Autophagy in Gastric Cancer Progression

Subjects: Pathology

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Gastric cancer is the fifth most common malignancy and the third leading cause of cancer-related death worldwide. The three entirely variable entities have distinct epidemiology, molecular characteristics, prognosis, and strategies for clinical management. However, many gastric tumors appear to be resistant to current chemotherapeutic agents.

Keywords: autophagy ; autophagy inducers ; autophagy inhibitors ; autophagy regulation ; chemotherapy ; gastric cancer

1. Introduction

Gastric cancer (GC) constitutes the fourth most frequent cause of death, due to malignancy and the fifth most commonly detected cancer worldwide [1]. A higher incidence is demonstrated in many countries among the continents such as in Western and Eastern Asia, Eastern Europe, and South America [2]. The gender disparity is reflected by the cumulative risk of mortality from birth till the age of 74 years, which is 0.57% for women and 1.36% for men. Despite the continuous amplification of GC cases in the last five decades, this trend is nowadays gradually decreasing due to the more efficacious treatment regimens for Helicobacter pylori (H. pylori) eradication, which composes a major factor for gastric carcinogenesis [3]. In view of the above, GC exhibits not only geographical variation, implying the influence of local environmental risk factors but also male predominance, with two-fold higher incidence for men [4][5], whereas the risk is equal for post-menopause women [6]. A familial predisposition for GC is demonstrated in the minority of GC cases (10%), while 1–3% of them are correlated with inherited syndromes such as gastric adenocarcinoma and proximal polyposis of the stomach syndrome (GAPPS), diffuse gastric cancer (HDGC), familial adenomatous polyposis (FAP), andhereditary non-polyposis colorectal cancer (HNPCC), and PeutzJegher’s syndrome [7].

The subdivision of GC is anatomically based, with two entities: the (i) non-cardia GC and the ii) cardia GC. The former is reported twice asfrequently asthe latter [8], constituting the majority of the cases (80–90%), and it is associated with H. pylori infection [9], as well as with dietary habits [10], economical, and sociological state, while the latter has an epidemiological background resembling that of esophageal adenocarcinoma (EAC), mostly in developed countries [11]. Different risk factors are taking part in gastric carcinogenesis based on the anatomical region. A stepping stone in distal, mainly antral, non-cardia GCs is H. pylori . Infection, resulting in gastritis and ulcers formation [12], increases almost six-fold the risk for GC in chronic infection in a span of ten years [13]. Based on AGA- 2020 Clinical practice guidelines, recommendation 1, patients with positive biopsies for pre-dysplasia stages asin gastric intestinal metaplasia (GIM) must be tested for H. pylori , and if infection occurs, it must be eradicated [14], which significantly reduces the risk for GC [15]. Obesity is linked with cardia GC, while esophageal pathologies such asBarrett’s esophagus and gastroesophageal reflux disease (GERD) are correlated with carcinogenesis in gastroesophageal junction [16]. Viral infection with EBV increases the risk of cancer development [17], while it accounts the 10% of the intestinal entity of GC, related also with microsatellite instability (MSI) [18][19]. Iatrogenic risk factors promote gastric carcinogenesis, such asthe long-term abuse of proton-pump inhibitors(PPIs) [19] and Bill Roth anastomosis [20][21].

Gastric carcinogenesis is a multifactorial event arising from deregulated pathways of signaling, mutated genes, and epigenetic aberrations, in combination with the influence of environmental factors. A huge range of natural products including tunicamycin, medicinal plants and microorganisms including flavonoids, coumarins, terpenoids, alkaloids, etc. have been identified as potential autophagy modulator and multidrug-resistance-reversal agents [22]. In addition, tunicamycin has been initially identified as a natural antibiotic and anticancer agent. It has suggested that tunicamycin inhibits N-glycosylation to aggravate endoplasmic reticulum stress, trigger autophagy, and increases the sensitivity of gastric cancer cells to Adriamycin and Vincristin. Moreover, the natural product genipin can induce p53 and DRAM expression and trigger apoptosis and autophagy in GC [22]. Out of all GC cases, 95% of them are adenocarcinomas, resulting in a multistep cancer progression (Correa Cascade) [23][24]. Based on the above, chronic gastritis followed by atrophic gastritis leads to intestinal gastric metaplasia, which further leads to dysplasia and adenocarcinoma [24]. There are two histological entities for GC—(i) the diffuse and (ii) the intestinal types of GC [25]—with the former being less differentiated than the latter, while the latter is well-differentiated with more frequent occurrence and a better outcome [26].

Based on a large number of preclinical studies, disturbances of autophagy machinery areclosely associated with tumorigenesis, as well as with metastasis and dismal outcomes, althoughit may act as a putative therapeutic approach for different cancer types, including gastric cancer. In this review, we gathered information from the current clinical and preclinical research data about autophagy modulation in gastric cancer and the therapeutic strategies for this highly invasive malignancy.

2. Targeted Autophagy as Putative Therapeutic Approach

Based on the qualities of autophagy as either a suppressor or stimulator of cancer growth, autophagy-based anticancer drugs are in the spotlight, including autophagy inhibitors and inducers. Autophagy inducers, such as mTOR inhibitors in cases of GC-disseminated-type or AMPK homeostatic pathway activators such as the antibiotic substance Tigecycline [27], could be used in cases of chemoresistant GC, in which other anti-cancer treatments failed to reduce the cancer progression. These are PI3K complex inhibitors and lysosome-specific targeted drugs, such as hydroxychloroquine (HCQ) and chloroquine (CQ) [28]. Lysosomes could be used as a therapeutic target via the blockage of the formation of autophagolysosome [28][29]. PI3K inhibitor and CQ could have a synergic role with other types of anti-cancer treatment, such as cisplatin, which reduces the chemoresistance of gastric cancer lines [30], andin case of its combination with oxaliplatin, they have enhanced anti-growth action for gastric cancer cells.

2.1. Autophagy Enhancer Agents

Numerous scientific research studies indicate the close relationship between the tumor micro-environment with autophagy pathway, as well as with the inducted anti-neoplastic immune reaction, in many malignancies, including GC.The influential characteristics of autophagy open up new horizons for the evolution of new anti-cancer substances.Some of the most remarkable autophagy inductors are Rapamycin inductors, including the inhibitors of mTOR, rapalogs, and Rapamycin analogs [31]. Some noteworthy rapalogs are everolimus, as well as temsirolimus, while deforolimus is a rapamycin analog, which activates the autophagy mechanism [31]. It is reported that the addition of Paclitaxel inEverolimus therapy has a significant suppressive effect on endometrial cancer cell progression [32]. There is a notable effect of Rapamycin as an anti-cancer treatment, which includes the activation of the autophagy pathway, the enhancement of radiationtherapy's effect on lung cancer cells of the -A549 type, and it also influences the DNA- repair process [33]. Although these autophagy inductors have a significant potential role in anti-neoplastic therapeutic schemes, further investigation is needed for their usage in clinical oncology [34].

Moreover, Metformin, a noteworthy substance for its pharmaceutical properties, constitutes an autophagy activator [35], such as in the case of pulmonary adenocarcinoma, which undergoes apoptosis through tumor-necrosis-factor (TNF-related-Apoptosis-Inducing-Ligand (TRAIL)) [36]. For breast malignancy, in the absence of mutant BRCA1 gene, metformin can be included in therapeutic schemes with spautin-1, which constitutes an autophagy suppressor, resulting in an altered mitochondrial functional state and inducing a notable reduction incancer cell survival and progression [35][37]. Furthermore, significant autophagy suppressors are mTOR inhibitors, such as alkaloids [37][38], including cepharanthine, liensinine, andisoliensinine [32], while they induce phosphorylation of the AMPK pathway. The above autophagy activators demonstrate great results in cases of resistant apoptosis in Mouse Embryonic Fibroblasts (MEFs) [39]. Another autophagy activator, a pan-inhibitor of anti-apoptotic Bcl-2 proteins that exhibits a cytotoxic effect on cancer cells through both apoptosis-dependent and -independent pathways, the so-called Obatoclax [40], is correlated with mitochondrial-pathway apoptosis via targeting the Bcl-2 protein family, and it is also linked with autophagy-complexes' death via necroptosis [35] [39]. Last but not least, the antioxidant omega-3polyunsaturated fatty acids have a key role in autophagy activation [41], constitute a potent adjuvant anti-cancer agent, such as in case of cholangiocarcinoma, while they do not have notable toxicity [32]. These agents activate 15-hydroxyprostaglandin dehydrogenase, which leads to the suppression of prostaglandin E2 (PGE2), which is a causative factor for the above malignancy [35]. In **Table 1**, we summarize some of the autophagy activators and the main mechanisms of action that are mostly known.

Table 1. Autophagy activators and their main mechanism of action.

Agents	Mechanism of Action	Target
Rapamycin	**mTORC1 inhibitor**	**Formation of Autophagosome**
Deforolimus	**mTORC1 inhibitor**	**Formation of Autophagosome**
Temsirolimus	**mTORC1 inhibitor**	**Formation of Autophagosome**
Everolimus	**mTORC1 inhibitor**	**Formation of Autophagosome**

Agents	Mechanism of Action	Target
GDC-0941	PI3K Class I inhibitor	Formation of Autophagosome
GDC-0980	PI3K and mTORC1 inhibitor	Formation of Autophagosome
Tat–Beclin-1 peptide	Releases Beclin-1 into cytoplasm	Formation of Autophagosome
Perifosine	AKT inhibitior	Formation of Autophagosome
Metformin	AMPK activator	Formation of Autophagosome
fluspirilene	Antagonists of L-type Ca^{2+} channels	Lysosome
cepharanthine	Natural alkaloid	Autophagic flux
isoliensinine	Natural alkaloid	Autophagic flux

mTORC1: mammalian target of rapamycin complex 1; AMPK: 5′ AMP-activated protein kinase; PI3K: phosphatidylinositol 3-kinases; AKT: Protein kinase B (PKB); Beclin-1: the mammalian ortholog of the yeast autophagy-related gene 6 (Atg6).

2.2. Autophagy Inhibitors

In the past few years, except for the conventional cancer therapies such as radiation therapy and chemo-immunotherapy, a new anti-cancer therapeutic strategy is in the spotlight, including autophagy-based treatments, such as autophagy inhibitors [42]. As was previously underlined, autophagy can serve as either a suppressor or promoter of carcinogenesis. These new regimens make use of the basic properties of the autophagy pathway and their influence on the metabolic state and the endurance of cancer cells [43]. Autophagy inhibitors that are broadly noted are HCQ, CQ, and Lys05 (dimeric of CQ), which are used in many cancers, interfering with the formation of the autophagolysosome. The latter exhibits a strong anti-neoplastic effect as a modifier of lysosomal function [44]. Despite the fact that they exhibit adequate effectiveness as a combination treatment with other anti-cancer regimens [45], as a monotherapy, they demonstrate a restricted performance as a consequence of their discontinuous inhibitory effect [46]. In animal models, the combination of CQ with Interleukin-2 has shown benefits in secondary hepatic cancer, with limited toxic effects and improved prognosis [42]. A great improvement in pancreatic cancer progression is also noted, in which Gemcitabine is combined with HCQ, with an important decrease inCA19-9 neoplastic marker (60%) [47]. Although these inhibitors show beneficial effects on cancer treatment, they can provoke interactions with other pharmaceutical agents, and they can induce alterations in the tumor microenvironment [44][46].

Due to the fact that their effect cannot be assessed by specific markers, other current inhibitors are used in therapeutic schemes [42]. The initiation step is highly regulated by many proteins such as the ULK1 as well as the Vps34-signaling pathway, including some critical proteins such as Vps34, Beclin-1 and Vps18, which have a significant role in the conveyance of the vesicles, as well as the lysosomes [48]. Inhibition of the above key-proteins for the initiationstep of autophagy exhibits an intense anti-neoplastic effect, starting with SBI-0206965, a highly selective ULK1 inhibitor [49], as well as Beclin-1 suppressors, which induce cancer cell death via the stimulation of more CCL5 expression in cancer cells that attract Natural-Killer cells to them [42]. Moreover, suppressors such as SAR405 inhibit Vp34 and lead to the alteration of lysosomal function [48], while spautin -1 inhibits USP10 and USP13 peptidases (ubiquitin-specific peptidases) [50]. Additionally, the level of autophagolysosome formation is targeted by many medical substances such as clomipramine, desmethylclomipramine (DCMI), and [51], with the enhancement of DCMI efficiency by adding doxorubicin, as was demonstrated in in vitro studies [52].

In some cases, inhibition of the autophagy pathway could limit the immune response to carcinogenesis and could lead to cancer cell progression and survival.However, this hypothesis has proved wrong based on studies for breast cancer and melanoma. Subsequently, for the intensification of the anti-neoplastic immune response, autophagy suppressors are used in combination with other chemotherapeutic substances [27][53]. In **Table 2**, we summarize some of the autophagy inhibitorsand the main mechanism of action that are mostly known.

Table 2. Autophagy inhibitors and their main mechanism of action.

Agents	Mechanism of Action	Target
Chloroquine (CQ)	Neutralizes the acidic pH of intracellular vesicles	Lysosome
Hydroxy-chloroquine (HCQ)	CQ derivative	Lysosome
Bafilomycin A1	Inhibition of lysosomal acidification	Lysosome

Agents	Mechanism of Action	Target
Azithromycin	Inhibition of lysosomal acidification	Lysosome
Concanamycin A	Inhibition of lysosomal acidification	Lysosome
3-Methyladenine (3-MA)	PI3K- Class III inhibitor	Formation of Autophagosome
Wortmannin	PI3K- Class III inhibitor	Formation of Autophagosome
LY294002	PI3K- Class III inhibitor	Formation of Autophagosome
LY3023414	PI3K- Class III inhibitor	Formation of Autophagosome
SAR405	Vps18 and Vps34) inhibitor	Formation of Autophagosome
SB203580	Inhibit trafficking of Atg9	Formation of Autophagosome
Paclitaxel	Microtubule stabilizer inhbits phosphorylation of VPS34	Formation of Autophagosome
SAHA	Inhibit fusion of autophagosome and lysosome	Formation of Autophagosome
Sputin-1	(USP10) and (USP13) inhibitor	Formation of Autophagosome
NSC185058	ATG4 inhibitor	Formation of Autophagosome
Verteporfin	Alter lysosomes accedification	Formation of Autophagosome

VPS34: vacuolar protein sorting-associated protein 34; mTORC1: mammalian target of rapamycin complex 1; PI3K- Class III: Phosphoinositide 3-kinases (PI3Ks) class III.

Finally, the utilization of autophagy properties opened new horizons for developing new anti-cancer therapeutic agents and intensifying the effect of other conventional anti-neoplastic treatments for many malignancies.For example, the inactivation of AKT can succeed via Perifosine, which constitutes an alkylphospholipid that demonstrates anti-cancer activity. Combinational treatment with Perifosine and NH4Cl or CQ induces apoptosis, as well as limitation of tumor progression and expansion [54]. It is reported that a combinational therapeutic scheme with HCQ, an autophagy inactivator, and Temsirolimus, which is an mToR inactivator, has been utilized in late-stage solid tumors or in case of melanoma; however, this clinical trial is in phase I [55]. Moreover, in head and neck malignancies, such as in squamous cell carcinoma, the use of CQ with either oprozomib or carfilzomib, which are next-generation proteasome inhibitors, demonstrates activation of autophagy pathway and cancer cell destruction [56]. Another combinational therapy is propachlor with the mTOR inhibitor Everolimus, which act as autophagy activators and lead to malignant celldeath in prostate cancer. Another mTOR inhibitor, RAPA, when combined with temozolomide-treated, shows beneficial effects in cases of glioma, with the death of U251 cells [57]. Autophagy induction via isoliquiritigenin, in combination with 3-MA, leads to the enhancement of anti-cancer response in ES-2 cells [57]. Multiple events, such as the accumulation of proteins induced by CQ in lysosomes and protein aggregation in cytosol, induced by Bortezomib, possibly leads to mitochondrial function disturbances, followed by the activation of Apaf-1, which contains apoptotic complex and the release of cytochrome c [57]. Further research is needed for the handling of GC, which remains a difficult task in clinical practice.

3. Conclusions

Overall, management of GC remains a difficult task for clinical practitioners, mainlyattributed to the increased chemoresistance of this malignancy in conventional therapeutic approaches. The autophagypathway is the focus of many scientific studies with respect toits properties as a physiological cellular adaptation mechanism in stressful conditions, as well asits binary function in cancer, either as suppressor or inducer of cancer progression. Promisingopportunities have opened up for the development of new therapeutic strategies againstmany malignancies, including GC. The combination of conventional andautophagy-based anti-neoplastic agents are showing promising results in in vitro studies. However, there are limitations such as discontinuous inhibition of autophagy, interactions with other pharmaceutical agents, and alterations in tumor microenvironment and the anti-neoplastic immune response.Despite the abovelimitations, autophagymodulators open up new horizons as treatmentstrategies for GC, as well as a combinational treatment with other chemotherapeutic agents, promising better therapeutic results and elongated survival by enhancing chemosensitivity or restoring the drugresistance of GC.In conclusion, further investigation is required for the controversial role of autophagyand the manipulation of its multiphasic nature, with a wide variety of druggable targets, for the creation of a novel anti-neoplastic medical treatment.

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