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Autophagy and Digestive Disorders: Advances in Understanding and Therapeutic Approaches

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Abstract

The gastrointestinal (GI) tract is a series of hollow organs that is responsible for the digestion and absorption of ingested foods and the excretion of waste. Any changes in the GI tract can lead to GI disorders. GI disorders are highly prevalent in the population and account for substantial morbidity, mortality, and healthcare utilization. GI disorders can be functional, or organic with structural changes. Functional GI disorders include functional dyspepsia and irritable bowel syndrome. Organic GI disorders include inflammation of the GI tract due to chronic infection, drugs, trauma, and other causes. Recent studies have highlighted a new explanatory mechanism for GI disorders. It has been suggested that autophagy, an intracellular homeostatic mechanism, also plays an important role in the pathogenesis of GI disorders. Autophagy has three primary forms: macroautophagy, microautophagy, and chaperone-mediated autophagy. It may affect intestinal homeostasis, host defense against intestinal pathogens, regulation of the gut microbiota, and innate and adaptive immunity. Drugs targeting autophagy could, therefore, have therapeutic potential for treating GI disorders. In this review, we provide an overview of current understanding regarding the evidence for autophagy in GI diseases and updates on potential treatments, including drugs and complementary and alternative medicines.

Key Words: Functional dyspepsia, Irritable bowel syndrome, Inflammation, Autophagy

INTRODUCTION

Autophagy, which literally means self-eating, is an intracellular homeostatic mechanism. During stressful conditions, damaged cytoplasmic components and foreign antigens are degraded inside the autolysosome with the help of lysosomal hydrolases and lipases (Klionsky, 2007; Maes *et al*., 2013). Generally, autophagy can be classified into three primary forms: macroautophagy, microautophagy, and chaperone-mediated autophagy. These three forms show different morphologies (Klionsky, 2005). An autophagosome is formed by the sealing of a double-membrane compartment known as a phagophore. The autophagosome then fuses with a lysosome to form an autolysosome. This process is extensively modulated by various autophagy-related genes (ATG) and proteins (Wang and Klionsky, 2003). During stressful conditions, autophagy removes polyubiquitinated protein aggregates, degrades invading pathogens through a process called xenophagy, regulates the release of pathogen-induced pro-inflammatory cytokines, and participates in the development of lymphocytes and in antigen presentation (Boya *et al*., 2013).

Autophagy contributes substantially to intestinal homeostasis, host defense against intestinal pathogens, regulation of the gut microbiota, and innate and adaptive immunity (Mizushima, 2018). Changes in autophagy have been implicated in the pathogenesis of various diseases, including gastrointestinal (GI) diseases such as gastritis, inflammatory bowel disease (IBD), GI motility dysfunction, and GI cancers (Xavier and Podolsky, 2007; Castaño-Rodríguez *et al*., 2015). In this review, we provide an updated and comprehensive overview of the current understanding of autophagy in GI diseases, including the underlying mechanisms of autophagy in the pathophysiology of GI diseases, as well as several recent autophagy-related pharmacological interventions in the treatment of GI diseases.

AUTOPHAGY AND CHRONIC ATROPHIC GASTRITIS

Chronic atrophic gastritis (CAG) is a disorder characterized by the reduction or loss of the mucosal glands found in the fundus and the body of the stomach. These glands may be

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lost on account of fibrosis, or more frequently pseudo-pyloric or intestinal metaplasia, due to chronic inflammation (Correa, 1988; Rugge *et al*., 2002). The histological changes may be associated with *Helicobacter pylori* infection, or may be due to immune-mediated attacks on the gastric parietal cells. Autophagy plays a crucial role in the development of CAG (Wang *et al*., 2020b).

CAG FOLLOWING *H. PYLORI* INFECTION

Pathophysiology

H. pylori, a microaerophilic gram-negative bacterium, has been widely accepted as the major risk factor for the development of gastric cancer since the International Agency for Research and Cancer (IARC) of the World Health Organization designated it as a type 1 carcinogen in 1993 (International Agency for Research on Cancer, 1994). *H. pylori*-induced CAG is the first step in the multistep cascade of gastric cancer. The early phase of *H. pylori* infection elicits an acute inflammatory response with temporary clinical symptoms such as nausea and vomiting, which develops into chronic gastritis (Marshall and Warren, 1984). The two most important virulence factors secreted are vacuolating cytotoxin (VacA) and effector protein cytotoxin-associated gene A (CagA) (Xiang *et al*., 1995). In chronic infection, *H. pylori* adheres to the epithelial surface and degrades it, causing mucin loss, cytoplasmic basophilia, increased mitosis, and hyperchromatic nuclei (Carneiro *et al*., 1992; Leung *et al*., 1992). Generally, infiltration of the gastric mucosa with neutrophil granulocytes plays an important role in the immune response against *H. pylori*, triggered by high levels of interleukin-8 (IL-8). IL-8 attracts neutrophils, releasing oxyradicals to block oxidative stress (Hardbower *et al*., 2014).

Lymphocyte infiltration is also seen during the formation of lymphoid follicles, which is initiated by Th (T-helper cell)-1 and Th-17-responses involving the release of proinflammatory cytokines such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), interferon gamma (IFN-γ), and IL-17 (Khamri *et al*., 2010). *H. pylori* has been shown to regulate the autophagy pathway of the host by degrading the cytosolic proteins, organelles, and pathogens within the cell. The channel-forming activity of VacA is necessary and sufficient to induce autophagy in gastric epithelial cells (Terebiznik *et al*., 2009; Wang *et al*., 2009). VacA-mediated autophagy disrupts autophagosome maturation following prolonged exposure to the toxin (Raju *et al*., 2012). In chronic *H. pylori* infection, microRNA (miR)-30b is upregulated and compromised autophagy maintains the *H. pylori* infection (Xiao *et al*., 2009; Tang *et al*., 2012). A total of 28 autophagic genes, including *ATG16L1*, *ATG5*, *ATG4D*, and *ATG9A*, are downregulated in chronic *H. pylori* infection (Castaño-Rodríguez *et al*., 2015; Tanaka *et al*., 2017). $CagA^+$ *H. pylori* strains located in the gastric mucosa upregulate inflammatory cytokines, whereas autophagic proteins are downregulated by the c-Met/AKT (MET family receptor tyrosine kinase/protein kinase) signaling pathway, thereby inhibiting autophagy in chronic *H. pylori* infection (Li *et al*., 2017).

Therapeutic approaches

According to clinical guidelines and consensus statements (Sugano *et al*., 2015; Chey *et al*., 2017; Malfertheiner *et al*., 2017), all patients with CAG who have *H. pylori* infection should receive eradication therapy when there are no other considerations. The goals of treatment in such individuals include cure of infection, resolution of mucosal inflammation, recovery of gastric acid secretion, prevention of lesions in the gastric mucosa, and prevention of gastric cancer (Lee *et al*., 2016).

The first-line therapy for *H. pylori* eradication is antibiotics, which is limited in effectiveness due to antibiotic resistance. A non-antibiotic therapy is, therefore, required. Catechins, natural phenols found in tea, red wine, fruit, and some plants, have antioxidant and anti-microbial effects (Mabe *et al*., 1999). Sialic acid, mostly found in GI mucins and milk, has an anti-adhesive effect against *H. pylori* infection (Simon *et al*., 1997). The catechin and sialic acid combination therapy can eradicate *H. pylori* and ameliorate *H. pylori*-induced epithelial cell death by enhancing autophagy and decreasing the activation of caspase-1 and the secretion of IL-1 (Yang *et al*., 2013). Thus, this combination therapy could provide an alternative regimen for treating *H. pylori*-induced atrophic gastritis. Moreover, resveratrol, a potent anti-inflammatory and antioxidant polyphenol mostly found in berries, nuts, and grape skin, decreases the levels of IL-8 and inducible nitric oxide synthase and inhibits NF-κB activation in *H. pylori*-induced gastritis (Zhang *et al*., 2015). Resveratrol also exerts inhibitory effects on translation, outer membrane protein transport, ATP synthase, and other possible oxidative damage against *H. pylori* (Xia *et al*., 2020). Therefore, resveratrol has potential as a treatment option for *H. pylori*-induced gastritis.

Eudesmin, extracted from *Fatsia polycarpa* Hayata, efficiently eradicates *H. pylori* and enables recovery from the *H. pylori*-induced gastric epithelial cell death mediated by autophagy and apoptosis. This eradication is achieved by decreasing the expression of proteins involved in apoptosis and autophagy, such as LC-3B, caspase-3, caspase-9, caspase-8, Bax, and Bid (Yang *et al*., 2018). Eudesmin treatment provides a unique and promising non-antibiotic therapy for *H. pylori* infection. Furthermore, astaxanthin, a carotenoid pigment found in algae, yeast, and aquatic animals (Hussein *et al*., 2006), has been found to be effective against *H. pylori*-induced apoptosis in gastric epithelial cells. This inhibitory effect in gastric epithelial cells of the AGS line is produced by upregulation of phosphorylated AMPK, which in turn blocks mTOR activation, thereby activating Unc 51-like autophagy activating kinase 1 and inducing autophagosome formation by increasing the microtubule-associated protein expression of light chain 3B II (LC3B-II) (Lee *et al*., 2020).

AUTOIMMUNE GASTRITIS

Pathophysiology

Autoimmune gastritis (AIG) refers to a selective loss of parietal cells in the gastric mucosa due to autoimmune phenomenon. This condition is strongly associated with a severe form of vitamin B12-deficiency anemia, known as pernicious anemia, resulting from the increased pH of the stomach and a loss of intrinsic factors due to parietal cell destruction (De Block *et al*., 2000; Neumann *et al*., 2013). The pathophysiology of AIG is as yet poorly understood. Both the α-subunit (catalytic) and β-subunit (glycoprotein) of the gastric proton pump H^+/K^+ ATPase, located on parietal cells, are the major target autoantigens recognized by anti-parietal cell antibodies (Callaghan

et al., 1993). The H^+/K^+ ATPase induces the proliferation of $CD4^+$ T cell clones of the gastric mucosa, causing the production of tissue necrosis factor (TNF) and IFN-γ by Th-1 cells (D'Elios *et al*., 2001). Intrinsic factors, which are recognized by activated autoreactive $CD4^+$ T cells and activate cytotoxic T cells against parietal cells, are secreted in the gastric mucosa of patients with AIG (Troilo *et al*., 2019). Recent studies have suggested that AIG is associated with changes in autophagy. IFN-γ initiates autophagy in gastric epithelial cells by activating the transcription of Beclin-1 and increasing the expression levels of LC3-II (Tu *et al*., 2011).

Therapeutic approaches

Supplementation with micronutrients is essential in the management of AIG. Various oral iron supplements, including ferrous sulfate, ferrous fumarate, ferrous gluconate, ferrous glycine-sulfate, ferric protein-succinylate, ferric mannitol-ovalbumin, and ferric polymaltose complex, are available. These are used to overcome iron deficiency in patients with AIG (Pisani *et al*., 2015). Oral vitamin B_{12} supplements are prescribed when pernicious anemia is present (Andres *et al*., 2010). Parenteral injection of vitamin B_{12} is also important when neurological symptoms occur (Lenti *et al*., 2020). Additionally, eradication of *H. pylori* infection to decrease the levels of AIG-linked antibodies can be another therapeutic strategy, as co-infection with *H. pylori* is related to AIG (Stolte *et al*., 1998; Faller *et al*., 1999).

IFN-γ is an important component of the type 1 immune response produced by activated CD4+ and CD8+ T cells (Billiau *et al*., 1998). Some studies have shown the potential role of IFN-γ in the treatment of autoimmune gastritis. Overexpression of IFN-γ reduces gastric inflammation by inducing autophagy and decreasing gastric epithelial cell apoptosis (Tu *et al*., 2011). Conversely, in a mouse model of autoimmune gastritis, depletion of IFN-γ has positive outcomes in treating AIG because IFN-γ acts directly on gastric epithelial cells and is necessary for the development from gastritis to atrophic gastritis and metaplasia (Osaki *et al*., 2019). Therefore, it is important to note that cytokines can have either antagonistic or synergistic effects during an immune response. The relevant mechanisms should be explored in more detail to determine the potential role of IFN-γ in AIG prevention.

AUTOPHAGY AND IBD

Pathophysiology

IBD is a lifelong inflammatory disease of the GI tract. IBD encompasses Crohn's disease (CD), ulcerative colitis (UC), colonic IBD, and an unclassified type (IBDU). CD and UC are relatively common and can be considered the main subtypes of IBD. They differ in the extent of the affected site. In CD, submucosal or transmural inflammation and ulcers can be found at any location along the GI tract, whereas in UC, ulcers are localized to the colon and inflammation is limited to the mucosa and epithelial lining of the GI tract. The cause of IBD is not fully understood. In 2017, Choy *et al*. (2017) found that more than 160 genetic loci were associated with IBD; most of these are related to host immune response and microbial handling by the immune system. The disease is thought to be the result of an uncontrolled immune response to a trigger in genetically prone individuals, fueled by environmental factors (Alatab *et al*., 2020).

By 2020, more than 200 genetic loci had been found by large-scale, genome-wide studies to be associated with IBD (Jairath and Feagan, 2020), some of which were also associated with autophagy (Massey and Parkes, 2007; Parkes *et al*., 2007; Rioux *et al*., 2007). The ATG *ATG16L1* (autophagy related 16-like 1) and *IRGM* (immunity related GTPase M) are closely related to the occurrence and development of IBD (Massey and Parkes, 2007; Naser *et al*., 2012). ATG16L1 is involved in autophagosome formation; it also interacts with other key proteins such as ATG5 and ATG12. The *IRGM* gene is related to the processes of autophagy regulation, proinflammatory cytokine production, and apoptosis, and plays an important role in the body's immunity (Parkes *et al*., 2007). These genetic studies have given rise to a growing number of studies that aim to identify the connection between autophagy dysfunction and IBD pathogenesis.

Generally, the immune system of the body can be divided into three lines of defense. The first of these consists of structural barriers, such as the epithelium barrier in the intestine. The second line of defense includes the inflammatory response, involving antimicrobial proteins or phagocytic leukocytes. The third line of defense is adaptive immunity. According to several studies, autophagy has a direct impact on all three lines of defense in the intestinal tract.

Autophagy has been reported to enhance intestinal barrier function by downregulating the pore-forming tight junction protein claudin-2 through lysosomal degradation in Caco-2 intestinal epithelial cells (Nighot *et al*., 2015). Increased intestinal permeability caused by defective intestinal epithelium barrier function is a common diagnostic factor in active IBD patients (Hu *et al*., 2015; Chang *et al*., 2017) and the upregulation of claudin-2 has been reported to be associated with the disease (Luettig *et al*., 2015). Innate immune signaling pathways are initiated when microorganism-specific pathogen-associated molecular pattern molecules are recognized by host pattern recognition receptors (PRRs). PRRs, such as toll-like receptors (TLRs) and nucleotide oligomerization domain (NOD)-like receptors (NLRs), can regulate the autophagy pathway (Oh and Lee, 2014). *NOD2*, an NLR, was the first gene found to be associated with CD, and it remains one of the genetic factors that confer the greatest risk for the development of CD (Philpott *et al*., 2014). Stimulation of NOD2 induces autophagy in dendritic cells in a receptor-interacting serine-threonine kinase 2-dependent manner (Cooney *et al*., 2010); stimulation of TLRs with their specific ligands has also been shown to stimulate autophagy (Delgado and Deretic, 2009). CD-associated NOD2 variants cannot recruit ATG16L1 to the plasma membrane at the bacterial entry site, thereby resulting in defective bacterial clearance (Fritz *et al*., 2011). Moreover, the combination of disease-associated alleles of ATG16L1 and NOD2/caspase recruitment domain-containing protein 15 has been shown to synergistically increase susceptibility to CD, indicating possible crosstalk between NOD2- and ATG16L1-mediated processes in the pathogenesis of CD (Billmann-Born *et al*., 2011).

Furthermore, mutations in the ATG (*ATG16L1*, *IRGM*, and *NOD2*) in intestinal epithelial cells may result in defective bacterial clearance. Abnormal morphology and autophagy dysfunction in Paneth cells are among the major outcomes of polymorphisms in *ATG16L* and *IRGM*, which are associated with IBD. Paneth cells are secretory epithelial cells found pre-

dominantly in the intestinal crypts. Their function is to secrete antimicrobial peptides and immunomodulating proteins to regulate the intestinal microbiota, thereby providing protection to the intestinal stem cells that line the crypt walls (Lueschow and McElroy, 2020). It has been reported that Paneth cells are an original site for intestinal inflammation in diseases such as IBD (Wang *et al*., 2018a; Wehkamp and Stange, 2020). In both *ATG16L1* mutant mice and *IRGM1* knockout mice, impaired autophagy was seen, along with effects on both morphology and function of the Paneth cells; most notably, a marked alteration in the appearance of their secretory granules was observed (Cadwell *et al*., 2008; Liu *et al*., 2013). The impairment of ATG also allows adherent–invasive *E. coli* to replicate and survive, leading to CD progression (Lapaquette *et al*., 2010). Furthermore, increased expression of IRGM could reduce the inflammatory response by mediating selective autophagic degradation of inflammasomes targeting the NLR family pyrin domain 3 (NLRP3) and the adaptor molecule apoptosis-associated speck-like protein containing a CARD (ASC) (Mehto *et al*., 2019).

In addition to its essential role in innate immune defense against infection, autophagy plays a role in the adaptive immune response (Wang *et al*., 2018a). Autophagy contributes to the formation of antigenic peptides in antigen-presenting cells that link to T cells by means of major histocompatibility complex (MHC)-I or MHC-II to trigger an adaptive immune response (Henderson and Stevens, 2012). The dendritic cells from CD patients with risk-associated NOD2 and ATG16L1 variants are defective in autophagy induction and MHC class II antigen presentation (Cooney *et al*., 2010).

Therapeutic approaches

A report of autophagy induction via mammalian target of rapamycin complex 1 (mTORC1) and the unfolded protein response (UPR) by the IBD drug azathioprine suggests that stimulation of autophagy and UPR may contribute to the therapeutic efficacy of a drug (Hooper *et al*., 2019). Azathioprine is a commonly prescribed immunosuppressant in IBD and is used to maintain remission in moderate to severe CD/UC (Retnakumar and Muller, 2019). Cyclosporine, another immunosuppressant drug used in IBD therapy, also induces autophagy (Ciechomska *et al*., 2013; Kim *et al*., 2014).

Some autophagy regulators have shown promising effects in IBD, and research into autophagy-related targets is growing. Metformin, which has been found to regulate autophagy (Nazim *et al*., 2016; Wang *et al*., 2018b), also ameliorates IBD (Chen *et al*., 2018). In a dextran sulfate sodium (DSS)-induced murine colitis model, the mTOR inhibitor P2281 as well as torin1, were effective (Bhonde *et al*., 2008). Rapamycin, betanin, and trehalose induce autophagy and ameliorate intestinal inflammation and colitis by reversing the increased expression of M1 macrophage-associated markers (CC-chemokine receptor 7, and clusters of differentiation 11c and 86), and pro-inflammatory cytokines (cyclooxygenase-2 and IL-6), as well as affecting NF-κB signaling, while increasing the levels of anti-inflammatory cytokine IL-10 in mice with 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced colitis (Macias-Ceja *et al*., 2017). In addition, rapamycin has been effective in clinical studies. In a case report of a 37-year old woman with severe refractory colonic and perianal CD, rapamycin (sirolimus) treatment produced marked and sustained improvements in symptoms and endoscopic appearance, after treatment with azathioprine, methotrexate, and infliximab failed (Massey *et al*., 2008). Another study has also provided evidence that sirolimus can be effective as a rescue therapy in a subgroup of children with severe IBD refractory to conventional therapies, by inducing both clinical remission and mucosal healing (Mutalib *et al*., 2014). Selected investigations from the past five years that demonstrate the promising nature of IBD therapies targeting autophagy, are described in Table 1.

Table 1. Recent laboratory findings indicating links between autophagy and IBD

Treatment	Outcome	Reference
Slit2/Robo1	Activated autophagy in intestinal stem cells and mitigated DSS-induced UC	Xie *et al*., 2020
Dapagliflozin	Increased colonic autophagy and suppressed apoptosis in a TNBS-induced rat colitis model	Arab *et al*., 2021
TREM-1 inhibitor	Restored impaired autophagy and alleviated colitis in mice	Kokten *et al*., 2018
IL-33	Reduced TNBS-induced colitis in mice by promoting mitophagy	Wang *et al*., 2019
Celastrol	Augmented NLRP3 inhibitor (CP-456773) activity through heat shock protein-90 and increased autophagy in rats with DSS-induced colitis	Saber *et al*., 2020
Curcumin	Prevented the development of DSS-induced colitis in mice through inhibition of excessive autophagy and regulation of the subsequent cytokine network	Yue *et al*., 2019
Herb-Partitioned Moxibustion	Attenuated intestinal inflammation and promoted the repair of colon mucosal injury in rats with CD while downregulating the autophagy-associated *NOD2* and *IRGM* genes	Zhao *et al*., 2019
Resveratrol	Increased autophagosome levels, decreased inflammatory cytokine levels, and alleviated UC-related intestinal mucosal barrier dysfunction in mice with DSS-induced colitis	Pan *et al*., 2020

Robo1, roundabout homolog 1; DSS, dextran sulfate sodium; UC, ulcerative colitis; TNBS, 2,4,6-trinitrobenzene sulfonic acid; TREM1, triggering receptor expressed on myeloid cells 1; IL-33, interleukin-33; NLRP3, NOD-, LRR-, and pyrin domain-containing protein 3; CD, Crohn's disease.

AUTOPHAGY AND GI CANCER

Pathophysiology

In GI cancers, as in the general pathophysiology of cancer, once cells liable to cancer begin their neoplastic transformation, these genetically mutated oncogenic cells grow into abnormal shapes and divide uncontrollably and rapidly, yielding in-situ or malignant (invasive) tumors over time. GI cancers are those in which the neoplasms arise in any tissue of the GI tract or other organs involved in digestion, including the esophagus, stomach, biliary system, pancreas, small intestine, large intestine, rectum, and anus.

As a crucial homeostatic process, autophagy is increasingly recognized as an important factor in understanding and managing cancers. Mounting evidence highlights the complex and multifaceted role of autophagy in carcinogenesis (Folkerts *et al*., 2019). The neoplastic transformation in the early phase of carcinogenesis is associated with disruption of the autophagy process. This allows the accumulation of oncogenes and reactive oxygen species, thus favoring neoplasm formation. Correspondingly, blockading chaperone-mediated autophagy in fibroblasts has been shown to enhance the efficiency of cellular transformation driven by the proto-oncogene *MYC/c-Myc* (Gomes *et al*., 2017). In the same study, chaperone-mediated autophagy was reported to suppress c-Myc activity by promoting proteasomal degradation. Another study reported that an experimental frameshift mutation of the ATG *UVRAG* (UV radiation resistance-associated gene) in mice resulted in an increased inflammatory response and colitis-associated cancer (Quach *et al*., 2019). In addition, autophagy can selectively eliminate oncogenic pathogen infection (Sui *et al*., 2017). Chronic *H. pylori* infection is a well-known risk factor for gastric carcinogenesis. One effect of acute VacA exposure is the induction of xenophagy to counteract *H. pylori* infection. However, prolonged exposure to this toxin strongly disrupts xenophagy and promotes infection, eventually leading to gastric cancer (Greenfield and Jones, 2013). Correspondingly, individuals carrying the ATG polymorphism (*ATG16L1* rs2241880 variant) have an increased risk of developing *H. pylori* infection and gastric cancer, indicating that a xenophagy defect may be associated with the initiation of gastric cancer (Castaño-Rodríguez *et al*., 2015).

Conversely, increased autophagic flux is common in established cancers, perhaps as an energy supply to cancer cells, contributing to their survival during stress (Folkerts *et al*., 2019). Autophagy could also maintain tumor stemness and enable leveling up to the metastasis stage. In addition, it also influences the metabolism of cancer cells. Cancer cells can perform glycolysis even in the presence of oxygen, known as the Warburg effect, to offset nutrient stress. Autophagy can control this aerobic glycolysis at different levels by selectively degrading the rate-limiting enzymes involved in the glycolytic pathway, such as pyruvate kinase muscle isozyme M2 (PKM2) (Lv *et al*., 2011) and hexokinase 2 (Jiao *et al*., 2018), resulting in the accumulation of glycolytic intermediates that re-enter various branching biosynthetic pathways to support cancer growth. Furthermore, the stem cells of multiple cancer types have been shown to express relatively high levels of essential genes, and autophagy inhibition could augment their chemosensitivity (Li *et al*., 2018; Nazio *et al*., 2019).

Notably, sensitivity to and tolerance of autophagy regulation vary with cancer stage and cellular conditions (Lauzier *et al*., 2019). In addition to protective autophagy, autophagic cell death can occur. This refers to cell death induced by the activation of autophagy flux alone, with no other types of programmed cell death involved (Jung *et al*., 2020). Cancer cells at risk of cell death could stimulate protective autophagy against the stress induced by chemotherapy, resulting in chemoresistance (Jing *et al*., 2020; Xu *et al*., 2020a). Conversely, direct and strong stimulation of autophagy by specific chemotherapies could induce autophagic cell death, highlighting the dual nature of autophagy. Autophagy itself can reverse chemoresistance by mediating apoptosis and/or inhibiting epithelial-mesenchymal transition (EMT) (Xu *et al*., 2020a). The outcome of the autophagy process varies depending on the stress level and stage of the cancer. Alterations in ATG have been found to be associated with tumorigenesis in GI cancers (Burada *et al*., 2015; Qian and Yang, 2016); these alterations are summarized as follows. Frameshift mutations in *ATG2B*, *ATG5*, *ATG9B*, and *ATG12* are common in gastric cancers with high microsatellite instability, while other genes, such as *ATG6/Beclin1*, *ATG8/LC3*, *p62/SQSTM1* (sequestosome-1), or SIRT1 (sirtuin-1), are often upregulated in GI cancers. Furthermore, higher expression of *ATG10* is associated with tumor lymph node metastasis and poor survival in colorectal cancer (Choy *et al*., 2017). Additionally, researchers have reported that major ATG can be used as prognostic markers for GI cancers, including esophageal, gastric, colon, and pancreatic cancers, as well as hepatocellular carcinoma (Mo *et al*., 2019; Wang *et al*., 2020a; Xu *et al*., 2020b; Yue *et al*., 2020; Du *et al*., 2021; Li *et al*., 2021).

Therapeutic approaches

Several clinical trials of autophagy-inhibiting anti-cancer treatments have been conducted and published. Currently, chloroquine (CQ) and hydroxychloroquine (HCQ) are the only drugs available for clinical use (Xu *et al*., 2018). A meta-analysis based on various types of cancer, including pancreatic cancer, showed that both CQ and HCQ can significantly improve the overall response rate (ORR), 1-year overall survival (OS) rate, and 6-month progression-free survival (PFS) rate. HCQ-based therapy produces better 1-year OS and 6-month PFS rates than CQ-based therapy, and CQ-based therapy produces better ORR than HCQ-based therapy (Xu *et al*., 2018). Some clinical trials published in the last seven years are described in Table 2. The clinical value of targeting autophagy with the HCQ therapy remains controversial.

Nevertheless, the laboratory investigations into drugs targeting or mediating autophagy in cancer therapy are attracting increased attention; novel compounds and pathways have been discovered. For instance, natural products, such as flavonoids, alkaloids, terpenoids, and coumarins, have been reported as potential autophagy inhibitors and activators and as agents reversing multidrug resistance in gastric cancer (Xu *et al*., 2020a). Furthermore, pectolinarigenin, which is a natural flavonoid present in *Cirsium chanroenicum* and citrus fruits, has been shown to induce autophagy- and apoptosis-related cell death in the AGS and MKN28 human gastric cancer cell lines (Lee *et al*., 2018). Chrysin from propolis also inhibits colorectal cancer to an extent comparable to inhibition by the 5-fluorouracil+oxaliplatin combination through autophagy induction (Lin *et al*., 2018). High PKM2 expression has been reported in GI cancers, including esophageal, gastric, colorectal, and liver types (Su *et al*., 2011; Wu *et al*., 2016; Wang *et*

Table 2. Overview of phase I/II clinical trials in GI cancers with HCQ

Treatment	Cancer type	Outcome	Reference
HCQ+Gemcitabine (Phase II randomized)	Metastatic pancreatic adenocarcinoma	Addition of hydroxychloroquine to chemotherapy did not improve overall survival among patients with metastatic pancreatic cancer, but significantly increased the overall tumor response rate.	Karasic *et al*., 2019
HCQ (Phase II and pharmacodynamic study)	Metastatic pancreatic adenocarcinoma	In patients with previously treated metastatic pancreatic cancer, HCQ monotherapy achieved inconsistent autophagy inhibition and demonstrated negligible therapeutic efficacy.	Wolpin *et al*., 2014
HCQ+Gemcitabine (Phase II randomized)	Potentially resectable pancreatic cancer	Improved pathologic and biomarker response.	Zeh *et al*., 2020

HCQ, hydroxychloroquine.

Table 3. Recent laboratory findings indicating links between autophagy and human GI cancers

Treatments	Cancer type	Outcome	Reference
miR-133a-3p	Gastric cancer	Targeted GABARAPL1 to block autophagy-mediated glutaminolysis, further repressing gastric cancer growth and metastasis	Zhang *et al*., 2018
Caffeine and theophylline	Gastric cancer	Effectively induced gastric cancer cell apoptosis and autophagy by PTEN activation and PI3K/AKT/mTOR pathway suppression	Liu *et al*., 2019
Berberine	Gastric cancer	Repressed human gastric cancer cell growth *in vitro* and *in vivo* by inducing cytostatic autophagy via inhibition of mitogen-activated protein kinase /mTOR/70-kDa ribosomal protein S6 kinase and AKT	Zhang *et al*., 2020
Compound TDB	Gastric cancer	Induced autophagy-dependent apoptosis by regulating PI3K/AKT/mTOR	Xiao *et al*., 2021
Trifolirhizin	Colorectal cancer	Induced autophagy by activating the AMPK/mTOR pathway and positively contributed to extrinsic apoptosis both *in vivo* and *in vitro*	Sun *et al*., 2020
S130 (Atg4 inhibitor)	Colorectal cancer	Significantly attenuated the growth of xenografted colorectal cancer cells, especially when combined with caloric restriction	Fu *et al*., 2019
UBLA4	Pancreatic ductal adenocarcinoma	Inhibited autophagy-mediated proliferation and metastasis	Chen *et al*., 2019
miR-18-5p	Pancreatic cancer	Inhibited autophagy by targeting SIRT1	Tian *et al*., 2017

GABARAPL1, gamma-aminobutyric acid receptor-associated protein-like 1; PTEN, phosphatase and tensin homolog; TDB, tricyclic decyl benzoxazole; UBLA4, ubiquitin-like protein 4A; SIRT1, sirtuin-1.

al., 2017). Overexpression of the PKM2 enzyme can block the autophagy process by activating mTORC1 (He *et al*., 2016). ATG7 has been shown to reduce the Warburg effect by blocking the binding of PKM2 to its upstream kinase fibroblast growth factor receptor 1 and thereby suppressing the phosphorylation of the PKM2 Tyr105 site, leading to reduced EMT in tumor cells (Feng *et al*., 2018). Inhibition of the $PFKFB_3$ glycolytic enzyme (6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3) attenuates autophagy and increases the chemosensitivity of colorectal cancer cells to oxaliplatin treatment (Yan *et al*., 2019). Some new findings linking autophagy and GI cancer therapy published in the last five years, are described in Table 3.

AUTOPHAGY AND GASTROINTESTINAL MOTILITY DISORDERS

Pathophysiology

Gastrointestinal smooth muscles generate rhythmic electrical pulses, resulting in cycles of contraction and relaxation (Sanders *et al*., 2012). Contractility is driven by the phosphorylation of myosin light chain 20 (MLC20) by Ca^{2+}/calmodulin-dependent myosin light chain kinase or Ca^{2+}-independent kinases, including ρ-kinase, integrin-linked kinase, and zipper-interacting protein kinase. Relaxations are caused by the myosin light-chain phosphatase-containing myosin phosphatase target subunit, which dephosphorylates MLC20 (Somlyo and Somlyo, 2003; Ihara and MacDonald, 2007). Moreover, interstitial cells of Cajal (ICC) provide a pathway for the propagation of slow waves and are responsible for the motility pattern observed at the organ level (Iino *et al*., 2009). ICCs depend on the SCF/c Kit interaction for growth and development and respond to its signaling by upregulating neurotransmission

(Chai *et al*., 2017). Lack of ICC in the small intestine results in failure to generate pacemaker activity, leading to GI dysfunction in humans. ICC is, therefore, recognized as the pacemaker for the GI tract (Huizinga *et al*., 1995; Burns, 2007; Farrugia, 2008).

Slow waves propagate via the depolarization-induced activation of voltage-dependent Ca^{2+} channels, facilitating Ca^{2+} entry into the ICC and inducing Ca^{2+} release (Sanders *et al*., 2012). The Ca^{2+}/calmodulin-dependent protein kinase 2-AMPK pathway induces autophagy following an increase in cytosolic Ca^{2+} levels (Ghislat *et al*., 2012). Consequently, excessive autophagy can disrupt the ICC, which largely depends on SCF/c-kit signaling, leading to GI dysmotility disorders such as slow transit constipation. Moreover, irritable bowel syndrome (IBS) is also linked to SCF/c-kit signaling, which is activated by a mild inflammatory response (Chai *et al*., 2017). In rats with functional dyspepsia, the AMPK/TSC2 (tuberous sclerosis complex 2)/Rheb signaling pathway is inactivated, and ghrelin levels in rat tissues are reduced. The mTOR inhibitor rapamycin accelerates the positive effect of electro-acupuncture in rats with functional dyspepsia (Tang *et al*., 2020). Inhibition of mTOR leads to the activation of au-

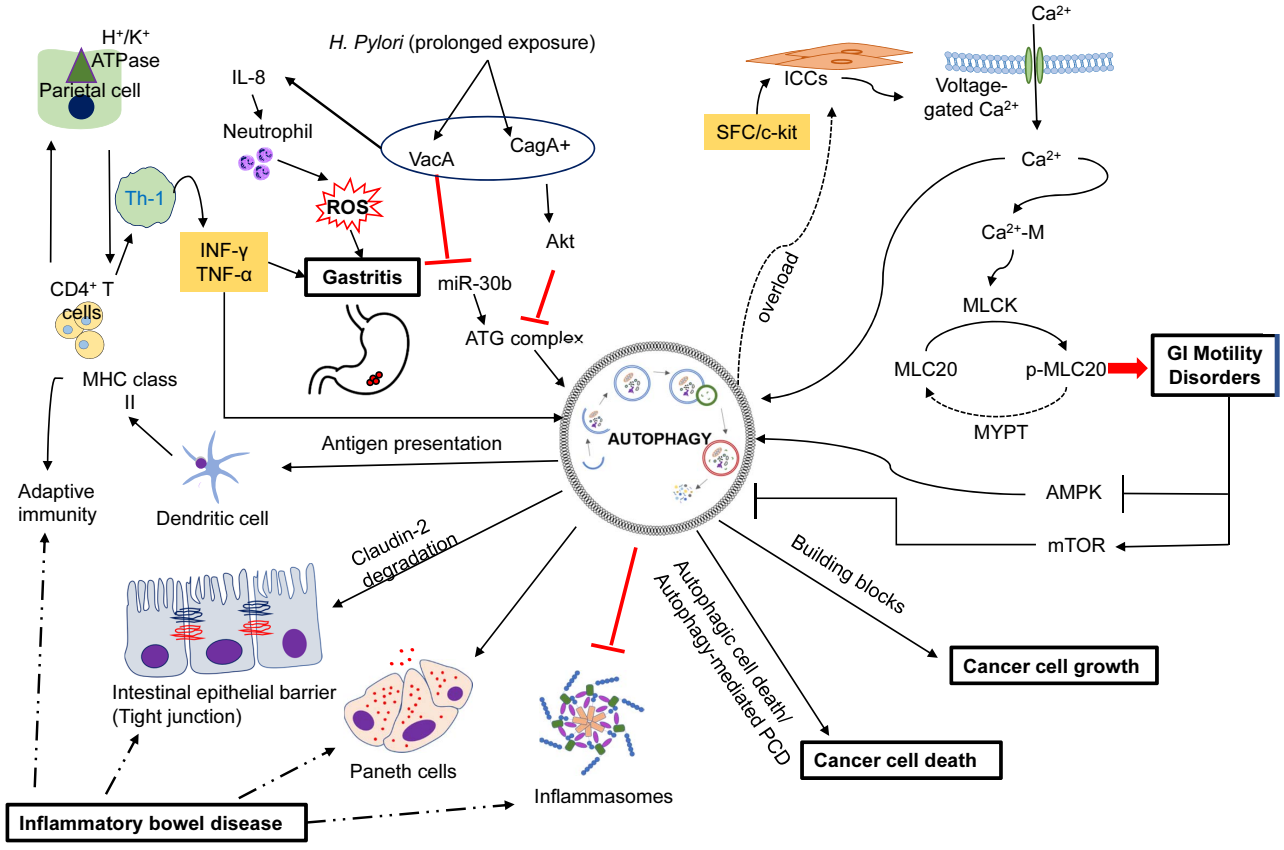

Fig. 1. Autophagy-related mechanisms of pathogenesis in gastrointestinal disorders. Virulence factors (VacA and $CagA^+$) from *Helicobacter pylori* cause the production of IL-8, which attracts neutrophils, thereby releasing reactive oxygen species (ROS). These attack the gastric mucosa, leading to chronic atrophic gastritis. The two virulence factors also block the autophagy process through the activation of AKT and inactivation of miR-30b, both of which regulate autophagy. When the dendritic cells in the gastric mucosa become activated, they cause the release of the proton pump H+/K+ ATPase from the parietal cells. H+/K+ ATPase is a major target for autoantigens during autoimmune activation. CD4+ T cells become activated in the presence of a proton pump and cause Th-1 cells to release INF-γ and TNF-α, thereby leading to autoimmune gastritis. INF-γ and TNF-α activate the autophagy process. Autophagy stimulates dendritic cells through antigen presentation. MHC class II is activated by the adaptive immune system in inflammatory bowel disease (IBD). IBD causes intestinal epithelial barrier destruction via claudin-2 degradation, which originates from the activation of autophagy. Autophagy can cause impairment of Paneth cells, which are the primary site for intestinal inflammation in IBD. Autophagy degrades the inflammasomes that are released during IBD. During cancer cell growth and cell death, autophagy stimulates the building blocks for cancer pathogenesis and causes autophagic cell death. In addition, the SCF/c-kit propagates the slow waves through the ICCs, which increases the entry of Ca^{2+} into the cells. Ca^{2+} binds to calmodulin and causes smooth muscle contraction via activation of MLCK, which phosphorylates MLC20 to p-MLC20. Autophagy overload causes the destruction of ICCs and leads to gastrointestinal motility disorders, in which AMPK is decreased and mTOR is activated. The entire autophagy process is thus impaired, as is gastrointestinal homeostasis. GI, gastrointestinal; IL-8, interleukin 8; MHC class II, major histocompatibility complex class II; VacA, vacuolating cytotoxin A; CagA+, cytotoxin-associated gene A; INF-γ, interferon gamma; TNF-α, tumor necrosis factor alpha; AKT, protein kinase B; SCF, stem cell factor; ICCs, interstitial cells of Cajal; Ca^{2+}-M, calcium calmodulin complex; MLCK, myosin light chain kinase; MLC20, myosin light chain 20; p-MLC, phosphorylated myosin light chain 20; MYPT, myosin light chain phosphatase; mTOR, mammalian target of rapamycin; AMPK, 5' adenosine monophosphate-activated protein kinase; Th-1, helper T cell 1; ATG, autophagy-related genes.

tophagy. Therefore, it can be assumed that autophagy plays an important role in functional dyspepsia.

Therapeutic approaches

Drugs targeting SCF/c-kit inhibition are potential treatments for the management of IBS due to autophagy. Pharmacological antagonists for SCF/c-kit signaling include imatinib, lapatinib, sunitinib, imatinib, and sorafenib. However, the use of these inhibitors is limited because blocking SCF/c-kit signaling may worsen the tissue damage (Milenkovic *et al*., 2007). Therefore, further research is needed to identify the detailed mechanisms, and to explore the efficacy and safety of the use of these inhibitors in IBS therapy.

In addition, some complementary and alternative medicines have beneficial effects in other GI disorders. Tong bian decoction, a Chinese medicinal herb, exerts a laxative effect in rats with slow transit constipation by inhibiting autophagy in the ICC, which in turn promotes its regeneration and repair abilities (Zhou *et al*., 2020). Applying electro-acupuncture in rats with functional dyspepsia increases their ghrelin levels and activates AMPK/TSC2/Rheb signaling by inhibiting mTOR, leading to the amelioration of dyspepsia (Tang *et al*., 2020).

Zhi Shi Xiao Pi Tang (ZSXPT), a Chinese traditional medicine formulation, consists of ten medicinal plants: immature bitter orange, *Magnolia officinalis*, coptis, *Pinellia ternate*, ginger rhizome, malt, rhizoma atractylodis macrocephalae, *Poria cocos*, *Codonopsis pilosula*, and liquorice. Pretreatment with ZSXPT in rats with functional dyspepsia accelerates autophagy and inhibits ROS generation and subsequent apoptosis via the blockade of mTOR signaling. The efficacy and safety of these medicinal herbs for treating functional dyspepsia should be further investigated.

CONCLUSION AND FUTURE DIRECTIONS

A number of factors must be considered in future studies. Firstly, the signaling pathway of autophagy can influence neighboring proteins for different purposes under certain circumstances. Secondly, as the original purpose of autophagy is homeostasis, it should be noted that all ATG also exist in normal cells, not only in the abnormal cells of our target. Thirdly, the known autophagy regulators therefore also have off-target or autophagy-independent effects and lack specificity to any particular cell type. Fourthly, our understanding of GI disease etiology is still incomplete, especially in IBD and cancer. Importantly, the IBD-associated ATG variants are also found in healthy individuals, and their presence alone is not sufficient to induce IBD. Fifthly, autophagy itself is flexible and varies in the different levels of stimuli it accepts; the outcome of the autophagic process may be beneficial or deleterious to the host cells. Therefore, logically, the development of novel strategies or compounds to precisely modulate the specific autophagic processes that are pathologically defective, without interfering with other autophagy processes, or the development of efficient personalized approaches depending on the specific pathological conditions, should form the basis of future research. Furthermore, studies enabling a clear understanding of the molecular mechanisms underlying the connection between autophagy and GI diseases are needed. The summary of the pathogenesis mechanisms of autophagy on disorders is provided in the Fig. 1.

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