaminase is upregulated in CF (74), and that there is a link between oxidative stress and inflammation in CF (75). Derived from these findings, treatment with cystamine – a transglutaminase inhibitor, and N-acetyl-L-cysteine – an antioxidant, increased Beclin-1 levels, rescued autophagy and ameliorated lung injury by preventing the damaging aggresome formation of the CFTR ΔF508 protein (73). However, it is not known whether deficient autophagy is involved in mediating the disease found in other organs such as the pancreas or the liver; this is an area of interest for future investigation.
ISCHEMIA-REPERFUSION INJURY

Ischemia-reperfusion injury (IRI) occurs during liver transplantation or hepatic resection when blood supply is disrupted and subsequently restored. Damaged mitochondria with impaired oxidative phosphorylation lose their membrane potential, produce reactive oxygen species and release proapoptotic factors. The innate immune system is especially activated during reperfusion and, thus, the inflammatory response leads to aggravated organ injury. In an in vivo model of ischemia-reperfusion with temporary clamping of liver vessels, pretreatment with the autophagy-inducing agent cisplatin led to a reduced expression of the inflammatory mediators tumor necrosis factor-alpha and interleukin-6, and protected mice from liver injury as assessed by transaminase levels (76). In the livers of the cisplatin-treated mice, Beclin-1 and LC3 levels were increased, and ultrastructural analysis with electron microscopy showed autophagy of mitochondria (also known as mitophagy). In contrast, in a study of prolonged cold ischemia of rat livers using University of Wisconsin solution, the inhibition of autophagy withwortmannin increased the survival rate of the rat liver recipients (77). Comparing these two studies highlights the difficulty of translating the results of cell culture or animal models into clinical practice. Many factors may explain the apparently conflicting results including the differences in modeling ischemia-reperfusion with variable durations of ischemia, warm versus cold ischemia, preservation solution with or without amino acids, and potential autophagy-independent effects of the pharmacological agents.

More insight into the role of autophagy in IRI may be obtained as our understanding of ischemic preconditioning (IP) evolves. The beneficial effects of IP were first described in the heart. IP with interruption of the blood supply followed by reperfusion protects the myocardium from a subsequent ischemic insult (78). In a study of patients who underwent hepatic resection of colon cancer metastasis (79), increased LC3 expression and autophagosome formation was detected in liver biopsies following IP compared with biopsies taken before IP. Conflicting results were found in studies that assessed the impact of IP on postoperative liver function, morbidity and mortality rates (80-82). Thus, additional studies are needed to determine whether the increase in autophagy after IP has a protective role in IRI (83).

THERAPEUTIC CONSIDERATIONS

As outlined above, given the importance of autophagy in a variety of diseases, a great deal of interest has centered on the development of potential therapies to modulate this pathway. Thus, the gastroenterological, hepatological and pancreatic disorders. There is a need for increased understanding of the mechanisms underlying these observations, suitable models to study the pathway in disease and the potential benefit of pharmacological manipulation of the pathway before clinical trials can be safely performed. Although initial reports about the benefit of autophagy-modulating drugs are promising, there is a need to develop highly selective drugs that target specific components of the autophagy pathway and are suitable for clinical use.

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