Chapter 38
Autophagy and Gastrointestinal Diseases

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Abstract Normal gastrointestinal physiology is fundamental for all the living beings. Gastrointestinal diseases mainly include gastrointestinal motility disorders, infectious inflammation (such as *Helicobacter pylori* infection, cholera, and intestinal parasites), non-infectious inflammation (such as chronic gastritis and Crohn's disease), and gastrointestinal cancers. In addition, intestinal microbial disorder is also an important cause of intestinal diseases, so intestinal microecological treatment (fecal microbiota transplantation) is an important mean of treating gastrointestinal diseases. In recent years, the role of autophagy in gastrointestinal diseases has been studied extensively. Autophagy is observed under various pathological processes of the gastrointestinal tract. For example, it has been demonstrated that autophagy plays an important role in maintaining the homeostasis and integrity of intestinal epithelium. Additionally, autophagy regulates host response to *H. pylori* infection and development of gastrointestinal cancers. Therefore, we will discuss pivotal roles of autophagy in various gastrointestinal diseases and analyze the underlying molecular mechanisms, which may provide new therapeutic targets applicable for the treatment of gastrointestinal diseases.

Keywords Autophagy · Chronic atrophic gastritis · Helicobacter pylori · Chronic non-atrophic gastritis · Inflammatory bowel disease · Gastrointestinal infections · Intestinal microecology · Gastrointestinal motility disorders

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38.1 Overview

The gastrointestinal system consists of stomach, small intestine, and large intestine. It is an important place for digesting nutrients and excreting metabolic products. The stomach is an important organ for storing and digesting food. Pepsin in the gastric juice digests a portion of the protein in the food, and the stomach acid in the stomach provides the acidic environment necessary for the function of the pepsin. The food that enters the small intestine from the stomach is further decomposed with the aid of pancreatic-derived pancreatic enzymes and liver-derived bile, and a large amount of fluff in the small intestine increases the absorption area of nutrients. The main function of the large intestine is to further absorb water and dielectrics, forming and storing feces. Since the gastrointestinal tract is directly connected to the outside world, intestinal microbes, which are called the ninth largest organ of the human body, are anchored in the gastrointestinal tract.

The normal function of digestive system is crucial for human beings. Gastrointestinal diseases mainly include infectious inflammation (such as intestinal parasites, *H. pylori* infection, and cholera), non-infectious inflammation (such as Crohn's disease and chronic gastritis), gastrointestinal motility disorders, and gastrointestinal cancers. In addition, turbulence of intestinal microbial composition also plays an important role in intestinal diseases. Importantly, autophagy is observed under various pathological processes of the gastrointestinal tract. For example, it has been demonstrated that autophagy is crucial for maintaining the homeostasis of intestinal epithelium. Meanwhile, autophagy regulates host response to *H. pylori* infection and development of gastrointestinal cancers.

38.2 Autophagy and Non-infectious Inflammation of Gastrointestinal Tract

38.2.1 Autophagy and Chronic Atrophic Gastritis

38.2.1.1 Changes in Autophagy During the Development of Chronic Atrophic Gastritis

Chronic atrophic gastritis (CAG) is a type of chronic gastritis characterized by a decrease in the intrinsic glandular mucosa. The main clinical manifestations are upper abdominal pain, abdominal distension, abdominal discomfort, loss of appetite, and occasionally anemia, weight loss or diarrhea. Chronic atrophic gastritis belongs to a pathological concept, including metaplastic atrophy and non-metaplastic atrophy. The former refers to the glandular glands that are replaced by intestinal metaplasia or pseudo-pyloric gland metaplasia. Replacement with fibrous or fibromuscular tissue, or infiltration of inflammatory cells causes a decrease in the number of intrinsic glands. CAG is an intermediate stage in the conversion of normal gastric mucosa

into cancer. It is a benign lesion. If it is not controlled, it may increase the chance of transformation into gastric cancer. In 1978, the World Health Organization listed CAG as a precancerous lesion of gastric cancer, and the cancer rate is 1–3%.

The cause of chronic atrophic gastritis mainly includes all pathological processes that can cause gastric mucosal damage and lead to a decrease in the number of glands and intestinal metaplasia. *H. pylori* (HP) infection and autoimmune injury are the main causes of chronic atrophic gastritis. Autophagy plays an important role in the process of chronic atrophic gastritis.

HP is one of the most common human gastrointestinal pathogens, mainly colonized in gastric mucosal epithelial cells. 50–60% of the world's population is infected with this disease. HP infection has been considered as an infectious disease. It is recognized as a class I carcinogen, and HP infection is a major factor in the development of chronic atrophic gastritis and the progression to intestinal and cancerous development. Studies have shown that autophagy changes throughout the process of precancerous lesions such as chronic atrophic gastritis, intestinal metaplasia, and dysplasia caused by HP. HP has a two-way regulation of autophagy. After HP infection, vacuolar toxin (vacuolating cytotoxin A, VacA), HP secretion-associated antigen (HP0175), and HP flagella can activate autophagy in gastric epithelial cells, and autophagy can degrade VacA and reduce HP toxicity to cells, which suggests that autophagy is a protective mechanism after HP infection. Some studies have also pointed out that the expression of autologous activating gene ATG16 is significantly downregulated after HP infection, and a variety of miRNAs are also involved in down-regulation of autophagy, which creates a suitable environment for HP infection, which leads to inflammation and precancerous lesions.

Autoimmune gastritis refers to diffuse atrophy of the stomach in the context of CD4+ T cell-mediated autoimmune diseases, specific autoantibodies (e.g., anti-internal factors antibody, anti-parietal cell antibody, anti-gastrin-secreting cells antibody) are the main cause of gastric gland atrophy; and the incidence rate in China is low. Recent studies have found that autoimmune gastritis is accompanied by changes in autophagy. The study found that IFN-γ, as a key molecule in the pathogenesis of autoimmune gastritis, activates the transcription of Beclin-1 and upregulates the expression level of LC3-II, thereby activating autophagy and inducing T cell apoptosis (Tu et al. 2011). IFN-γ initiates the autophagy process of gastric epithelial cells by inducing the formation of the autophagosome. Moreover, treatment of mice with specific expression of IFN-γ in gastric epithelial cells using the autophagy inhibitor chloroquine blocked cell lysosomal turnover.

In summary, the changes in autophagy accompanying the development of chronic atrophic gastritis, but the specific mechanism needs further study.

38.2.1.2 The Role of Autophagy in the Progression of Chronic Atrophic Gastritis

In 1988, Correa proposed a classic model of natural progression of intestinal type gastric cancer, namely, "normal mucosa—chronic non-atrophic gastritis—chronic atrophic gastritis—intestinal metaplasia—dysplasia—gastric cancer". Chronic atrophic gastritis is a critical stage in which gastritis is benign or malignant. Chronic atrophic gastritis gradually progresses to intestinal metaplasia, dysplasia, and gastric cancer. Therefore, chronic atrophic gastritis is recognized as a precancerous lesion of gastric cancer. Studies have found that about 4.41% of chronic atrophic gastritis progressed to gastric cancer after 8 years of follow-up. Studies have found that autophagy plays an important role in the progression of chronic atrophic gastritis.

(1) Autophagy and precancerous lesions (intestinal metaplasia and dysplasia)

Intestinal metaplasia and dysplasia are important stages in the progression of gastritis to gastric cancer, marking changes in cell morphology and structure, and gradually embodying "malignant" biological characteristics. Recent studies have found that autophagy plays an important role in the evolution of this state (Li et al. 2018). The study used MNNG reagent to induce precancerous lesions (intestinal metaplasia and heterosexual hyperplasia) in mice. The researchers found that autophagy-related factors ATG5, ATG12, Ambra1, Beclin1, LC3, and p62 were significantly upregulated during modeling (Cai et al. 2018). The researchers found that the traditional Chinese medicine "Astragaloside IV" has a significant therapeutic effect on precancerous lesions in mice, and it has been observed that the expression of autophagy-related genes in mice is gradually downregulated with the improvement of precancerous lesions. The results of this study clearly suggest that autophagy plays an important role in the progression of chronic atrophic gastritis to intestinal metaplasia or heterosexual hyperplasia, but the specific mechanism needs further study.

(2) Autophagy and gastric cancer

① There are many reports that the expression of autophagy-related genes in gastric cancer tissues is significantly upregulated: studies have used immunohistochemistry to detect the expression of LC3A in 188 patients with gastric cancer and adjacent tissues. The results suggest that LC3A is significantly expressed in gastric cancer tissues. Increased and high expression of LC3A is associated with increased risk of recurrence after radical resection of gastric cancer, and is also associated with decreased survival of gastric cancer. The above studies suggest that autophagy is activated during the development of gastric cancer. ② Autophagy can promote the progression of gastric cancer (growth and metastasis): autophagy can protect tumor cells from damage by exercising normal autophagy. Autophagy separates mitochondria and other organelles in cells, which can effectively prevent the spread of the apoptotic factor in the cell and help the tumor cell escape the threat of apoptosis. Studies have confirmed that the use of the anticancer agent MHY218 in gastric cancer can inhibit the progression of gastric cancer by inhibiting autophagy. Another study reported that autophagy promotes gastric cancer progression by activating

AKT phosphorylation and inhibiting PI3K-AKT signaling pathway in human gastric cancer cell lines. Meanwhile, some studies have found that the upregulation of ATG5 with the emergence of drug resistance, the formation of residual cancer stem cell resistance, often lead to reduced sensitivity to certain drugs, and cause tumor recurrence and even metastasis. ③ However, autophagy also can inhibit the formation of early tumors in gastric cancer: in general, autophagy plays a role in inhibiting the development of cancer by limiting inflammatory reactions, tissue destruction, and genomic instability. When autophagy is activated, it inhibits the growth of gastric cancer cells and induces apoptosis. Studies have shown that NF-κB-specific inhibitors can effectively promote the death of gastric cancer cells by upregulating the expression of LC3 and Beclin1, so the anti-inhibition between NF-κB inhibitor, autophagy activation, and apoptosis may become a new direction for the diagnosis and treatment of gastric cancer.

38.2.2 Autophagy in Chronic Non-atrophic Gastritis and Special Gastritis

38.2.2.1 Autophagy and Chronic Non-atrophic Gastritis

According to the consensus opinions on chronic gastritis in China, chronic non-atrophic gastritis (CNAG) is the most common type of chronic gastritis diagnosed by upper gastrointestinal endoscopy in China, accounting for 49.4% of all types of chronic gastritis. The pathological features of CNAG include chronic non-atrophic inflammatory lesions in the gastric mucosa, which are mainly infiltrated by lymphocytes and plasma cells in the gastric mucosa.

H. pylori (Hp) infection is the main cause of CNAG. In fact, CNAG can be regarded as an infectious disease to some extent. It is currently believed that autophagy plays an important role in Hp infection as well as gastric mucosal epithelial injury and repairing (see Sect. 3 of the current chapter for details).

Non-steroidal anti-inflammatory drugs (NSAIDs)-related gastric mucosa injury is also the common factor of CNAG. According to the classical theory, NSAIDs reduce the biosynthesis of prostaglandin (PG) of gastric mucosa by reducing the activity of cyclooxygenase (Cox), leading to gastric epithelial injury. However, recent studies have shown that autophagy-related gastrointestinal epithelial cell death may be an important way of NSAIDs-related mucosa injury. Lee et al. found that the autophagy protein 5 (ATG5) level in gastric mucosa was significantly upregulated after 6 h of indomethacin treatment. Within 24 h, the expression of autophagy markers such as microtubule-associated protein light chain 3B-II (LC3B-II) and Beclin-1 was also significantly increased, which led to autophagy and death of gastric epithelial cells. In addition, Smad-7 plays a protective role in indomethacin-induced gastric epithelial autophagy. Overexpression of Smad-7 inhibits autophagy and the expression of LC3B-II by activating the p38-MAPK signaling pathway. Moreover, Lee et al. also

found that indomethacin-induced gastric mucosa injury was significantly alleviated after the treatment of autophagy inhibitor chloroquine. Another study demonstrates that compared with wild type mice, gastric mucosal epithelial damage of gastrointestinal mucosa epithelial cells-specific *Atg5*-knockout mice was significantly alleviated after indomethacin induction, accompanied by enhanced ERK/Nrf2/HO-1 pathway activity (Harada et al. 2015). These evidences show that autophagy plays a key role in the damage of gastric epithelial cells in CNAG caused by NSAIDs such as indopexin, in which decreased activity of Smad-7 and ERK/Nrf2/Ho-1 pathways may be an important mechanism involved.

38.2.2.2 Autoimmune Gastritis and Autophagy

Autoimmune gastritis is a chronic gastritis caused by autoimmune mechanism. Due to the presence of antibodies against endogenous antigen of gastric tissue, such as anti-internin antibody, anti-parietal cell antibody, anti-gastrin-secreted cell antibody, the corresponding tissue damage or dysfunction can lead to vitamin B12 absorption disorders, gastrin secretion disorders, decreased gastric acid secretion, and so on (New and Thomas 2019).

Autoimmune gastritis is a rare chronic gastritis with low incidence in China. Recent studies have found that IFN-γ can directly attack gastric epithelial cells, playing an important role in the pathogenesis and destruction of autoimmune gastritis. In 3D culture model of gastric somatic cell organs (gastroids culture), the IFN-γ containing supernatant of the immune cells culture medium could lead to the death of gastroids by its cytotoxicity. Therefore, the damage of gastric epithelial cells in autoimmune gastritis is closely related to IFN-γ. Recently, some scholars have further found that IFN-γ could trans-activate the transcription of Beclin-1 and upregulate the expression level of LC3B-II, thus to initiate the autophagy process of gastric epithelial cells by increased formation of autophagosomes. Moreover, the lysosomal turnover of gastric epithelial cells-specific IFN-γ overexpression mice was blocked when treated with the autophagy inhibitor chloroquine. These results suggest that IFN-γ-mediated autophagy may be an important pathogenesis of autoimmune gastritis, but direct evidence is still lacking. The relationship between autoimmune gastritis and autophagy remains to be further explored.

38.2.2.3 Special Types of Chronic Gastritis and Autophagy

In the Kyoto classification of gastritis, the special types of gastritis mainly include Ménétrier disease, allergic gastritis, lymphocytic gastritis, and eosinophilic gastritis. The mucosal lesions caused by these types of chronic gastritis are often associated with immune cell activation and inflammatory cytokine attack. Although there is no literature that directly aim to the role of autophagy in the occurrence and development of the above special types of gastritis, as an important mechanism that mediate and regulate inflammatory responses, autophagy may still participate in the damage process of gastric mucosa and submucosa by specific or non-specific manner.

For example, lymphocytic gastritis is closely related to Hp infection. Its pathological feature is the lymphocytes accumulation within gastric mucosa epithelium and pit cells, which is related to the high concentration of CXCL or CCL family chemokines in this area. The autophagy-dependent secretion may be the crucial mechanism for the enrichment of chemokines in these immune cells. The immune cell chemotaxis mechanism may also exist in allergic gastritis and eosinophilic gastritis that are associated with immune cell aggregation. Moreover, the pathogenesis of Ménétrier disease is closely related to cytomegalovirus infection or TGF-α secretion. Studies have shown that TGF-α may promote autophagy and regulate apoptosis by increasing LC-3 II/LC-3 I ratio. However, the above hypothesis still needs to be verified through in-depth studies in specific animal models or patient tissues.

In conclusion, the studies on the mechanism of autophagy in chronic non-atrophic gastritis and special gastritis are scarce. For these gastritis is closely related to exogenous factors such as Hp infection and cytomegalovirus infection, as well as endogenous factors such as immune cell aggregation and activation, it is very important to study the mechanism of autophagy in this field. The elucidation of the pathogenesis of the special type of gastritis is also helpful to change the current hormone-based clinical treatment, so as to truly benefit the patients.

38.2.3 Role of Autophagy in the Pathogenesis of Inflammatory Bowel Disease

38.2.3.1 Introduction

Inflammatory bowel disease (IBD) results from a complex series of interactions between susceptibility genes, the environment, and the immune system, including ulcerative colon (ulcerative colitis, UC) and Crohn's disease (Crohn's diseases, CD) and undifferentiated colitis (intermediate colitis, IC). Recent studies have shown that the pathogenesis of IBD is closely related to environment, genetics, immunity, microbial infection, and other factors, which leads to immune abnormalities, intestinal flora disorders, oxidative stress effects, and ultimately the occurrence and development of IBD (Baumgart and Sandborn 2012). Current studies suggest that the pathogenesis of IBD may be related to abnormal autophagy in addition to excessive inflammation, changes in immune response, and imbalance of intestinal flora. With the development and application of genome wide association studies (GWAS), more than 160 genetic loci associated with the pathogenesis of IBD have been identified. Single-nucleotide polymorphisms (SNP) of various autophagy-related genes have been found to be related to the sensibility of IBD, making people begin to pay attention to the relationship between autophagy and IBD.

38.2.3.2 IBD-Relevant Autophagy Genes

Studies have found that the pathogenesis of IBD is closely related to gene polymorphism, such as Crohn's disease susceptibility genes NOD2(CARD15), ATG16L1, IR-GM and IL-23R. ATG16L1 and IRGM are autophagy-related proteins, which are closely related to the occurrence and development of IBD.

(1) ATG16L1: ATG16L1 is a key protein in the process of autophagosome formation, located on human chromosome 2q37.1, which plays an important role in the immune response induced by intracellular pathogens and is mainly expressed in intestinal epithelial cells and lymphocytes of the small intestine and colon. ATG16L1 can interact with ATG12 and ATG5 to form atg12-atg5-atg16l1 conjugated complex, and recruit microtubule-associated protein 1 light chain 3 (microtubule-associated protein 1 light chain 3), which can be connected with phosphatidyl ethanolamine and contribute to the extension of autophagy membrane and the formation of autophagosome. The single-nucleotide polymorphism (SNP) sites of ATG16L1 gene are strongly associated with CD, and are important risk factors of CD pathogenesis. Mice with ATG16L1 deficiency showed more sensitization to colitis induced by sodium glucan sulfate (DSS), and showed significantly more leukin (IL-1 and IL-18) than the control group.

The effects of ATG16L1 genetic polymorphism on CD are mainly reflected in the abnormal cell morphology and secretion of Paneth, the defective clearance of intracellular pathogens by intestinal epithelial cells (IEC), and the promotion of inflammation. Paneth cells are characteristic cells of the small intestine, secreting antibacterial substances such as defensin and antibacterial peptide, which play an important role in the innate immune process and inflammatory response of the mucosa. Studies have found that when the expression of ATG16L1 gene in mice is inhibited, the number of particles in Paneth cells in the intestinal tract decreases and the morphology is abnormal. Similarly, Paneth cells of CD patients with ATG16L1 gene mutation also have similar changes. All the above studies indicate that ATG16L1 has a direct impact on the function of Paneth cells.

Saitoh et al. studied the mechanism of ATG16L1 in the regulation of inflammation, and found that ATG16L1 can regulate the activation of endotoxin-mediated inflammatory complexes through the autophagy process, and further regulate the production of proinflammatory cytokines interleukin-1 and il-18. Nguyen et al. studied the regulatory mechanism of ATG16L1. Under physiological conditions, upregulation of mir-30c, mir-130a, mir-106b, and mir-93 can reduce the level of ATG16L1 and inhibit autophagy, thereby blocking autophagy-dependent intracellular bacterial clearance. Lassen et al. found that the inflammatory environment of CD induces cell stress and apoptotic protease activation, which in turn increases caspase-3-mediated ATG16L1 lysis, reduces ATG16L1 level and leads to autophagy abnormalities. ATG16L1 not only plays a role in the process of autophagy, but also plays an important role in other related pathways, which is expected to be a new target for the future treatment of CD.

(2) NOD2/CARD15: NOD2, the first CD-related susceptibility gene, belongs to the NOD like receptor family and is named as CARD15. It is an important pattern recognition receptor that can recognize pathogen-related molecular patterns and thus play a role in innate immunity. NOD2 recognizes the cell wall of bacteria acyl dipeptide, induction of inflammatory corpuscle NLRP 3 formation, activation of MAPK, downstream the nf-kappa B pathway, then raise structure through own caspase activation domain (caspase-activating and recruitment domain, CARD) recruitment and activation of caspase 1, release of inflammatory factors such as IL-1β and IL-18, further causing an immune reaction. NOD2 plays an important role in the autophagy clearance of bacteria, but this process requires the participation of ATG16L1, which is manifested in the interaction between NOD2 and the WD40 domain of ATG16L1 through the CARD domain, thus inducing the autophagy process of cells against the invading pathogen. After NOD2 recognizes intracellular bacteria, ATG16L1 can be collected to the entrance of bacteria in the plasma membrane, while mutation NOD2 cannot complete the above process, resulting in impaired autophagy clearance of bacteria. Sorbara et al. found that ATG16L1 knockout can promote NOD2-induced proinflammatory cytokines production through autophagy-independent pathway, suggesting a close relationship between ATG16L1 and NOD2.

In European and American populations, the common SNP loci of NOD2/CARD15 gene currently mainly include rs2066844 (R702W), rs2066845 (G908R), rs41450053 (L1007fsC), etc. Seidere study found that the risk of CD of the mutant homozygous NOD2 is 20–40 times higher than that of normal people, and the onset age is younger, and the clinical manifestations are more serious, and the probability of ileal stenosis and the need for surgical intervention is significantly increased. However, the above SNP loci were not found to be significantly associated with IBD in Asian and southern African countries, and the incidence of IBD in these countries was much lower than that in northern European and North American countries, which may also confirm the promoting effect of abnormal NOD2/CARD15 gene on the incidence of IBD.

(3) IRGM: IRGM gene is located on human chromosome 5q33.1, and its encoded protein belongs to the immune-related GTPase family, which is expressed in a variety of human cells, and is related to the processes of allogeneic autophagy, proinflammatory cytokine production and apoptosis, etc., playing an important role in the body's immunity. McCarroll showed that the polymorphism of IRGM gene was closely related to CD through GWAS analysis, such as rs10065172 and rs13361189. Liu et al. found that the intestinal Paneth cells of IRGM knockout mice had abnormal localization and morphological changes of secretory particles, and such mice were more susceptible to dss-induced colitis. The above studies suggest that IRGM variation may affect the formation of autophagosomes and the function and morphology of Paneth cells, thereby promoting the occurrence of intestinal inflammation. Studies have found that cd-related SNP can be found at the missing sites upstream of IRGM, and its deletion is correlated with the risk of CD. Subsequent studies have confirmed that mir-196 with

high expression in inflammatory intestinal epithelial cells of patients with CD protects the body by downregulating the expression of IRGM gene. In addition, NOD2 and IRGM interact with each other. NOD2 can promote the oligomerization of IRGM, and promote the interaction between IRGM, and the autophagy regulating molecules ULK1 and BECN1.

38.2.3.3 Pathology and Pathogenesis of IBD

(1) Autophagy and innate immunity

Autophagy is an important part of innate immunity, which can decompose damaged organelles and proteins, decompose pathogens to eliminate them, activate innate immune cells, and regulate innate immune functions. Studies on Paneth cells in the small intestine have shown that Paneth cells can secrete antimicrobial substances to resist intestinal bacterial infection. When the expression of ATG16L1 gene in mice is restricted, the number of particles in Paneth cells decreases and the morphology is abnormal, indicating that ATG16L1 plays an important role in the exocrine process of Paneth cells. Multiple studies have shown that defects in the autophagy process can lead to excessive secretion of IL-1, IL-6, and IL-18. The mechanism is that autophagy-deficient cells produce high levels of reactive oxygen species (ROS) and activate caspase-1, which is dependent on TIR binding protein (Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF), leading to the increase of IL-1 and IL-18. In addition, ATG16L1 knockout mice are more likely to be glucohexaose sodium sulfate (dextran sulfate sodium, DSS)-induced acute colitis, which show the ulcer and lymphocyte infiltration is aggravating, serum inflammatory cytokines IL-1β, IL-6, IL-18. Remission can be achieved by injecting antibodies against IL-1β and IL-18. Intracellular abundance of IL-1β, IL-6, and IL-18 induces apoptosis and inflammatory responses in a range of responses.

(2) Autophagy and adaptive immunity

Autophagy plays an important role in adaptive immune regulation. An important bridge between innate and adaptive immunity is the antigen-presenting cell (APC). In histocompatibility complex-II (MHC-II)-dependent APC, autophagy plays an important role in the process of antigen processing and presentation. ATG16L and IRGM mutations can be involved in the occurrence of CD through the following mechanisms. First, intracellular soluble antigens can be degraded into antigenic peptides by autophagy, recognized by MHC class II molecules and presented to T cells to activate adaptive immunity. Autophagy defects can affect bacterial antigen peptide presentation. Second, the body normally maintains the homeostasis of the immune response by regulating the number of T cells. The number of T cells increased with antigen stimulation and decreased with antigen removal. The sustained activation of Th1, Th2, and Th17 at CD may be related to the autophagy disorder and the decreased ability of the body to regulate the duration and intensity of the adaptive

immune response by controlling the survival time of T cells. Overstabilization of the immune synapse may be one of the mechanisms responsible for the excessive activation of T lymphocytes. Knockout of the expression of ATG16L1 and IRGM in dendritic cells (DCs) causes too close interaction between DCs and T cells, resulting in stable immune synapses and increased activation of T cells, especially Th17 cells. Immune synaptic overstabilization was also observed in DCs isolated from CD patients with ATG16L1 mutation. Adaptive immunity may be one of the reasons for CD susceptibility in ATG16L1 mutation. Third, autophagy defects may reduce the immune tolerance of the body to intestinal symbiotic bacteria and autoantigens, resulting in enhanced intestinal adaptive immunity. Autophagy can not only deal with exogenous antigens, but also remove apoptotic and necrotic autosomal cells, so as to prevent the occurrence of autoimmunity. In addition, autophagy promotes the proliferation and survival of memory T cells by removing excess mitochondria. In the ATG5 deficient mouse model, the number of memory T cells was greatly reduced, and the mice showed enteritis, indicating that autophagy plays an important role in the process of immune homeostasis.

(3) Autophagy and endoplasmic reticulum stress

Endoplasmic reticulum is an important cell that is involved in the folding, modification, transport, and function of intracellular proteins. Under the stimulation of various stress factors, the normal function of endoplasmic reticulum can be affected, resulting in unfolded or misfolded proteins. This process is called endoplasmic reticulum stress. Intestinal endoplasmic reticulum stress can lead to abnormalities in the function of Paneth cells and goblet cells (such as antimicrobial peptides and mucous eggs), and damage the barrier of normal intestinal antimicrobial and mucous proteins, thereby causing continuous inflammatory stimulation and promoting the occurrence of IBD. Autophagy helps to degrade the abnormally folded proteins produced by endoplasmic reticulum and maintain homeostasis. Endoplasmic reticulum stress and NF-kB and TNF-signaling pathways increased in autophagy-deficient mice, leading to spontaneous ileitis similar to CD. These results suggest that autophagy and ER stress may be closely related to the occurrence and development of IBD.

(4) Autophagy and intracellular bacterial infection

Autophagy is one of the immune defense mechanisms of the body to eliminate invading bacteria, and the deficiency of autophagy will affect the cells to remove invading pathogens. In addition to promoting adaptive immune clearance of infection through the mhc-ii presentation pathway, autophagy can also be directly cleared by phagocytosis. Intestinal flora imbalance and persistent bacterial infection may be the cause of IBD. Adherent-invasive Escherichia coli (AIEC) infection was identified in CD patients with ileum involvement. Lapaquette et al. found that autophagy at the physiological level can effectively inhibit the proliferation of AIEC, while there is a large amount of AIEC proliferation in cells with defective IRGM and ATG16L1 genes. Murthy et al. conducted an in-depth study on the mechanism of ATG16L1 mutation leading to bacterial clearance obstacles, and found that caspase3 played an important

role in this process, and the ATG16L1 T300A mutation made it degraded by caspase3. Without activation of caspase3, the autophagy level of the mutant ATG16L1 T300A is not affected. Caspase3 can be activated after the stress state caused by Yersinia enterocolitica infection or the binding of TNF- with the death receptor on the cell membrane, leading to the decomposition of ATG16L1 T300A and the decrease of the autophagy level. The increased secretion of TNF- and il-1 during *E. coli* infection may explain why anti-TNF—therapy is effective in patients with Crohn's disease. The reason may be that TNF—can activate caspase3 to degrade ATG16L1, which instead leads to more TNF—secretion. Studies have shown that AIEC can upregulate the microRNA-30c and microRNA-130a levels of intestinal epithelial cells through the NF-kb pathway, thereby reducing the expression of ATG16L1 and ATG5, inhibiting the autophagy process, and contributing to the survival and further invasion of AIEC. All the appeal studies demonstrated the relationship between autophagy defects and AIEC infection and the pathogenesis of C. Autophagy defects lead to decreased clear ability of intracellular bacteria, persistent intracellular infection, recruitment of more inflammatory cell infiltration, and excessive secretion of cytokines to form chronic granuloma, all of which may be related to the pathogenesis of CD.

(5) Autophagy and abnormal function of Panth cells

Panth cells are a kind of cells located at the base of intestinal gland. As an important part of intestinal antimicrobial biological barrier, they can dissolve the cell wall of intestinal bacteria by secreting bacteriolytic enzymes, defense elements, and other bactericidal substances. Some scholars found that the ability of Panth cells to secrete bactericidal substances in ATG16L1 and ATG5 deficient mice was significantly decreased. In CD patients with ATG16L1 defect, the secretory function of Panth cells is abnormal, which may change the normal intestinal flora by weakening the intestinal antimicrobial biological barrier and cause intestinal injury and chronic inflammation.

(6) Autophagy and intestinal mucosal barrier function

The intestinal mucosal barrier is composed of four parts: mechanical barrier, biological barrier, chemical barrier, and immune barrier, with the function of preventing bacterial and endotoxin migration and pathogenic substances from entering the systemic circulation. Any part of the defect or damage may lead to intestinal homeostasis imbalance, causing intestinal flora migration, enterogenous infection and other diseases. Intestinal mucosal barrier dysfunction is an important basis for the pathogenesis of IBD. Studies have found that autophagy mediates intestinal mucosal barrier injury in IBD patients through multiple pathways, such as regulating the tight connection between intestinal epithelial cells, regulating the expression of inflammatory signals, regulating endoplasmic reticulum stress, and participating in the clearance of pathogenic microorganisms. Nighot et al. found that autophagy could induce the degradation of claudin-2 perforin, thus enhancing the function of intestinal mucosal barrier. Xavier et al. demonstrated that IFN-gamma and other helper T cell type 1 (Th1) cytokines secreted during bacterial infection can induce autophagy, while Th2

cytokines inhibit autophagy. The role of inflammatory cytokines in the pathogenesis of IBD is clear. Autophagy can regulate the secretion of proinflammatory factors and anti-inflammatory factors in both directions, thus participating in the regulation of intestinal mucosal inflammation. Wang's study indicated that ER stress can initiate autophagy in Paneth cells through various pathways. Autophagy defects can lead to excessive activation of endoplasmic reticulum stress in DSS-induced colitis animal models and IBD patients, thus aggravating the severity of IBD (Kaser and Blumberg 2014).

38.2.3.4 Role of Autophagy in IBD and Future Prospects

The pathogenesis of inflammatory bowel disease is complex, and the etiology has not been fully elucidated. Studies have shown that abnormal autophagy may be involved in the pathogenesis of IBD. The regulatory role of autophagy in IBD is also interactive. Under normal conditions, autophagy-related proteins interact with intestinal mucosal immunity and mucosal barrier to jointly maintain intracellular homeostasis. When cells are stimulated by the outside world, the intracellular equilibrium state is broken, and a series of signaling factors are stimulated to regulate autophagy or endogenous protective substances, so as to exert endogenous regulation, promote metabolism, and jointly maintain the survival of cells. When the external stimulation is too strong, the dynamic balance of cells is broken, leading to the occurrence of disease. How to reasonably and effectively induce or inhibit autophagy to maintain or repair the function of intestinal mucosal barrier, protect the integrity of intestinal mucosal barrier, maintain intestinal homeostasis, and thus prevent and control IBD still needs further research.

38.3 Autophagy and Gastrointestinal Infections

38.3.1 Autophagy and Gastrointestinal Bacterial Infectious Diseases

38.3.1.1 Autophagy and Chronic Infection of *H. pylori*

H. pylori is a kind of engraftment in the stomach and duodenum gram-negative, micro-aerobic bacteria can secrete a variety of virulence factors such as urease and lipopolysaccharide, adhesion factor, cytotoxin genes (CagA), a protein cavitating toxin (VacA), such as peptic ulcer, chronic gastritis, the stomach with people closely related diseases such as malignant tumor. At least 75% of gastric cancer is due to *H. pylori* infection, so *H. pylori* is classified as class I carcinogen. Some studies have found that *H. pylori* can re-enter the extracellular environment after completely eliminating extracellular bacteria with gentamicin, suggesting that *H. pylori* is not

only an extracellular pathogen, but also a facultative intracellular bacteria. It can survive not only in epithelial cells, but also in immune cells. *H. pylori's* sojourn in host cells not only increases its resistance to antibiotics, but also enables it to escape the attack of humoral immunity, which may be closely related to the continuous infection of *H. pylori* in the body.

(1) *H. pylori* can induce autophagy

VacA is one of the important substances that induces autophagy. Autophagy was induced by co-culture of purified VacA toxin with AGS cells. On the other hand, autophagy degrades VacA and reduces the toxicity of *H. pylori*. HP0175 is a kind of peptide-based proline isomerase of *H. pylori*. The study of Halder et al. (Halder et al. 2015) found that compared with the cells treated by the wild strain, AGS infected by the mutant strain that eliminated HP0175 showed decreased autophagy, suggesting that HP0175 can upregulate the expression of autophagy-related genes. This may play an important role in autophagy mediated by unfolded protein reactions.

(2) *H. pylori* can also inhibit the occurrence of autophagy

Tanaka et al. (2017) conducted microarray analysis of autophagy-related genes (ATG) in the gastric mucosa of 266 patients infected with *H. pylori*, and found that 16 genes were upregulated and 9 genes were downregulated. Among them, the level of ATG16L1 mRNA of autophagy core component was significantly downregulated, and it was negatively correlated with the colonization density of *H. pylori* and the atrophy degree of gastric mucosa. It suggests that *H. pylori* infection can provide a good living environment for *H. pylori* colonization and promote the cytotoxicity of *H. pylori* by inhibiting autophagy. Microtubule-associated protein 1 light chain 3 (MAP1LC3A) is the main regulator of autophagosome formation. Muhammad et al. found that MAP1LC3A variant strain 1 (MAP1LC3Av1) was methylated and silenced in the gastric cancer tissues infected by *H. pylori* and adjacent non-cancerous tissues, but not in the gastric tissues infected by *H. pylori*. In addition, in vitro studies, MAP1LC3Av knockdown cells showed stronger proliferation and invasiveness. All the above evidences suggest that the inactivation of MAP1LC3Av1 destroys the autophagy pathway, which may lead to carcinogenesis of gastric epithelial cells.

38.3.1.2 Autophagy in Intestinal Tuberculosis

Intestinal tuberculosis (ITB) is a chronic intestinal-specific infection caused by Mycobacterium tuberculosis (MTB). Once occurs, often leads to abdominal pain, diarrhea, hematochezia, and other non-specific gastrointestinal clinical manifestations. Macrophages are the main target cells of MTB during the occurrence and development of ITB, as well as the main defense cells of the body. Autophagy, as an important immune regulatory mechanism, can participate in the intestinal infection and defense process of MTB from multiple aspects, and is also reversely regulated by MTB.

(1) Mechanism of autophagy promoting MTB clearance

Autophagy can directly promote MTB clearance. It has been found that the use of vitamin D and lipopolysaccharide can induce autophagy of macrophages and enhance the direct clearance of MTB by macrophages. On the one hand, autophagy activation can promote the maturation of MTB phagocytes in macrophages, which is conducive to MTB clearance. On the other hand, autophagy plays a bactericidal role by promoting the secretion of antimicrobial peptides in lysosomes.

(2) Activation of the natural immune system in the progression of intestinal tuberculosis is involved in the occurrence of autophagy

During the occurrence of intestinal tuberculosis, activation of the natural immune system can induce autophagy of macrophages. After the invasion of MTB into the intestinal tract, macrophages recruit and activate the infected area, and recognize the pathogen-associated molecular patterns (PAMP) of MTB through the pattern recognition receptor (PRR) on its surface, thus activating autophagy and initiating immune defense. Among them, toll-like receptor (TLRs), c-type lectin receptor, scavenger receptor, NOD1 and NOD2 receptor in the PRR family of macrophages are all involved in the recognition of MTB by macrophages, and then initiate autophagy to resist the infection of MTB. Studies have found that TLR4 on the surface of macrophages can recognize lipids, glycoproteins, and secreted proteins in the cell wall of MTB, activate downstream signaling pathways, and promote beclin-1 to form initiation complexes with PI3KC3 and PI3P, thus forming autophagy initiation reactions. PI3KC3 complex plays an important role in the formation and maturation of autophagosomes and promotes the clearance of MTB. Meanwhile, activation of TLR4-related signaling pathway can regulate the stability of autophagy-related signaling molecules beclin-1 and ULK1 through TRAF6, thereby regulating autophagy. Other studies have found that in macrophages infected with MTB, NOD2 receptors on the surface of macrophages can activate downstream signaling pathways by recognizing bacterial MDP, and produce a large number of inflammatory factors such as il-1b and tnf-a, inducing the expression of autophagy markers such as LC3, thereby promoting the occurrence of autophagy (Shaw et al. 2011).

(3) MTB can also reverse regulate the occurrence of autophagy

MTB infection can inhibit autophagy by inducing activation of mTOR signaling pathway. In addition, MTB can express autophagy inhibiting factors such as MTB Eis protein, which can specifically regulate the activity of JNK and inhibit the activation of beclin-1, thus inhibiting the occurrence of autophagy. In addition, at the early stage of MTB infection, antigenic target proteins can be secreted to block the maturation of phagocytes and their fusion with autophagic lysosomes.

38.3.2 Autophagy and Enterovirus Infectious Diseases

Autophagy plays an important role in the process of virus infection. Autophagy can eliminate viruses by degrading viruses, presenting viral antigens and activating the immune response. Meanwhile, viruses can also evade autophagy and maintain their own survival and replication. RNA viruses, in particular, can rapidly alter the genome and evolve, thus inhibiting the autophagy of host cells, which is conducive to the replication of viruses themselves.

Human enteroviruses belong to the enterovirus genus of the small ribonucleic acid viridae family, including poliovirus, coxsackievirus, Ecovirus, and new enteroviruses. There are more than 100 kinds or subspecies of viruses. Poliovirus, Ecovirus 7, Coxsackievirus A16, Coxsackievirus B3, Coxsackievirus B4, and enterovirus 71 infections have been shown to promote autophagy formation, and autophagy promotes viral self-replication and protein expression.

38.3.2.1 Viral Infection and Cell Autophagy

(1) The mechanism of autophagy induced by virus

Virus is an intracellular infectious microorganism, and autophagy is an effective process to maintain cell homeostasis. Several events in viral replication, such as receptor interaction and endoplasmic reticulum stress, can trigger downstream autophagy. It has been found that GTPase family M protein (IRGM) is a common target protein of five RNA viruses, such as retroviruses, Flaviviridae, paramyxoviruses, orthomyxoviruses, and tunica viruses, which interact with autophagy-related proteins. When the expression of IRGM was inhibited, autophages induced by virus particles such as measles virus, human immunodeficiency virus type 1, and hepatitis C virus decreased significantly.

① Endoplasmic reticulum stress is involved in the activation of autophagy pathway. Endoplasmic reticulum stress can prevent proteins from entering the endoplasmic reticulum by processing misfolded proteins in the endoplasmic reticulum through unfolded protein response (UPR). UPR is the main protective and compensatory mechanism of endoplasmic reticulum stress. Unfolded protein response has three signaling pathways: protein kinase-like endoplasmic reticulum kinase (PERK), endoplasmic reticulum transmembrane protein inositol-requiring enzyme 1 (IRE1) and transcription activator 6 (ATF6). When the virus is infected, a large number of viral proteins are synthesized in the cells, which increases the burden of endoplasmic reticulum, and then increases the accumulation of unfolded proteins and misfolded proteins to cause unfolded protein reaction, thus activating endoplasmic reticulum stress in host cells and inducing autophagy.

② Receptor interaction Virus binding to cell surface receptor can directly induce autophagy and initiate cell anti-infection mechanism. CD46 receptors are ubiquitous on the cell surface and can bind to a variety of viruses to induce autophagy.

CD46 can be divided into two domains, Cyt-1 and Cyt-2, according to the carboxyl end. CD46-Cyt-1 can induce autophagy by interacting with the skeleton protein GOPC and coupling with the autophagy initiation complex Vps-34/Beclin. It has been reported that measles virus can induce autophagy through CD46-Cyt-1/GOPC pathway by binding to CD46 molecule on cell membrane. Human immunodeficiency virus type 1 envelope glycoprotein gp120 and cardiomyocyte *N*-methyl-D-aspartate receptor (NMDA) induce autophagy, which involves c-JUN amino-terminal kinase (JNK) and PI3K.

(2) Interaction between Virus and Cell Autophagy

① The antiviral effect of autophagy: Autophagy is the process of maintaining homeostasis in cells. Viral infection can lead to the disorder of intracellular environment. When the virus is infected, the body will activate the autophagy mechanism to resist the virus infection. Autophagy can activate innate immunity by transporting viral nucleic acid to intracellular receptors and presenting viral antigens to MHC-I and MHC-II molecules to activate adaptive immune response. At the same time, autophages can transfer viruses from cytoplasm to lysosomes, fuse with lysosomes, phagocytize and degrade viruses.

② Cell autophagy promotes virus infection: Autophagy is an inherent metabolic process in eukaryotic cells. However, in the process of virus infection and evolution, viruses may adapt to cell autophagy through some mechanisms, which is conducive to their own replication. Viruses may adapt to autophagy in the following ways: (1) Autophages can be the place where viruses replicate. The formation of viral replication sites often leads to membrane rearrangement and cytoskeleton remodeling. Similar rearrangements occur during the formation of aggregates and autophages in cells to promote protein degradation. Autophagy provides an assembly platform for the replication complexes of positive-stranded RNA viruses; (2) Autophagy is beneficial to the replication and expression of viral genes. Non-structural protein 4 (NSP4) of rotavirus releases calcium ions from endoplasmic reticulum into cytoplasm, activates calcium ion/calmodulin kinase-β and 5′-adenosine monophosphate-activated protein kinase (AMPK) signaling pathways, thus initiating autophagy and transporting viral proteins from endoplasmic reticulum to viral replication through autophagic membrane transport process, causing rotavirus infection.

38.3.2.2 Human Enterovirus and Autophagy

(1) Coxsackievirus and Autophagy

When coxsackievirus B3 (CB3) infects Hela and HEK293T cells, the expression of GFP-LC3 increases, and the proportion of LC3-II/LC3-I increases, suggesting that

CB3 infection induces autophagy formation. However, autophagy-mediated protein degradation marker p62 did not change significantly after CB3 infection, suggesting that CB3 infection did not promote protein degradation in lysosomes. Autophagy promotes viral replication in CB3 infection. Rapamycin and starvation enhanced CB3 replication, while CB3 replication products decreased when 3-MA or RNA interfered with the expression of Beclin 1, Vps34, and Atg7. BPIFP3, a novel autophagy regulator, has been reported to inhibit CB3 replication by inhibiting key links in the autophagy process. When BPIFP3 is absent, CB3 replication is greatly enhanced. Receptor-Interacting Protein Kinase-3 (RIP3) is a necrosis regulator, which promotes autophagy formation during CB3 infection. In the late stage of CB3 infection, RIP3 is cleaved by CB3-encoded cysteine protease 3c, eliminating RIP3-mediated necrosis signal, inducing non-necrotic cell death, inhibiting cell necrosis and benefiting CB3 from autophagy regulation.

Infection of primary rat neurons with Coxsackievirus B4 (CB4) induces autophagy and LC3-II accumulation; 3-MA inhibits autophagy by inhibiting the activation of calpain, thus reducing CB4 replication. Coxsackievirus A16 (CA16) infection has been shown to promote autophagy formation and enhance self-replication through autophagy (Song et al. 2018). The expression of CA16 non-structural virus protein 2C can enhance the activation of IRGM promoter and induce autophagy. In addition, CA16 infection can inhibit the AKT/mTOR signaling pathway that negatively regulates the formation of autophages, and activate the extracellular regulated protein kinase (ERK) signaling pathway to induce autophagy.

(2) Poliovirus and Autophagy

Poliovirus (PV) induces the formation of intracellular vesicles at the early stage of infection and bilayer membrane vesicles at the late stage of infection. These bilayer vesicles and autophages exhibit many similar characteristics, including the division of bilayer membranes enclosing the intracytoplasmic cavity, the acquisition of intracellular labeled lysosome-associated membrane protein-1 (LAMP-1) and the aggregation of host protein LC3. The GFP-LC3 construct was used to study the vesicles induced by PV. The results showed that the GFP-LC3 signal was co-localized with the PV3A protein and the PV double-stranded RNA replication intermediates. At the early stage of PV replication, when PV2BC and 3A proteins were co-expressed in 293T cells of human embryonic kidney (HEK), the co-localization of autophagy marker GFP-LC3 and LAMP-1 indicated that PV infection-induced autophagy maturation. When autophages are transported to lysosomes, vesicles acidify, and PV proliferates vigorously in mature acidic vesicles, and vesicle acidification can promote the maturation of infectious PV particles.

(3) Ecovirus 7 and autophagy

When E7 enters Caco-2 polarized intestinal epithelial cells through endocytosis, autophagy-related proteins are required. Silencing autophagy-related genes such as Atg12, Atg14, Atg16, Beclin1, and LC3 had no effect on the adsorption of virus on cell surface, but could affect the upstream mechanism of E7 dehulling, suggesting that autophagy played an important role in the viral penetration stage.

(4) Enterovirus 71 and autophagy

Enterovirus 71 (EV71) infection promotes the occurrence of autophagy. With the prolongation of infection time and the increase of infection dose, the level of autophagy gradually increases. Autophagy inducer rapamycin enhanced EV71 replication, while autophagy inhibitor 3-MA inhibited EV71 replication. Non-structural protein 2BC of EV71 can trigger autophagic lysosome formation and facilitate virus replication. After blocking autophagic lysosome production with chloroquine, the titer, copy, and protein of EV71 decreased. It has been reported that Beclin1, Vps34, NGLY1, and VCP, which are beneficial to autophagy formation, promote EV71 replication. EV71 can induce multiple autophagy steps to complete its own replication.

(5) Human cytomegalovirus and autophagy

Human cytomegalovirus (HCMV) belongs to herpes virus and has a high infection rate in the population. About 90% of the population showed HCMV positive reaction. Although HCMV infection in healthy individuals is usually asymptomatic, for individuals with immunodeficiency, HCMV is the main cause of disease and death. At the same time, HCMV has the characteristics of latency-activation of herpes virus, and can exist in host cells for a long time. HCMV can evade the antiviral mechanism of autophagy by expressing a variety of specific anti-autophagic proteins. However, in order to replicate, proliferate, and establish latent infection, HCMV can also use autophagy instead of just fighting it. Autophagy can be used wholly or partially by HCMV, which can optimize the transmission or persistence of the virus.

(6) Summary and Prospect

Autophagy is a common programmed cell death mechanism in eukaryotic cells. Viruses, as a specific intracellular parasite, must interact with autophagy in the course of infection. In the process of virus infection, the interaction between autophagy and virus may be that autophagy successfully prevents virus replication, or that virus uses or inhibits autophagy of host cells to serve its own replication. At present, antiviral drugs mainly act on viral proteins. Because enteroviruses are easy to mutate and recombine, there are no effective therapeutic drugs. Understanding the role of autophagy in enterovirus infection can develop new fields for the treatment of enteroviruses.

38.4 Autophagy and Intestinal Microecology

38.4.1 The Role of Autophagy in Maintaining Intestinal Microbiota Homeostasis

Autophagy, a self-eating process, is an important mechanism for cells to maintain material turnover. When aging proteins, damaged organelles, and other wastes occur

in the cell, autophagic vesicles will wrap them up and send them to lysosomes for degradation and recycling, so as to ensure the metabolism of the cell itself and the renewal of some organelles. Intestinal autophagy plays an important role in regulating the diversity and composition of intestinal microbiota.

The autophagy process relies on proteins encoded by autophagy-related genes (Atg). So far, more than 40 autophagy-related genes have been found. Autophagy related genes are crucial for the formation of autophagosomes and the promotion of cell survival. Multiple ATGs play important roles in intestinal mucosal barrier function and intestinal flora homeostasis. ATG16L1, ATG5, and ATG7 play an important role in maintaining the morphology of pan cells and secreting antimicrobial peptides regulating the intestinal microenvironment. When ATG16L1, ATG5, and ATG7 are defective, they will lead to the destruction of the structure of mouse pantoli cells, the disorder of secretion, and the abnormality of granulosa exocytosis pathway, resulting in the peroxisomal value-added activation of receptor gene, which will directly damage the intestinal mucosal barrier function and make intestinal flora translocation. Studies have reported that the absence of intestinal epithelial Atg5 leads to significant changes in the intestinal microbiota of mice, resulting in a decrease in the diversity, as indicated by a decrease in the number of Akk bacteria, rumen coccaceae, and spirospiaceae, while an increase in the number of proinflammatory bacteria (candidatus athromitus) and potential pathogens (pasteuriaceae). Autophagy genes ATG16L1 and phosphatidyl ethanolamine (PE) and type of ubiquitin molecules LC3 connection, affect autophagy body form and function of the key step, by building contains ATG16L1 and allele (ATG16L1HM) in mice, and reduce ATG16L1 expression decreased and autophagy, found that mice intestinal ZhongGe gram-negative bacteria rat citric acid bacillus (intestinal pathogenic bacteria) decreased, thus protecting intestinal damage (Oh and Lee 2014).

38.4.2 Autophagy Mediated by Microbe

The primary function of the autophagy pathway is to adapt to nutrient deprivation by reusing energy and small molecules. However, recent studies have found that autophagy plays an important role in the innate antibacterial immune defense of the intestinal tract of eukaryotes (Girardin et al. 2003). In the event of pathogen invasion, autophagy can directly remove microorganisms in cells by targeting phagocytic microorganism degradation lysosomes. Intestinal flora can also promote autophagy in the body by producing polyamines and vitamin B6.

38.4.2.1 Microbe-Mediated Autophagy Initiation Signaling Activation

When intracellular bacteria destroy cell membranes and cause acute amino acid deficiency, some cell membrane receptors or cytoplasmic receptors are activated. More

importantly, the downstream signaling pathway of these receptors can activate multiple stages of autophagy, such as phagocytic vesicle nucleation, cargo loading and phagocytic vesicle extension. Studies have shown that group A streptococcus and measles virus can activate autophagy through CD46/GOPC/Beclin1 signaling pathway. In addition, the intestinal macrophages showed toll-like receptors (TLRs) that can identify specific microorganisms on the surface of the intestinal flora associated molecular patterns (MAMPs) (including the lipopolysaccharide (LPS) and teichoic acid (LTA) and peptidoglycan (PGN), mannose, bacterial DNA, glucan, etc.) and its downstream MyD88 and TRIF via combined with autophagy genes Beclin1 mutually competitive and reduce the combination of the Bcl—2 and Beclin1, so as to activate autophagy (Xu et al. 2007).

38.4.2.2 Microbe Activates the Autophagic Vacuole

Cytosolic nod-like receptor (NLRs) is a kind of protein family that can recognize intestinal bacterial peptidoglycan. Nod1 and Nod2 belong to the NLRs family. It has been reported that Nod1 and Nod2 can activate the autophagy defense system by binding to atg-5-atg12-atg16l1 complex during autophagy phagocytic vesicle elongation.

38.4.2.3 Microbe Activates the Fusion of Autophagosome and Lysosome

DNA damage-regulated autophagy modulator protein 1 (Dram1) is a lysosomal protein co-located with autophagosome. The number of intestinal mycobacteria was regulated by Dram1 protein. Other studies have confirmed that Dram1 mediates p62-dependent selective antimicrobial autophagy downstream of the tlr-myd88-nf-b signaling pathway. Dram1 was required for autophagosome formation and fusion of autophagosome and lysosome regulated by mycobacterium. Dram1 has been shown to play an important role in autophagosome maturation in response to intracellular pathogen invasion.

Whether bacterial infection can activate the mitochondrial autophagy pathway, the researchers tried a variety of bacteria, including listeria, salmonella, escherichia coli, and citrobacter, to systematically analyze the occurrence of mitochondrial autophagy. Studies have found that listeria and salmonella have the function of inducing mitochondrial autophagy. Further research on listeria also found that listeria can produce a protein called hemolysin O, which can cause mitochondrial damage in cells and thus induce mitochondrial autophagy.

38.5 Autophagy and Gastrointestinal Motility Disorders

38.5.1 The Relationship Between Cajal Interstitial Cells (ICC) and Gastrointestinal Motility—ICC Plays an Important Role in Maintaining Normal Gastrointestinal Motility and Is an Important Link in Regulating Gastrointestinal Motility

In 1893, Santiago Ramóny Cajal described for the first time a special cell type, which is located between endings of autonomic neurons and muscular cells. They were structurally and functionally further characterized in the gut musculature and named mesenchymal cells of Cajal (ICC). In the last 100 years, studies have focused on ICC were profoundly understood. As the pacemaker activity, ICC was arising from the interstitial cell of gastrointestinal tract. ICCs are known to provide pacemaker activity, propagation pathways for slow waves, transduction of inputs from motor neurons, the contraction of gastrointestinal smooth muscle, and neurotransmission. It is considered that ICC network loss and pathological damage may be related to the many types of gastrointestinal motility disorders. Currently, gastrointestinal motility disorders may be related to the disorders of development and differentiation, abnormal structure, quantity, and distribution of ICC. Because ICCs have unique features that are specific for the gut musculature, they are an ideal target for pharmacological action. Many studies have been performed in recent years that bring us closer to our understanding of the physiological role of ICC in controlling GI motility and their role in pathophysiology of motility disorders.

Different ICC populations were found in the gut muscle coat with region-specific location, region-specific ultrastructural features, and function. ICC was classified according to cell morphology, location, and function: (1) Classification according to cell morphology and location: Myenteric ICC (ICC-MY) is a form of a cellular network around the myenteric plexus in the space between the circular and longitudinal layers of muscle; Submucosal ICC (ICC-SM) distributes along the submucosal layer of the gastrointestinal muscular layer; Deep muscular ICC (ICC-DMP) designates the nerve plexus between the thick outer and thin inner subdivision of the circular muscle layer; Intramuscular ICC (ICC-IM), located in the muscular layer. (2) Classification according to ICC cell function: The network of IC-MY in small intestine and ICC-SMP in colon which is believed to be the origin of electrical slow waves is morphologically independent from but associated with the myenteric plexus. They are involved in regulation of intestinal smooth muscle nerve signals, which includes ICC-IM distributed in the gastrointestinal tract. There are three main functions for ICC that have been proposed: to pace slow waves and regulate their propagation; to mediate enteric neuronal signals to smooth muscle cells; and to act as mechanoreceptors coupled with ICC via gap junctions, and the functional unit thus formed enables rhythmically synchronized contractions and relaxations (Klein et al. 2013).

Studies have confirmed that abnormal ICC structure and loss of ICC lead to gastrointestinal dysmotility in humans and animals, including idiopathic achalasia, diabetic gastrointestinal disease, Hirschsprung's disease, chronic intestinal pseudo-obstruction, anorectal malformation, and slow transit constipation. Research suggests that in the pathogenesis of achalasia, especially in the development of the LES high-pressure zone, depletion of ICC networks and potential changes in the electrical activity of smooth muscle cells may play a crucial role. Research findings have shown that immature ultrastructural features of ICCs in infantile hypertrophic pyloric stenosis. Their findings were supported by studies, which provided substantial evidence that ICCs might have a role in the pathogenesis of hypertrophic pyloric stenosis. Diabetic gastrointestinal disease is one of the common complications of diabetes mellitus. There is increasing evidence for specific cellular changes in the stomach of patients with diabetic (DG) and idiopathic (IG) gastroparesis. The most significant findings are loss of interstitial cells of Cajal (ICC), neuronal abnormalities, and an immune cellular infiltrate. Chronic intestinal pseudo-obstruction (CIIP) is characterized by alteration of the ICC network. Decreased ICC density along with loss of processes and damaged intracellular cytoskeleton and organelles have been reported in patients with CIIP. Several investigators have studied the distribution of ICCs in the ganglionic and aganglionic bowel of patients with Hirschsprung's disease (HD), which described reduced number of ICCs with disrupted network. Recent research findings defects in the population of intestinal pacemaker cells may underlie the colonic hypomotility seen in high anorectal malformations and hence may contribute to refractory constipation. The present study suggests decrease in ICC and in neuronal cells in the slow transit constipation. A decrease in c-kit positive cells was noted in all regions of the sigmoid colon. It seems that ICC plays an important role in generation of the smooth muscle electrical slow wave that determines contractile activity.

38.5.2 Association Between Autophagy and Abnormalities in the Structure and Number of ICC Cells—Excessive Autophagy in ICC May be One of the Key Factors in Gastrointestinal Motility Disorders

Autophagy, also known as type II cell programmed death, is widely existed in eukaryotic cells and is involved in cell growth, development, and pathophysiological processes. Autophagy is not only a ubiquitous normal physiological process, but also one of the cell's defense mechanisms against adverse environment, and it is also a self-protection mechanism of cells. The nature of autophagy is a cup-shaped segmentation membrane derived from the endoplasmic reticulum, Golgi, or endosomal lipid bilayer. With a gradually prolonged structure, the segmentation membrane engulfs damaged organelles and some cytoplasm and forms autophagosomes. Autophagy has dual effect on cells. In normal cells, basal levels of autophagy are maintained at a lower level to keep cell homeostasis, and to ensure normal physiological function

of cells, with a similar effect as scavengers. When homeostasis changes, such as developmental differentiation, aging, or effects induced by external adverse stimuli, autophagy is immediately induced, and excessive autophagy will destructively degrade a large number of useful proteins and organelles, resulting in cells' incapacity to perform normal function, which will lead to cell disruption and programmed cell death. The current gold standard for measuring the level of autophagy in cells is to directly count the number of autophagic vacuoles in the cells using a transmission electron microscope.

The generation of ICC pacing potential and slow-wave propagation is affected by biological activities such as ion environment, neurotransmitters, and hormones, among which Ca^{2+} has the closest relationship. Studies have shown that ICC expresses a low-threshold Ca^{2+} channel, which causes periodic depolarization of ex vivo ICC and is closely related to changes in extracellular Ca^{2+} concentration. Intracellular Ca^{2+} overload is one of the initiation factors of autophagy and is the ultimate common pathway leading to cell damage and death. Because intracellular Ca^{2+} concentration changes are closely related to systolic activity of gastrointestinal smooth muscle, high concentration of Ca^{2+} can cause smooth muscle contraction, while low concentration of Ca^{2+} can cause smooth muscle relaxation. Intracellular Ca^{2+} overload is one of the initiation factors of ICC autophagy. Increased intracellular $Ca2^{+}$ concentration can directly activate some proteolytic enzymes, or act as a third messenger to influence cellular gene expression and induce autophagy. Therefore, excessive autophagy in ICC may be one of the key factors in gastrointestinal motility disorders.

38.5.3 Mechanism of Regulating Gastrointestinal Motility Disorders by Excessive Autophagy in ICC

Studies have shown that excessive autophagy in ICC may be one of the mechanisms in the pathogenesis of gastrointestinal motility disorders. Under the stimulation of the signal, autophagy begins in cells, firstly forming a bilayer membrane vacuole in the cytoplasm wrapping materials to be degraded, such as mitochondria and endoplasmic reticulum fragments, which is called autophagosomes. Autophagy is a complex process involving multiple factors. Among them, Beclin1 gene, also known as BECN1 gene, is a key factor involved in the regulation of autophagy, and is an important condition in the formation of autophagosome. After autophagy is induced, it is first combined with phosphatidylinositol 3-kinase (type III P13K) to form a complex that regulates the localization of other ATG proteins in the autophagy precursor structure, and regulates the synthesis of autophagosome membranes, which is a key gene in the initiation of autophagy. The BECN1 gene also regulates autophagy activity, and the number of the gene represents the activity of autophagy and is an important indicator for evaluating autophagy activity. Microtubule-associated protein 1 light chain 3 (LC3) is a homolog of the yeast ATG8 gene in mammalian cells. LC3A is routinely

expressed and freely present in the cytoplasm. During autophagy, LC3A is processed and modified by ubiquitin-like system to produce LC3B. LC3B is covalently linked to phosphatidylethanolamine (PE) on the surface of autophagy membrane to form liposoluble LC3B-PE, which is involved in the extension of autophagosome membrane, until the formation of autophagolysosome. The LC3B protein binds and is always localized on the membrane of autophagic vacuoles, and its number is directly proportional to the activity and the level of autophagy, and has been used as one of the specific methods for detecting the expression level of autophagy (Mizushima et al. 2010). It is suggested that the autophagy process is mainly regulated by the PI3K-Akt-mTOR signaling pathway and the Beclin 1 complex.

When the level of intracellular autophagy is elevated, excessive autophagy will occur, which may be manifested by an increase in the number of autophagic vacuoles in the cells, an increase in Beclin1 and LC3B proteins, changes in intracellular structure and the cell numbers due to programmed cell deaths. Studies have confirmed that abnormalities in the structure and number of ICC in intestinal motility disorders may be associated with excessive autophagy in ICC. The berberine hydrochloride was used to construct a rat model of slow transit constipation, detected colonic transit time in rats, and observed morphological changes of ICC and c-Kit protein expression in rat intestinal tract by IF technique, and detected ICC-specific proteins c-kit and Beclin1, LC3B with immunoblotting. The results showed that the colonic transit time of rats with slow transit constipation was significantly prolonged and the exercise capacity was significantly reduced, indicating abnormalities of intestinal myoelectric activity; in addition, compared with the normal group, the number of ICC cells in the intestine is significantly reduced, the ICC interaction network structure was obviously thinner, and the expression levels of Beclin1 and LC3B in the intestinal tissues were significantly higher, indicating that autophagy occurred in the ICC. Therefore, autophagy phenomenon occurred in the ICC in the intestinal tract of the slow transit constipation rat model, which may be one of the reasons leading to the decrease in the number of ICC and slow transit constipation. Based on ICC autophagy, the cause of intestinal motility abnormality in colitis mice was induced by glucose sodium sulfate solution, which measured the tension of isolated smooth muscle strips, observed the ultrastructure of ICC cells under high power transmission electron microscope, and detected the expression levels of c-kit, Beclin1, LC3B protein, and mRNA in intestinal tissues. Results showed that, in colitis mice, the contraction amplitude of colon smooth muscle strips decreased, the frequency of contraction increased, which is similar to the kinetic abnormality of intestinal tract in patients with colitis. And the structure of ICC was abnormal compared with the normal group, with excessive expression of Beclin1 and LC3B protein and excessive autophagy in the colon tissue, which leading to programmed cell death, with a decrease in c-kit protein expression, that is, a decrease in the number of ICC.

38.5.4 Relationship Between Autophagy and Gastrointestinal Motility Disorders/Functional Gastrointestinal Disorders

38.5.4.1 Autophagy Mediates the Protective Effect of Epithelial Cells in Eosinophilic Esophagitis (EoE)

Study found that EoE-associated inflammation promotes autophagy and basal cell proliferation in mice EoE and esophageal organelle models (Whelan et al. 2017). Chloroquine enhances basal cell proliferation and inhibits autophagic fluxes of esophageal keratinocytes to stimulate EoE-associated cytokines, including tumor necrosis factor and interleukin-13α, which are characterized by reactive oxygen species-dependent autophagic flux activation. Oxidative stress in EoE mice, esophageal cells, and human esophageal cancer cells can be enhanced by chloroquine treatment or autophagy inhibition by Beclin-1 or ATG-7 depletion. Compared with healthy subjects and patients with EOE remission, active infection and number of autophagic vacuoles are increased in pediatric esophageal epithelial EoE patients.

38.5.4.2 ICC Autophagy and Functional Dyspepsia

ICC was quantified by ICC-specific membrane protein c-kit. The results showed that the number of gastric ICC in FD rats was significantly lower than that in normal rats. There were a large number of autophagic vacuoles with bilayer membrane structure, which is consistent with excessive autophagy in cells, suggesting that excessive autophagy occurs in gastric ICC in FD rats and excessive autophagy in ICC may be one of the mechanisms leading to decreased gastric motility in FD. ICC-MY is distributed between the circular muscle and the longitudinal muscle in gastric antrum, and is mainly involved in gastrointestinal pacing. In the FD rat model, excessive autophagy in ICC and structural disorder and decreased number of ICC-MY were observed, suggesting the presence of excessive autophagy. At the same time, it is suggested that the pathogenesis of functional dyspepsia may be related to increased mRNA expression of Beclin1 protein and LC3B, which leads to increased autophagy activity of ICC and increased expression of autophagic vacuoles.

38.5.4.3 ICC Autophagy and Slow Transit Constipation (STC)

Recent studies have found that ICC autophagy may be associated with the pathogenesis of STC. The Cajal interstitial cells between the longitudinal and circular muscles can produce slow waves that cause automatic rhythmic movement of the gastrointestinal smooth muscle. The slow wave generated by ICC is closely related to the change of intracellular Ca^{2+}. The main cause of increased intracellular Ca^{2+}

is extracellular Ca^{2+} influx and release from calcium store. When Ca^{2+} in the calcium store is depleted, the calcium channel controlling the calcium store is activated, and leads to a large amount of extracellular Ca^{2+} influx and intracellular Ca^{2+} overload, which induces autophagic apoptosis. Intracellular Ca^{2+} overload can induce autophagy, which is one of the initiating factors of autophagy in cells. Therefore, excessive autophagy in ICC may be one of the key factors in the pathogenesis of STC.

38.5.4.4 ICC Autophagy and Diabetic Gastroparesis

At present, autophagy has been widely studied in many complications of diabetes. In a study of insulin resistance in diabetes, it is found that inhibition of JNK pathway can downregulate autophagy, thereby improving insulin resistance in diabetes. It has been found that highly expressed autophagy proteins can be detected in gastric tissues of diabetic rats. Meanwhile, the reduced contractility was found in isolated gastric smooth muscle cells cultured in high glucose, suggesting that high glucose may induce autophagy of gastric smooth muscle cells by activating JNK signaling pathway. Therefore, the abnormal function of autophagy may play an important role in the pathogenesis of diabetic gastroparesis.

38.5.4.5 Regulation of ICC Autophagy—A New Target for the Treatment of Gastrointestinal Motility Disorders

All in all, ICC has become a therapeutic target for gastrointestinal motility disorders, and autophagy regulation is a potential therapeutic measure for many diseases, which can be realized through multilevel and multipath interference. To further study the relationship between gastrointestinal motility disorder and autophagy of ICC, and to explore ways to strengthen this beneficial autophagy by regulating upstream signaling pathway, may be beneficial to the prevention and treatment of gastrointestinal motility disorder diseases.

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