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Review

Autophagy and its role in gastric cancer

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ABSTRACT

Autophagy, which is tightly regulated by a series of autophagy-related genes (ATGs), is a vital intracellular homeostatic process through which defective proteins and organelles are degraded and recycled under starvation, hypoxia or other specific cellular stress conditions. For both normal cells and tumour cells, autophagy not only sustains cell survival but can also promote cell death. Autophagy-related signalling pathways include mTOR-dependent pathways, such as the AMPK/mTOR and PI3K/Akt/mTOR pathways, and non-mTOR dependent pathways, such as the P53 pathway. Additionally, autophagy plays a dual role in gastric carcinoma (GC), including a tumour-suppressor role and a tumour-promoter role. Long-term *Helicobacter pylori* infection can impair autophagy, which may eventually promote tumourigenesis of the gastric mucosa. Moreover, Beclin1, LC3 and P62/SQSTM1 are regarded as autophagy-related markers with GC prognostic value. Autophagy inhibitors and autophagy inducers show promise for GC treatment. This review describes research progress regarding autophagy and its significant role in gastric cancer.

1. Introduction

Autophagy, from the Greek roots “auto” (meaning self) and “phagy” (meaning eat), is the phenomenon of cell self-digestion, first proposed by de Duve in 1963 [1]. Autophagy is a highly conserved multi-step catabolic pathway in which double-membrane cytosolic vesicles wrap around part of the cytoplasm, damaged organelles, long-lived proteins and other components (e.g., invading microbial pathogens) to form autophagosomes, and then the outer membranes fuse with lysosomal membranes to form autophagolysosomes. The intracellular cargoes are degraded by the hydrolytic enzymes in autophagolysosomes to achieve cellular metabolic needs and the turnover of some organelles, such as the mitochondria, endoplasmic reticulum and ribosomes [2,3]. Although autophagy normally occurs at low levels in almost all cells, it can be rapidly activated to provide cells with energy and nutrients and maintain cellular homeostasis under various stress conditions, including nutrient starvation (the most common stress), growth factor deprivation, hypoxia and inflammatory stimulation, by recycling degraded cargoes and regenerating ATP and materials [4]. With the deepening of autophagy research, there has been gradual recognition that autophagy is not only a survival mechanism but participates in the process of cell death, which is different from apoptosis (type I

Abbreviations: ATGs, autophagy-related genes; GC, gastric carcinoma; CMA, chaperone-mediated autophagy; Hsc70, 70 kDa heat shock homologue protein; LAMP-2A, lysosome-associated membrane protein-2A; mTOR, mammalian target of rapamycin; ULK, Unc-51-like kinases; FIP200, family interacting protein of 200 Kd; PI3KC, class III phosphatidylinositol 3-kinase; AMBRA1, activating molecule in BECN1-regulated autophagy protein 1; PI3P, phosphatidylinositol 3-phosphate; DFCP1, double FYVE-containing protein 1; WIPIs, WD-repeat protein interacting with phosphoinositides; ER, endoplasmic reticulum; MAP1LC3, microtubule-associated protein 1 light chain 3; PE, phosphatidylethanolamine; SQSTM1, sequestosome; mTORC1, mTOR complex 1; mTORC2, mTOR

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programmed cell death); thus, autophagy is also called type II programmed cell death [5,6].

The relationship between autophagy and gastric carcinoma (GC) has become a research hot spot in recent years. Various studies have shown that autophagy-related molecules have great clinical value and may serve as promising prognostic biomarkers of GC. Advances in targeting autophagy during GC treatment have also attracted increasing attention. Therefore, in this review, we aimed to elucidate the significance of autophagy in the prognosis and clinical therapy of gastric cancer in order to control tumourigenesis and improve disease management.

2. An overview of autophagy

Autophagy, a common biological phenomenon that exists in all eukaryotic cells, has been shown to play an important role both in pathophysiological responses, including energy metabolism, organelle turnover, growth regulation and aging, and in human diseases, such as cancer, cardiovascular diseases and neurodegenerative disorders [7,8].

2.1. Autophagy classification

Depending on the intracellular components transported to the lysosome for degradation in different pathways, autophagy can be categorized into the following three major forms (Fig. 1): macroautophagy, microautophagy and chaperone-mediated autophagy (CMA), which all have different mechanisms [9]. Macroautophagy, the most universal subtype and widely studied form of autophagy, is accepted as the main type of autophagy in this review (hereafter simply referred to as autophagy). During the process of macroautophagy, intracellular cargoes are engulfed in a double-membraned structure to generate autophagosomes and undergo fusion with lysosomes. The contents are degraded into fatty acids, amino acids, sugars, nucleotides and other components and then released into the cytoplasm for recycling and re-entry into biosynthesis [10]. Microautophagy is the pathway through which lysosomal membranes themselves invaginate and directly envelop cytoplasmic contents, followed by the delivery of cargoes into the lysosomes for degradation by lysosomal hydrolases, a process that does not involve autophagosome formation [11]. Finally, chaperone-mediated autophagy, which occurs mainly in mammalian cells, is a selective process in which cytoplasmic unfolded substrate proteins with a particular KFERQ-motif pentapeptide sequence recognize and bind to the cytosolic chaperone complex (e.g., the 70 kDa heat shock homologue protein, Hsc70), and then, through the action of the integral membrane receptor lysosome-associated membrane protein-2A (LAMP-2A), directly enter the lysosomal lumen for degradation [12].

2.2. The processes and molecular mechanisms of autophagy

The overall process of autophagy is roughly divided into the following distinct stages (Fig. 2): initiation and nucleation of the autophagosome, recognition of intracellular cargoes, elongation and maturation of the autophagosome, autophagosome fusion with the lysosome, and cargo degradation [13]. The specific and complex molecular mechanisms of autophagy are not fully understood but are thought to be encoded by more than 35 autophagy-related genes (ATGs) [14].

At the autophagosome initiation stage, the downregulation of mammalian target of rapamycin (mTOR) is stimulated by intracellular stress and then activates the downstream serine/threonine Unc-51-like kinases 1 and 2 (ULK1 and ULK2, the two mammalian homologs of ATG1). Subsequently, ULK1/2 forms the ULK1 complex with ATG13, family interacting protein of 200 kDa (FIP200, the mammalian homologue of ATG17) and ATG101 (also called C12orf44), to regulate autophagosome formation by activating the class III phosphatidylinositol 3-kinase (PIK3C3, also known as vacuolar protein sorting 34 or Vps34) complex comprising VPS34, ATG14 L, VPS15 (also known as PI3K regulatory subunit 4, PIK3R4), the activating molecule in BECN1-regulated autophagy protein 1 (AMBRA1), and the scaffold protein Beclin1 [13]. The resulting PIK3C3 complex produces phosphatidylinositol 3-phosphate (PI3P) to recruit certain effector proteins, such as double FYVE-containing protein 1 (DFCP1) and WD-repeat protein interacting with phosphoinositides (WIPIs), that are required for the formation of omegasomes, which are nucleation sites [14,15]. Thus far, the source of the autophagosomal membrane is not yet clear, and more

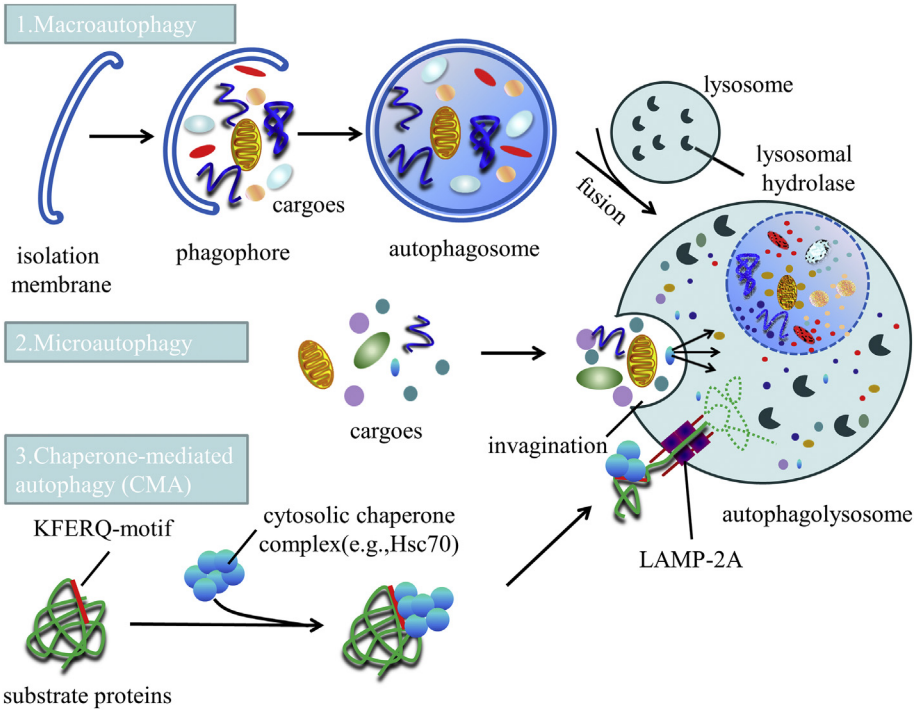

Fig. 1. The three different forms of autophagy.

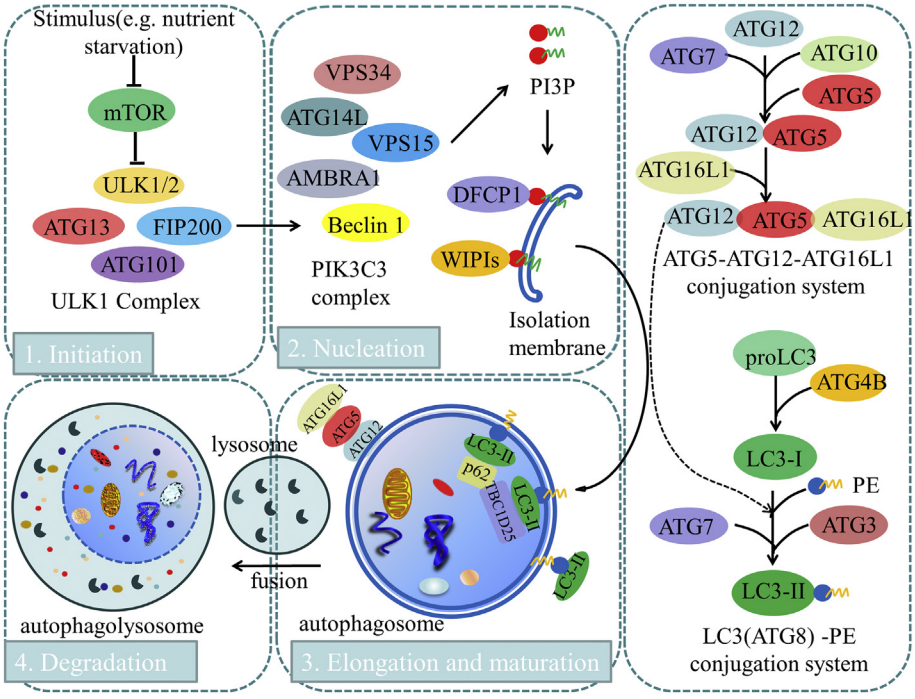

Fig. 2. The main processes of autophagy and their molecular mechanisms.

than one source is likely involved; potential sources include the endoplasmic reticulum (ER), which may be the main membrane source for autophagosome formation [16], mitochondria, Golgi apparatus, plasma membrane and recycling endosomes [17].

Autophagosome elongation and maturation requires two critical ubiquitin-like conjugation systems: first, the ATG5–ATG12 complex conjugates with ATG16L1; second, microtubule-associated protein 1 light chain 3 (MAP1LC3, commonly called LC3), which is a mammalian homologue of the yeast ATG8, is conjugated to the lipid phosphatidylethanolamine (PE). ATG12 is a ubiquitin-like protein that is covalently bound to ATG5 by the sequential action of the E1-like enzyme ATG7 and the E2-like enzyme ATG10. The resulting ATG5-ATG12 complex is recruited into the autophagic membrane and promotes its elongation by interacting with ATG16L1 [18,19]. It has also been reported that the ATG12-ATG5 complex associated with ATG16L1, which promotes the conjugation of LC3 to PE, may have the biological activity of an E3-like enzyme [18]. In another ubiquitin-like system involving LC3, the early synthesized inactive proLC3 (the unprocessed form of LC3) is proteolytically cleaved by the ATG4B cysteine peptidase to form cytoplasmic soluble LC3-I, which exposes its C-terminal glycine groups [20]. After coordinated activation by the E1-like enzyme ATG7, the E2-like enzyme ATG3, and the E3-like enzyme ATG12-ATG5-ATG16L1 multimers, the C-terminal glycine of LC3-I is tightly linked to the PE-specific amino acid site to generate the LC3-PE complex; this form of LC3 is termed membrane bound LC3-II [21,22]. The high lipophilicity of PE promotes LC3-II recruitment to the autophagosome and its distribution over both faces of the autophagosomal membrane, which plays a decisive role in autophagosome elongation [2]. LC3-II further acts as a binding receptor, interacting with adaptor proteins such as p62/SQSTM1 and TBC1D25/OATL1, which are closely associated with the selective degradation of autophagy [23].

After fusion of the autophagosomes with lysosomes to generate autophagolysosomes, LC3-II, located outside the autophagosomal membrane, is deconjugated from PE by Atg4 to form LC3-I and is released into the cytoplasm for recycling, whereas LC3-II, located inside the autophagosomal membrane, is ultimately degraded by lysosomal hydrolases. By contrast, the ATG12-ATG5-ATG16 L1 complex is dissociated from the autophagosomal membrane during later stages of the elongation process [24]. The intracellular components are rapidly degraded by autophagolysosomal hydrolytic enzymes such as cathepsins B, D and L [25].

2.3. Autophagy-related signalling pathways

The regulation of autophagy is closely linked to distinct autophagy-related signalling pathways, including mTOR-dependent pathways, such as the AMPK/mTOR and PI3K/Akt/mTOR pathways, and non-mTOR dependent pathways, such as the P53 pathway.

2.3.1. AMPK/mTOR pathway

Mammalian target of rapamycin (mTOR), a serine/threonine protein kinase that is highly conserved from yeast to mammals, plays an important role in distinct cellular processes, including protein synthesis, lipid synthesis and cell growth and proliferation [26]. mTOR exists in two protein complexes, mTORC1 (mTOR complex 1) and mTORC2 (mTOR complex 2), which exhibit different functions. The mTORC1 complex is composed of mTOR, G protein subunit-like (G L, also called mLST8) and regulatory-associated protein of mTOR (Raptor), and mTORC2 is composed of mTOR, mLST8, and rapamycin-insensitive companion of mTOR (Rictor) [27]. mTORC1, which is sensitive to inhibition by rapamycin, is a well-characterized autophagy regulator, whereas mTORC2 is not. In spite of this, mTORC2 is also involved in autophagy regulation by phosphorylating Akt (also referred to as protein kinase B, PKB) on Ser473 to promote its maximal activation [28,29]. A variety of growth factors, cytokines and amino acids (AAs) can maintain mTOR activity. Upon activation, mTORC1 negatively regulates the initiation of autophagy via ULK1 phosphorylation at Ser757, which is one of the major protein kinases that trigger autophagosome formation [30]. Conversely, under metabolic stress conditions, such as nutrient deprivation and energy depletion, adenosine monophosphate-activated protein kinase (AMPK), which is stimulated by an increased AMP/ATP ratio, can inhibit mTORC1 activity by activating the TSC1/TSC2 protein heterodimer (see the next paragraph), consequently inducing autophagy [31]. AMPK also directly phosphorylates Raptor, one of the protein components of mTORC1, which leads to the inhibition of mTORC1 [32]. In addition, the AMPK signalling

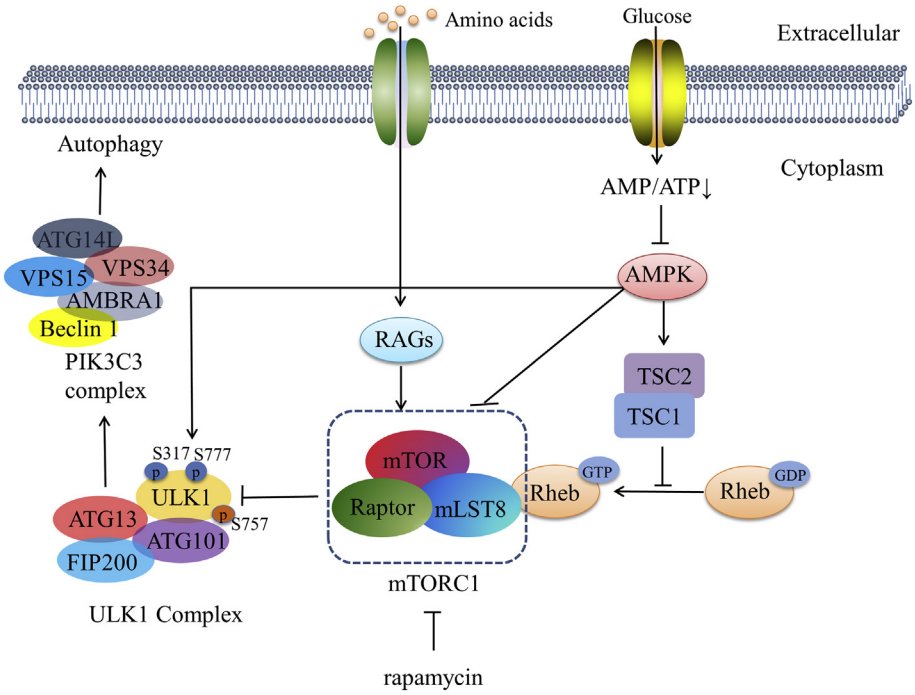

Fig. 3. AMPK/mTOR signalling pathway.

pathway regulates autophagy through an alternative mechanism, whereby AMPK directly activates ULK1 and thereby promotes autophagy via the phosphorylation of Ser317 and Ser777 [33]. A recent study demonstrated that compound C, a pharmacological inhibitor of AMPK, markedly increases the expression of mTOR, decreases the expression of ULK1 and obviously reduces the expression of the autophagy markers Beclin1 and LC3II, resulting in the downregulation of Tris (1, 3-dichloro-2-propyl) phosphate (TDCIPP)-induced autophagy in SH-SY5Y cells [34] (Fig. 3).

2.3.2. PI3K/Akt/mTOR pathway

The class I PI3K (PIK3C1) is a class of lipid kinase whose main biochemical function is to catalyse phosphatidylinositol (PI) phosphorylation at the D3 position and convert phosphatidylinositol 4,5-bisphosphate (PI-4,5-P2 or PIP2) into phosphatidylinositol 3,4,5-triphosphate (PI-3,4,5-P3 or PIP3) [35]. PIP3 is widely accepted as an important second messenger that recruits molecules containing conserved pleckstrin homology (PH) domains to achieve their activation at the plasma membrane [36]. Akt and its activator phosphoinositide-dependent protein kinase 1 (PDK1, which phosphorylates Akt at Thr308) bind PIP3 via their PH domains, giving rise to full Akt activation [28,36]. Activation of the PI3K/Akt pathway contributes to cell survival by inhibiting apoptosis and accelerating cell cycle progression, which has been verified by the decreased expression of the pro-apoptotic factors Bad and Fas and increased expression of cyclin-E [37]. Furthermore, activated Akt directly phosphorylates and stimulates downstream mTORC1, ultimately resulting in the suppression of autophagy. On the other hand, Akt activation can also phosphorylate and inactivate tuberous sclerosis complex 2 (TSC2) to disrupt the formation of the TSC1/TSC2 heterodimer and then contribute to mTORC1 activation [38]. The TSC1/TSC2 heterodimer is a critical negative regulator of Ras homologue enriched in brain (termed Rheb) that facilitates mTORC1 activation [39]. Thus, there is an opposing change in the mTORC1 activity regulated by AMPK and PKB through the TSC1/TSC2 heterodimer. In addition to inhibiting the ULK1 complex, activated mTORC1 directly phosphorylates ribosomal protein S6 kinase (S6K, also known as p70S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1), which play fundamental roles in both mRNA translation initiation and progression [40]. Conversely, mTORC1 elicits a negative feedback loop to downregulate the activity of Akt through S6K [41]. Peng et al. [42] discovered that blocking the PI3K/Akt/mTOR signalling pathway strongly inhibited the proliferation of colorectal cancer (CRC) cells by enhancing apoptosis and autophagy and inducing cell cycle arrest at the G1 phase to exert antitumour effects on HCT116 cells. PTEN, an important class of tumour suppressor gene, participates in cell autophagy through its built-in lipid phosphatase, which negatively regulates the PI3K-AKT-mTOR pathway in the cytoplasm [43] (Fig. 4).

2.3.3. Crosstalk between AMPK and Akt

Growing evidence reveals that crosstalk is present between AMPK and Akt, and additionally, Akt is an effective antagonist of AMPK. The AMPK/mTOR and Akt/mTOR pathways are involved in autophagy induction in cardiomyocytes, thereby reducing myocardial ischaemia reperfusion injury [44]. Irbesartan induces hepatic autophagy by enhancing PPAR-γ expression and activating the AMPK/Akt/mTOR pathway [45]. The AKT/mTOR/AMPK/p38 pathway acts as a target in apoptosis and autophagy induced by alisma [46]. 4-Nonylphenol also enhances apoptosis, autophagy and necrosis in Sertoli cells and may involve the ROS-dependent JNK and Akt/AMPK/mTOR pathways [47]. It has been reported that the Akt activator IGF-1 can weaken vitamin E succinate (VES)-induced AMPK activation, leading to autophagy inhibition in the human gastric carcinoma SGC7901 cell line, whereas treatment with the Akt inhibitor LY294002 can strengthen VES-induced AMPK activation and consequently trigger the initiation of autophagy [38,48]. After curcumin pretreatment, 5-Fu may undergo autophagy conversion through AMPK/ULK1-dependent autophagy inhibition and AKT regulation in vivo and in vitro, which may result in the increased sensitivity of colon cancer cells/xenogeneic transplantation to 5-Fu cell toxicity [48]. IL-12 increases the expression of the autophagy-associated protein LC3 and induces the formation of autophagy bodies in human breast cancer cells, which are related to AMPK pathway activation and PI3K/Akt pathway suppression [49]. AMPK and Akt/mTOR signal transduction play key roles in the resveratrol-induced autophagy and apoptosis of cisplatin-resistant human oral cancer cells [50]. AMPK/mTOR/AKT signalling is involved in inducing autophagy to

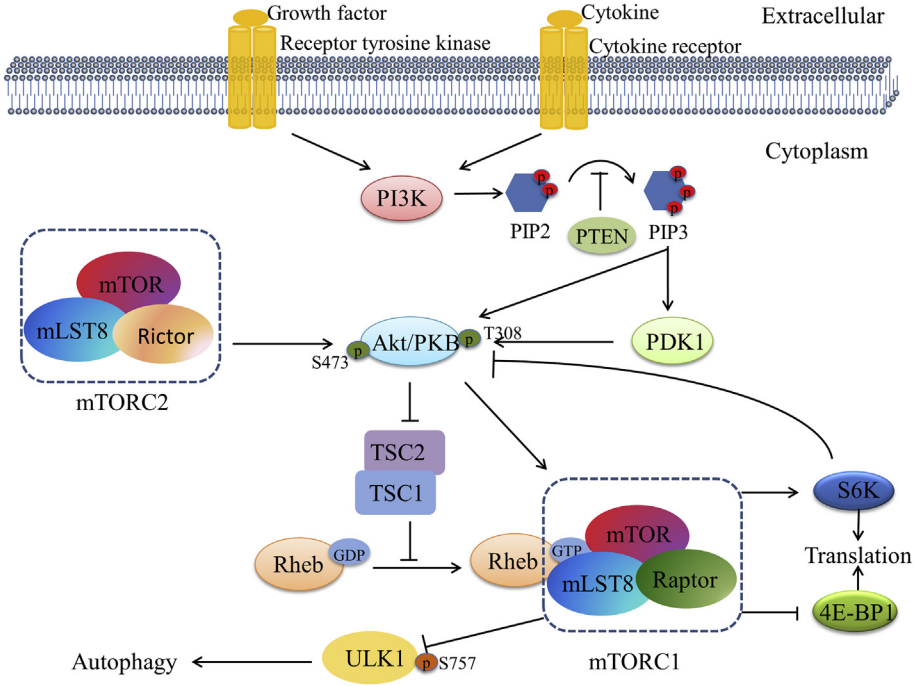

Fig. 4. PI3K/Akt/mTOR signalling pathway.

enhance the death of the human lung cancer A549 cell line [51]. Autophagy of human hepatoma cells is induced by kaempferol via AMPK and AKT signalling molecules [52].

3. The dual role of autophagy in GC

Although growing evidence has demonstrated that autophagy is largely associated with tumourigenesis and development, it is unclear whether autophagy has a tumour-suppressor role or a tumour-promoter role. Here, we take gastric carcinoma as an example to illustrate the dual role of autophagy in cancer.

3.1. Tumour-suppressor role in GC

Autophagy stimulation can serve as a protective mechanism to prevent cancer initiation, which is responsible for degrading dysfunctional proteins and organelles, preventing the toxic build-up of cellular unnecessary products, and inhibiting tissue damage and maintaining host defences [53]. Abnormal expression of autophagy genes may cause some cancer-related pathology [54,55]. Most known autophagy activators or effectors are located in cancer-related regions, which are frequently mutated or absent in many human cancers [56–58]. Under some stress conditions, autophagy is strongly triggered and acts like a battery that makes cells adapt to stress and sustains their survival for a certain period of time until the cellular stress is removed. Moreover, in gastric cancer cells, aberrant or excessive autophagic activity may produce cytotoxicity and contribute to the improper degradation of intracellular components that are essential for maintaining tumour cell survival, ultimately resulting in autophagic cell death and suppressing tumour development [59]. Some data favour the idea that autophagy fulfils a tumour-suppressive function. According to Yang et al. [60], 5-fluorouracil (5-FU) may induce cell proliferation arrest and autophagic cell death in GC cells by upregulating Beclin1 (mammalian Atg6 homologue), ultimately inhibiting GC development. Enhanced autophagy may significantly reduce the growth of cancer cells. Therefore, 5-Fu can inhibit GC cells by inducing autophagy and specifically autophagy cell death [60].

3.2. Tumour-promoter role in GC

On the other hand, autophagy is considered to play an oncogenetic function that promotes tumour progression and is related to chemoresistance in a variety of cancers. In tumours, autophagy is stimulated and required to protect some tumour cells against nutrient deprivation and low-oxygen conditions, which are the main characteristics of harsh tumour microenvironments and other anticancer treatments [61,62]. Autophagy is a key process regulating tumourigenesis, interstitial interactions and tumour therapy, the latter of which inhibits tumours at an early stage of cancer development but promotes growth in established tumours and is also a cause of tumour metastasis, recurrence and chemical resistance [61]. Some chemotherapeutics can promote protective autophagy in cancer cells, thereby preventing cancer cells from undergoing drug-induced apoptosis. One study demonstrated that protective autophagy induced by oxaliplatin can partially antagonize apoptotic cell death in gastric cancer MGC803 cells [63]. Recently, autophagy was shown to facilitate AQP3-mediated chemoresistance to cisplatin (cDDP) in gastric cancer cells, whereas chloroquine (CQ), an autophagy inhibitor, significantly enhanced cDDP chemosensitivity in GC cells [64]. Taken collectively, autophagy is viewed as a double-edged sword for both normal cell and tumour cell survival.

4. Autophagy and *Helicobacter pylori* infection

Helicobacter pylori (also termed *H. pylori*), a Gram-negative bacterial pathogen, colonizes the gastric epithelium of approximately half the global population and is classified by the WHO (World Health Organization) as a class I carcinogen [65,66]. *H. pylori* infection is considered a major aetiological factor in the pathogenesis of gastric cancer, but the specific mechanism involved is still not completely clear. *H. pylori* releases multiple virulence factors, such as vacuolating cytotoxin A (VacA) and the effector protein cytotoxin associated gene A (CagA), which modulate a series of host cell responses and contribute to gastric carcinoma development [67].

Autophagy is regarded as a major defence mechanism for the elimination of several pathogenic microorganisms, including *H. pylori*, by autophagy-dependent degradative pathways [68]. Growing evidence

reveals that *H. pylori* invasion interferes with the autophagy of gastric epithelial cells [69]. According to Terebiznik et al. [70], *H. pylori* invasion induces autophagy in AGS gastric cancer cells, an observation that was validated by the conversion of GFP-LC3 I to GFP-LC3 II in lysates prepared from *H. pylori*-infected AGS cells using immunoblot analysis. This shows that *H. pylori* infection can induce cellular autophagy in AGS cells. *H. pylori* infects gastric epithelial cells and induces autophagy in a VacA-dependent manner, and autophagosome formation is induced by regulating the level of intracellular VacA [70]. Moreover, the virulence factor VacA is required and sufficient for *H. pylori*-induced autophagy based on observations of the increased abundance of GFP-LC3 puncta and the production of LC3 II in AGS cells treated with purified VacA toxin (pVacA) [70]. During *H. pylori* invasion, autophagy activation might act as a cytoprotective response to protect gastric mucosal cells against the invading *H. pylori* and/or exposure to VacA. The Autophagy gene ATG16L1 has been identified as a candidate host gene for *H. pylori* infection. In vivo and in vitro studies have revealed a mechanism by which *H. pylori* usurps the autophagy pathway, resulting in increased levels of reactive oxygen species (ROS) and p62/SQSTM1. According to Raju et al. [71], up to 24 h of exposure to VacA leads to the accumulation of defective autophagosomes and subsequently causes the elevated production of ROS and overexpression of the oncoprotein p62/SQSTM1, which both contribute to *H. pylori*-mediated gastric tumourigenesis. Taken together, these results suggest that short-term exposure to VacA can trigger autophagy to protect gastric mucosal cells against bacteria and toxins, whereas long-term exposure to VacA can disrupt autophagy, subsequently promote chronic inflammatory damage caused by *H. pylori* infection, and may eventually promote carcinogenesis. Apart from this, Tang et al. [72] observed that the upregulation of miRNA30B (also called MIR30B) compromises gastric epithelial cell autophagy during *H. pylori* infection by inhibiting the expression of BECN1 and ATG12, providing the conditions for persistent colonization by *H. pylori*.

Generally, although acute *H. pylori* infection triggers the occurrence of autophagy that is conducive to the clearance of organelles and proteins damaged by VacA, it is clear that long-term exposure to *H. pylori* infection may disrupt autophagy by preventing maturation of the autolysosome. Additionally, coinciding with the persistent effects of virulence factors and chronic inflammation, impaired autophagy may facilitate the establishment of an environment favouring the malignant transformation of gastric mucosa cells and tumourigenesis through a variety of pathways.

5. Prognostic value of autophagy-related markers in GC

5.1. Beclin1 and GC prognosis

Beclin1, a protein mammalian homologue of the yeast autophagy-related gene 6 (ATG6), is encoded by the BECN1 gene, which is located on chromosome 17q21 in humans. Beclin1 is an indispensable factor in the initiation of autophagy and participates in various physiological and pathological processes, including tumour formation, the immune response, heart disease, and neurodegeneration [73]. Beclin1 plays a crucial role in regulating autophagy vesicle formation by activating PI3KC3 (also known as Vps34) and promoting the formation of the PI3K complex (also known as VPS34 complex) [74,75]. Levels of Beclin1 were quantitatively detected via enzyme-linked immunosorbent assay (ELISA) in sera collected from 45 breast cancer patients and 30 apparently healthy controls before and after chemotherapy. The results revealed that Beclin1 serum concentration levels were significantly increased after chemotherapy [76]. There is controversy regarding the expression of Beclin1 in gastric carcinoma and its prognostic value. Qu et al. [74] found that 50.9% of gastric cancer tissues overexpress Beclin1 protein, whereas nonmalignant gastric tissues do not express or weakly express beclin1 protein. Furthermore, high expression of Beclin1 predicts a worse prognosis for gastric carcinomas, which already are characterized by poor overall survival (OS). Yu et al. [77] also confirmed that Beclin1 mRNA and protein were distinctively expressed in various gastric carcinoma cell lines and tissue specimens. Beclin1 expression was negatively linked to lymph node metastasis, venous invasion, distant metastasis and tumour–node–metastasis (TNM) staging with intestinal-type carcinoma and could be used as a potential biomarker to predict the favourable prognosis of GC patients. In contrast, Beclin1 expression downregulation was associated with poor prognosis in gastric cancer, which is different from the results reported by many previous studies [75]. However, it has also been shown that Beclin1 expression is often lower in other tumour cells than in normal cells, such as liver, breast and cervical carcinoma [78–80]. This discovery may be related to the dual role of autophagy in tumour development. Ueno et al. demonstrated that beclin1 immunoreactivity increased in breast cancer patients following neoadjuvant endocrine therapy and that pretreatment beclin1 status in stromal cells was correlated significantly with increased carcinoma cell proliferation and poor clinical and pathological responses to exemestane treatment [81]. The expression of Beclin1 could be used as an independent prognostic factor for gastric cancer. However, further research is helpful to verify the biological function of Beclin1 in gastric cancer.

5.2. LC3 and GC prognosis

LC3 is associated with autophagosome biosynthesis in mammals and has been extensively used as an autophagosomal biomarker. LC3 includes three isoforms (LC3A, LC3B, and LC3C) distributed in distinct tissues, among which LC3B (hereafter referred to as LC3) is the most widely studied and commonly detected isoform in cancers [82]. Each of the different isoforms has two forms in the cell, namely, LC3-I and LC3-II. During autophagy, LC3-I undergoes ubiquitination and is covalently bound to PE to generate LC3-II. Since the amount of LC3-II corresponds to the level of autophagy, autophagic activity can be monitored by detecting LC3-II levels, either through visualization of GFP-LC3 puncta using fluorescence microscopy or through the conversion of LC3-I to LC3-II using Western blotting [2]. Boone et al. observed that patients with more than a 51% increase in LC3, determined by evaluating LC3 puncta staining in circulating peripheral blood mononuclear cells of patients with pancreatic adenocarcinoma before and following gemcitabine/hydroxychloroquine (HCQ) treatment, showed improvement in overall survival (34.83 vs. 10.83 months) and disease-free survival (15.03 vs. 6.9 months) [83]. High LC3 expression was observed in 58% of the cytoplasm of gastric cancer cells (22 of 38 cases), whereas it was undetectable in noncancerous gastric epithelial cells. In many cases, LC3 expression was higher in tumour cells located in the central part than in the front of cancer nests, indicating that LC3 expression was advantageous to the early development of gastric cancer [84]. The correlation of upregulated LC3 expression with adverse prognosis of gastric carcinoma has been investigated extensively in a variety of studies [85–87]. Interestingly, Liao et al. [88] indicated that a high number of LC3A-positive “stone-like” structures were associated with increased invasiveness and poor prognosis of GC patients from stages I to IV. However, the mean concentration level of LC3 was sequentially reduced in serum samples from healthy subjects and patients with hepatitis C virus (HCV) and HCV-hepatocellular carcinoma (HCC) [89]. Similarly, a recent study demonstrated that low expression of LC3, closely linked to the low expression of Beclin1, significantly predicted lymph node metastasis and worse prognosis of GC [75]. Hence, LC3 may be considered an independent indicator of the prognosis of patients with gastric cancer.

5.3. P62/SQSTM1 and GC prognosis

Another promising novel prognostic hallmark of gastric cancer is proposed to be p62/SQSTM1, a multi-functional adapter protein that is a well-characterized substrate of ubiquitinated proteins in selective

autophagy [90]. P62/SQSTM1 localizes at the outer surface of autophagosomes by directly interacting with LC3. Then, p62/SQSTM1 is constantly eliminated via incorporation into mature autophagosomes. Defective autophagy is accompanied by insufficient degradation of the p62/SQSTM1 protein; therefore, p62/SQSTM1 and LC3 are ubiquitously used together as markers of autophagic flux in cancer research. In addition, p62/SQSTM1 functions as a scaffold in various intracellular signalling cascades, such as mTOR and nuclear factor kappa-B (NF-κB) signalling [91]. Immunohistochemical analysis of gastric carcinoma tissues revealed that 57% (35 of 61) showed moderate- to high-intensity p62/SQSTM1 protein staining in the nucleus, and 61% (37 of 61) exhibited low to moderate-intensity staining in the cytoplasm [92]. In a recent study of oesophageal adenocarcinomas, Olivia et al. reported that low p62 nuclear staining and high p62 dot-like/cytoplasmic staining were correlated with worse overall survival after neoadjuvant treatment. In vitro, cytoplasmic p62 showed stronger resistance to chemotherapy relative to nuclear p62 [93]. The clinical implications of autophagic flux impairment in nonalcoholic fatty liver disease (NAFLD) patients were determined by evaluating serum p62/SQSTM1 levels using an ELISA. The results showed that the expression level of serum p62/SQSTM1 was markedly increased in NAFLD patients relative to control subjects and positively correlated with lobular inflammation and steatosis [94]. Emerging lines of evidence have revealed that p62/SQSTM1 protein levels are upregulated, and the abundant accumulation of p62/SQSTM1 is correlated with adverse clinical outcomes in several types of malignant tumours, including gastric cancer [95], prostate cancer [96], triple negative breast cancer [97] and lung adenocarcinoma [98]. Consistent with this conclusion, Sun et al. [99] showed that p62/SQSTM1 protein levels were more significantly upregulated in GC samples than in normal gastric mucosae, and according to Kaplan-Meier curves, overall survival for GC patients with high p62/SQSTM1 expression was strongly worse relative to those in the low-expression group. Moreover, in gastric carcinomas, increased expression of p62/SQSTM1 tends to be correlated with less lymph node metastasis and poor differentiation [92].

6. Autophagy in GC treatment

Targeting autophagy in gastric cancers has been proposed to be a promising novel therapeutic strategy. Both autophagy inhibitors and autophagy inducers lead to tumour cell death and achieve antitumour effects, but currently they are only in the clinical development stage for the treatment of GCs.

6.1. Autophagy inhibitors in GC treatment

Autophagy can maintain the survival of gastric cancer cells for a period of time and potentiate the resistance of cancer cells to anticancer drugs. Thus, inhibition of autophagy destroys the protective mechanism and thereby promotes gastric cancer cell death induced by therapeutic drugs. Commonly used autophagy inhibitors include the lysosomal inhibitor chloroquine (CQ), the PI3K inhibitor 3-methyladenine (3-MA) and others.

CQ has been well-established as an autophagy inhibitor in cancer treatment, and its mechanism occurs mainly through lysosome and late endosome (LE) alkalinization, which blocks the fusion of autophagosomes with lysosomes, thereby leading to the accumulation of autophagic and endosomal cargoes [61,100]. In a phase II clinical trial, seventy-three patients with brain metastases from solid tumours were randomized to receive CQ (150 mg daily for 4 weeks) concomitant with whole-brain irradiation (WBI) or a matching placebo concomitant with WBI. One-year brain metastasis progression-free survival rates were 83.9% for the patients who received CQ and 55.1% for the placebo arm [101]. CQ enhances the chemosensitivity of MGC803 and SGC7901 cells to cisplatin (cDDP), with synergistic antitumour activity [64]. As shown by Xu et al. [63], the administration of CQ distinctly increased apoptotic cell death induced by oxaliplatin and improved oxaliplatin's inhibitory effect on the proliferation of gastric cancer cells in vitro. 3-MA, another widely used autophagy inhibitor, mainly inhibits the activity of PI3K and reduces the production of PI3P. A recent study [102] confirmed that curcumin alone induces protective autophagy to prevent apoptotic cell death in the gastric cancer cell lines BGC-823, SGC-7901 and MKN-28, but the combination of 3-MA and curcumin significantly promotes the induction of cell apoptosis, thereby enhancing the anticancer effect.

6.2. Autophagy inducers in GC treatment

In recent years, many studies have found that the induction of autophagy can also play an antitumour role in certain cases, which indirectly reflects the dual role of autophagy. The reason for this is that excessive autophagy leads to autophagic cell death, thereby inhibiting the proliferation of tumour cells. mTOR inhibitors and AMPK inducers are probably the most extensively used autophagy inducers.

mTOR inhibitors have also been shown to be effective as antitumour drugs that are responsible for activating the ULK complex to induce autophagy and inhibit angiogenesis [103]. Hashimoto et al. [104] reported that the chemokine CXCL12 induced activation of the mTOR pathway and played an important role in gastric cancer cell peritoneal metastasis. Thus, administration of the mTOR-specific inhibitor rapamycin induced autophagic cell death, which might represent a novel therapeutic strategy for peritoneal disseminated GC. Everolimus (RAD001), a derivative of rapamycin, has been approved by the FDA for the treatment of various tumours, including renal cell carcinoma [105], breast cancer [106], glioblastoma [107] and pancreatic neuroendocrine tumours [108]. A phase II clinical trial of 40 patients investigated low-dose everolimus in combination with cisplatin-HDFL (high dose 5-fluorouracil and leucovorin) chemotherapy in advanced gastric cancer patients and failed to observe an increased objective response in a preplanned statistical assumption but did observe prolonged progression-free survival in treatment-naïve patients [109]. Chen et al. [110] demonstrated that treatment of gastric cancer cell lines with everolimus inhibited cell proliferation by decreasing the phosphorylation of p70S6K and ERK (extracellular regulated protein kinases). In addition, as a glycylcycline class antibiotic drug, tigecycline also has been shown to have anticancer properties. Based on the observations of Tang et al. [111], tigecycline induces autophagy by activating the AMPK pathway and then decreasing the phosphorylation of mTOR and p70S6K, ultimately inhibiting human gastric cancer cell growth.

7. Conclusions and prospects

Autophagy is proposed to be a self-defensive pathway against certain stress conditions that plays not only a tumour-suppressive role but also a tumour-promoter role. On the whole, the biphasic function of autophagy contributes differentially to tumourigenesis depending on the developmental stage of the tumour and its type. The tumour-suppressor rationale is based on studies indicating that mice missing one allele of Beclin1 suppress autophagy and develop spontaneous tumours [112]. Low expression of beclin1 has been demonstrated in multiple tumours, including hepatocellular carcinomas [113], cervical squamous cell carcinomas [114] and osteosarcomas [115]. Support for the tumour-promoter mechanism comes from research in which inhibition of autophagy leads to tumour regression and prolonged lifespan in pancreatic cancer mouse models [116]. In addition, the analysis of numerous cancers has demonstrated that the core autophagy proteins, ATG5 and ATG7, are involved in tumour development and progression. For instance, upregulation of ATG7 expression was observed in bladder cancer [117], while upregulation of ATG5 expression was observed in prostate [118] and gastric cancers [119].

Although the exact molecular mechanisms of autophagy in gastric cancer remain to be clarified unequivocally, there is no doubt that

autophagy is associated with oncogenesis, development, prognosis and treatment of this malignancy, and further investigation is quite necessary.

Taken collectively, future studies are expected to focus on the following aspects. First, autophagy and apoptosis are two different types of programmed cell death (PCD) that execute gastric cancer cell fate. There are some differences between apoptosis and autophagy: these two different self-destructive programmes determine the turnover of entire cells and cytoplasmic organelles, respectively [120]. Autophagy exerts conflicting effects on the regulation of cell death, protecting cells from stress conditions to sustain cell survival or inducing programmed cell death. However, the regulatory effects of apoptosis on cell death seem unidirectional and can actively remove aging and abnormal cells to play a scavenger role. Of note, a great deal of evidence suggests complex crosstalk between the regulatory circuits governing apoptosis and autophagy, but the exact mechanisms of this crosstalk are still not entirely clear. The interconnection between these two processes resides in the PI3K/AKT/mTOR signalling pathway and the regulatory protein Beclin1 [121]. A number of studies have documented that in gastric cancer cells, autophagy determines cell fate with apoptosis via independent [122] or cooperative [123] routes. However, to some extent, these two routes may interact. The inhibition of autophagy, coinciding with the promotion of apoptosis, can jointly enhance the sensitivity of MGC-803 cells to chemotherapy drugs [124]. Second, related proteins involved in autophagy, such as Beclin1, LC3 and p62/SQSTM1, are expected to become novel prognostic biomarkers of gastric cancer. Nevertheless, according to a comparative analysis of previous studies, there are a few discrepancies in the expression and prognostic value of these autophagy-related proteins. Such discrepancies may be explained by the dichotomous role of autophagy in tumour development, and most discrepancies still need to be further verified by standardizing the sample source, sample size and detection methods. Third, both autophagy inhibitors and autophagy activators have achieved evident clinical trial results in the treatment of gastric cancer and broad clinical application prospects. Furthermore, the two opposing effects of autophagy in the treatment of gastric cancer also need to be considered. The next clinical trial design will focus on how to maintain balance between the dual roles and maximize the efficacy of chemotherapy drugs in gastric cancer therapy. Finally, autophagy-related drugs mostly act by activating or blocking signalling pathways, and AMPK/mTOR and PI3K/Akt/mTOR have been identified as the main signalling pathways regulating autophagy. Therefore, thorough research on the molecular mechanisms of signal transduction in autophagy is helpful to better target autophagy to kill cancer cells and improve clinical outcomes in patients with gastric cancer.

In general, there is still a long way to go to clarify all of the issues described above, so further research regarding the specific role of autophagy in gastric cancer is necessary in the future. We believe that, along with gradual deepening of our understanding of autophagy, autophagy will become a novel target for the clinical treatment of gastric cancer.

Disclosure

Conflicts of interest: The authors report no conflicts of interest.

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