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Review Article

Autophagy in pancreatic cancer pathogenesis and treatment

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Abstract: Pancreatic cancer is the fourth most common cancer to cause death due to advanced stage at diagnosis and poor response to current treatment. Autophagy is the lysosome-mediated degradation pathway which plays a critical role in cellular defense, quality control, and energy metabolism. Targeting autophagy is now an exciting field for translational cancer research, as autophagy dysfunction is among the hallmarks of cancer. Pancreatic tumors have elevated autophagy under basal conditions when compared with other epithelial cancers. This review describes our current understanding of the interaction between autophagy and pancreatic cancer development, including risk factors (e.g., pancreatitis, smoking, and alcohol use), tumor microenvironment (e.g., hypoxia and stromal cells), and molecular biology (e.g., K-Ras and p53) of pancreatic cancer. The importance of the HMGB1-RAGE pathway in regulation of autophagy and pancreatic cancer is also presented. Finally, we describe current studies involving autophagy inhibition using either pharmacological inhibitors (e.g., chloroquine) or RNA interference of essential autophagy genes that regulate chemotherapy sensitivity in pancreatic cancer. Summarily, autophagy plays multiple roles in the regulation of pancreatic cancer pathogenesis and treatment, although the exact mechanisms remain unknown.

Kewords: Autophagy, pancreatic cancer, oncogene, hypoxia, pancreatitis, HMGB1, RAGE, p53, HIF1α, AMPK

Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the USA for both men and women; 30,500 new cases will occur annually [1]. It is responsible for 5% of all cancer-related deaths; the overall five year survival rate is 3~5% because of advanced stage at diagnosis and poor response to current treatment [2]. Eighty percent of all pancreatic cancers are pancreatic ductal adenocarcinoma (PDAC) [3, 4]. Morphologically, PDAC progresses from non-invasive pancreatic lesions termed "Pancreas Intraepithelial Neoplasias" (PanINs) (**Figure 1**) [5]. PanINs show glandular patterns with duct-like structures and varying degrees of cellular atypia and differentiation. PanINs undergo a neoplastic transformation ranging from early mucinous change (PanIN-1) to frank carcinoma *in situ* (PanIN-3). Genetically, PDAC progresses as a result of the accumulation of genetic alterations in cellular cancer-causing genes, such as encoding of the small GTPase protein *K-Ras* and tumor suppressors such as *INK4A /p16*, *p53*, *BRCA2*, and *DPC4/SMAD4* (**Figure 1**) [3]. Of these genetic alterations, mutations of the *K-Ras* gene occur in over 90% of pancreatic carcinomas and are proposed to be the initiating genetic lesion in PDAC. Epidemiologically, factors such as smoking, alcohol use, diet, obesity, and chronic pancreatitis can increase the risk of acquiring the genetic mutations that may potentially result in pancreatic cancer [6]. Moreover, developmental regulatory pathways, in particular the Wnt, Notch, and Hedgehog pathways, are often activated in PDAC [7]. In addition, deregulation of high mobility group protein box 1 (HMGB1) - the receptor for advanced glycation end products (RAGE) signaling pathway [8, 9] and the tumor microenvironment of PDAC (e.g., inflammation and stromal cells) [10, 11] are closely associated with pancreatic cancer development. Autophagy, a lysosomal degradation pathway, has been recently demonstrated as active in PDAC and regulates chemotherapy sensitivity during pancreatic treatment [12-19].

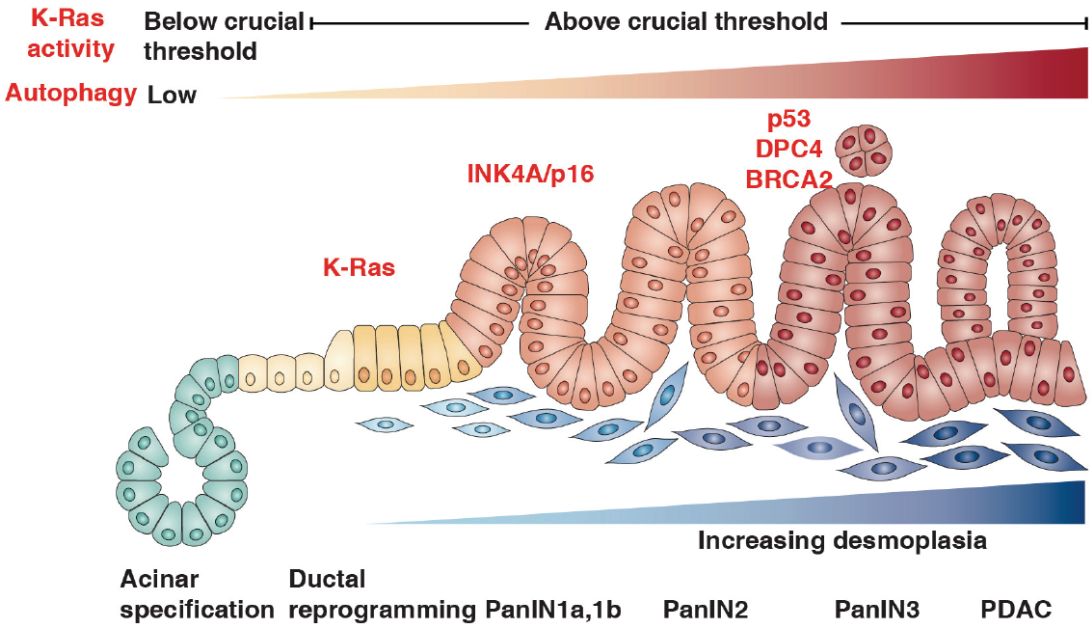

Figure 1. Progression model for pancreatic ductal adenocarcinoma. PDAC progresses from PanINs. The progression from histologically normal ductal epithelium to low-grade PanIN to high-grade PanIN is associated with the accumulation of specific genetic changes. Early changes include K-ras mutations; intermediate changes include INK4A/p16 mutations; and changes associated with either *in situ* or early invasive cancer include p53, BRCA2, and DPC4/SMAD4. Elevated expression of autophagy in cancer cells has been implicated in the development of PDAC.

Our findings suggest that the HMGB1-RAGE pathway plays critical roles in induction of autophagy, and inhibition of HMGB1-RAGE leads to increased apoptosis and decreased autophagy in pancreatic cancer cells [9, 20-23]. Current studies aim to fully understand the emerging role of autophagy in pancreatic cancer formation and progression and determine whether targeting autophagy represents a valid approach to pancreatic cancer treatment in the clinic.

Autophagy and cancer

Lysosomes are cellular organelles that contain acid hydrolase enzymes to break down waste materials and cellular debris. Autophagy is a lysosomal degradation pathway by which cytoplasmic components, such as damaged organelles and unused long-lived proteins, are digested within lysosome [24]. There are at least three distinct types of autophagy pathways, including macroautophagy, chaperone-mediated autophagy, and microautophagy [25]. Macroautophagy (referred to hereafter as autophagy) is a dynamic process by which cytoplasmic components are engulfed by an isolation membrane structure termed the phagophore, and then are sequestered in a double or multimembrane structure termed the autophagosome, which is finally delivered to the lysosome to form an autolysosome where their contents are degraded (**Figure 2**). Potential sources for the phagophore include the Golgi complex, endosomes, ER, mitochondria, and plasma membrane [26]. Although nonselective autophagy is the main autophagic degradation process, evidence of a more selective degradation of particular organelles (e.g. mitochondria) and intracellular bacteria/virus has been found. These processes have been termed mitophagy [27], and xenophagy [28, 29], respectively.

The molecular machinery that controls the autophagy pathway is extremely complex [30, 31]. Over 30 different autophagy-related genes (ATGs) have been identified in yeast, and many of these are functionally conserved in higher eukaryotes. Autophagy is induced by the inhibition of mammalian target of rapamycin (mTOR) [32], resulting in the formation of class III phosphoinositide 3-kinase (PI3KC3) -Beclin 1 (also known as Atg6 in yeast) core complex [33], which mediates nucleation of the phagophore in

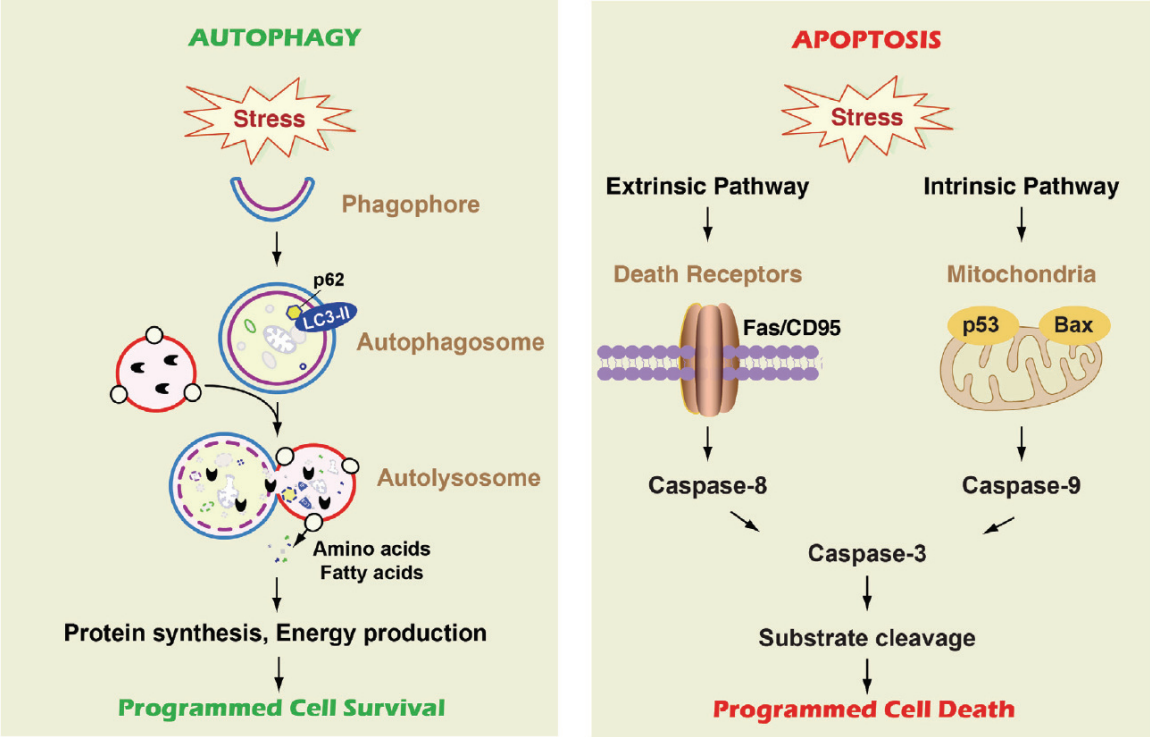

Figure 2. Autophagy and apoptosis: process and function. Autophagy and apoptosis may be triggered by common stimuli. As programmed cell survival, autophagy is an intracellular bulk degradation system, through which cytoplasmic component is delivered to lysosomes to be degraded. The main process of autophagy includes formation and maturation of the phagophore, autophagosome, and autolysosome. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing to LC3-II and can be degraded by autolysosome after binding adaptor protein p62. Autophagy provides amino acids and fatty acids for the synthesis of protein and ATP. In contrast, apoptosis is programmed cell death. There are two main pathways to control apoptosis. The extrinsic pathway is mediated by death receptors such as Fas/CD95. The intrinsic mitochondrial pathway is controlled by Bcl-2 family proteins such as Bax and other regulators, such as p53. Caspase 3 is a caspase protein that is activated by caspase 8 and caspase 9, and in turn, mediates substrate cleavage and cell destruction.

autophagy. After that, two ubiquitin-like conjugation systems (Atg8 and Atg5-Atg12-Atg16) are essential for autophagosome formation. Microtubule-associated protein 1 light chain 3 (LC3), a homologue of Atg8 in yeast, is widely used to monitor autophagy by western blot analysis of turnover of LC3-I to LC3-II, and imaging analysis of LC3 puncta formation under co-treatment with inhibitors of early (e.g., 3-methyladenine) and late (e.g., chloroquine and bafilomycin-A1) autophagy [34]. The lysosomal-associated membrane protein 1 (LAMP1) and LAPM2 and the small GTPase RAB7 are involved in autolyosomal formation.

As programmed cell survival mechanism, autophagy is critical in maintaining a range of normal human physiological processes, such as cellular homeostasis, energetic balance, development, and cellular defense against danger signals [35, 36]. Conversely, autophagy is a double-aged sword, implicated in the pathogenesis of diseases including cancer, neurodegenerative diseases, aging, muscle diseases, infection, and immunity diseases [37-41].

The role of autophagy in cancer is extremely complex [42-46]. Decreased, increased, and mutant ATGs expressions are detected in various tumors. Autophagy was initially as an anti-oncogenic mechanism in the tumor, because heterozygous Beclin1$^{+/-}$ mice develop spontaneous tumors [47]. Moreover, deletion of other autophagy genes such as *UVRAG* [48], *Atg5* [49] and *Bif* [50] in mice also increase tumorigenesis. It is believed that autophagy partly pro-

motes cancer development through its ability to shape inflammatory reaction [51], metabolic requirements [52], and oxidative stress [53]. In addition, autophagy has a pro-tumor growth effect in some cancers such as pancreatic [12] and *BCR-Abl*-mediated leukemogenesis [54]. Thus, autophagy may play opposite roles in the early and late cancer development stages. One possible explanation for this difference is that the content of metabolic stress, immune response, and microenvironment is different. Autophagy and apoptosis share common stimuli, signal pathways, and regulators (**Figure 2**) [55]. Interestingly, apoptosis, as a programmed cell death process, also has dual roles in regulation of tumorigenesis [56]. The inter-relationship between autophagy and apoptosis might decide tumor cell's fate. In many cases, blockage of autophagy sensitizes tumor cells to anticancer therapy, including chemotherapy, radiation, and immunotherapy [57]. However, under certain conditions such as apoptosis deficiency, autophagy can also be pro-death, termed "autophagic cell death" [58, 59].

Autophagy and pancreatic cancer development

Pancreatic tumors have more elevated autophagy under basal conditions than those of other epithelial cancers [60]. A clinicopathological study of 71 archival pancreatic cancer tissues demonstrated that autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome [19]. They found a stronger LC3 intensity expression level in the peripheral area of the pancreatic cancer tissue by immunohistochemical staining with LC3 antibody. Moreover, strong expression of LC3 is associated with enhanced expression of the hypoxia marker carbonic anhydrase IX at the peripheral area, which correlates with poor outcome and a short disease-free period. Another study showed that LC3 is low or absent in normal exocrine pancreas and in low-grade PanIN-1 and PanIN-2 lesions, whereas staining is upregulated and exhibits a vesicular staining pattern in all high-grade PanIN-3 and PDAC (**Figure 1**). These studies suggested that autophagy may contribute to pancreatic tumor progress. Basal autophagy is significantly elevated compared with non-transformed human pancreatic ductal and other tumor cells, confirmed by monitoring LC3 spot formation in PDAC cell lines and primary tumors [12]. Moreover, knockdown of autophagy genes such as ATG5 and ATG3 by RNA interference significantly inhibits cell growth and colony formation of PDAC cell lines *in vitro* [12]. Furthermore, chloroquine, an anti-malaria drug with autophagy inhibition activity, suppressed pancreatic tumor growth in xenograft mice [12]. These studies suggest that autophagy can contribute to pancreatic tumor growth and development. *In vitro*, inhibition of autophagy in pancreatic tumor cells augments production of reactive oxygen species, increases DNA damage, and limits effective metabolism via decreased mitochondrial oxidative phosphorylation [12], suggesting a unique role of autophagy in pancreatic tumorigenesis through regulation of mitochondrial function and energy production. Notably, chloroquine has multiple functions independent from autophagy [61]. Thus, specific autophagic gene deficient mice will confirm whether autophagy promotes pancreatic tumorigenesis. Our recent study indicates that global knockout of RAGE in mice inhibits interleukin-6 (IL-6) / signal transducer and activator of transcription 3 (STAT3) induced autophagy and K-Ras mutant driven PanINs development [62].

Autophagy and pancreatic beta-cell

The pancreas is comprised of separate functional units that regulate two major physiological processes: digestion and glucose metabolism. Blood glucose metabolism is controlled by the pancreatic beta-cells, which are located in the islets of Langerhans. Deficiency in the number of pancreatic beta cells causes diabetes. Beta cell growth is disordered in pancreatic cancer [63]. K-ras in islet cells leads to tumor only with inflammation [64, 65]. Recent studies suggest that basal levels of autophagy are also required for maintenance of beta cells homeostasis in the pancreas [66]. Beta cell -specific Atg7 knockout mice had increased apoptosis and reduced proliferation of beta cells, which resulted in reduced beta cell mass and pancreatic insulin content [66]. Moreover, pancreatic islets from mice with Beta cell-specific Atg7 knockout mice had decreased adenosine triphosphate (ATP) production [66]. Islet cell destruction had functional consequences, because the remaining cells were depleted of insulin and the mice developed hyperglycemia that was worsened by a high-fat diet [67]. Autophagy and vacuole membrane protein 1 (VMP1) expression are early cellular events in experimental diabetes [68].

Autophagy and risk factors for pancreatic cancer

Acute pancreatitis precedes chronic pancreatitis, which is a significant risk factor for the development of pancreatic carcinoma [69, 70]. Several experimental models of severe acute pancreatitis have been produced in rats or mice by administration of cerulein, bile acid (e.g. taurocholate) or L-arginine, or by feeding mice a choline-deficient, ethionine supplemented diet [71]. Cerulein-induced pancreatitis promotes oncogenic transformation of adult pancreatic acinar cells by oncogenic K-Ras in a mice model [10]. In the early stage of acute pancreatitis, autodigestion is provoked by inappropriate activation of proteolytic enzymes within the acinar cells. These early events are followed by the generation of local inflammatory reaction, as well as systemic inflammatory response syndrome. Recent studies suggest that the impaired autophagic and lysosomal dysfunctions mediate key pathologic responses of pancreatitis, such as acinar cell vacuolation and trypsinogen activation [72-78]. Pancreatitis stimulates autophagy induction and at the same time impairs late stages of autophagy [78]. Inhibition of autophagy by genetic or pharmacologic means markedly improved cerulein-induced pancreatitis. It was indicated that autophagic flux is reduced in pancreatitis due to deficient lysosomal degradation caused by impaired cathepsin processing and activation, and decreased levels of LAMP-1 and -2 [72]. Moreover, there is a decrease in the fully mature ("double-chain") cathepsin forms and accumulation of their immature forms in pancreatitis [78]. It is unclear how cathepsin processing becomes defective or how this relates to reported decreases in other lysosomal proteins. It is indicated that pancreatitis-like injury spontaneously develops in LAMP-2 knockout mice [79]. Of note, LAMP-2 is up-regulated in many cancer cell lines and human tumors, including pancreatic cancer [80]. Knockdown of LAMP-2 resulted in tumor shrinkage by regulation of p53-mediated glycolytic metabolism [81].

Currently, there are no studies exploring the direct relationship between autophagy and other risk factors factor for pancreatic cancer, only pancreatitis. Alcohol use, smoking, and diet are all implicated as possible pancreatic cancer risk factors. Autophagy is induced by alcohol and prevents liver injury [82]. Cigarette smoke-induced autophagy is involved in early stages of chronic obstructive pulmonary disease progression [83]. A high-fat diet stimulates autophagy in β cell and regulates glucose metabolism [67]. In addition, high-fat diet-induced hepatic steatosis was associated with autophagy [84]. Thus it is interesting to explore whether these factors also induce autophagy in the pancreatic cancer cell.

Autophagy and microenvironment of pancreatic cancer

Accumulating evidence suggests that the tumor microenvironment is in a hypoxic condition [85, 86]. The tumor microenvironment includes immune cells (lymphocytes, natural killer cells, and antigen presenting cells), stromal cells (including fibroblasts/stellate cells), and the vasculature (pericytes and the endothelium). The tumor microenvironment directs pancreatic tumor growth [87]. Stromal cells stimulate cancer cell growth and invasion through the chemokine-chemokine receptor axis. The tumor vasculature allows nutrients and oxygen uptake by tumors and tumor-infiltrating immune cells and damage associated molecular pattern molecules (DAMPs) anergize the immune effectors.

Autophagy can be induced by hypoxia (**Figure 3A**). Hypoxia-inducible factor 1 (HIF) is a master regulator of oxygen homeostasis. In many case, hypoxia-induced autophagy is mediated through HIF-1α induction of Bcl-2/adenovirus E1B 19-kDa protein-interacting protein 3 (Bnip3) and BNIP3 like protein (BNIP3L) in several cells, including cancer cells [88-90]. Bnip3 is a member of the Bcl-2 homology domain 3-only subfamily of proapoptotic Bcl-2 proteins and is associated with cell death. However, another study suggests that hypoxia-induced autophagy in tumor cells is AMP-activated protein kinase (AMPK)-mTOR dependent, but not HIF-1α, BNIP3, and BNIP3L dependent [91]. In addition, autophagy may be induced during hypoxia as a result of signals generated by the unfolded protein response in the endoplasmic reticulum. The PKR-like endoplasmic reticulum kinase (PERK)-activating transcription factor 4 (ATF4) pathway is involved in unfolded protein response associated autophagy. Recent studies demonstrate that autophagy mediates survival of pancreatic tumor-initiating cells in a hypoxic microenvironment [92]. They found that physiological en-

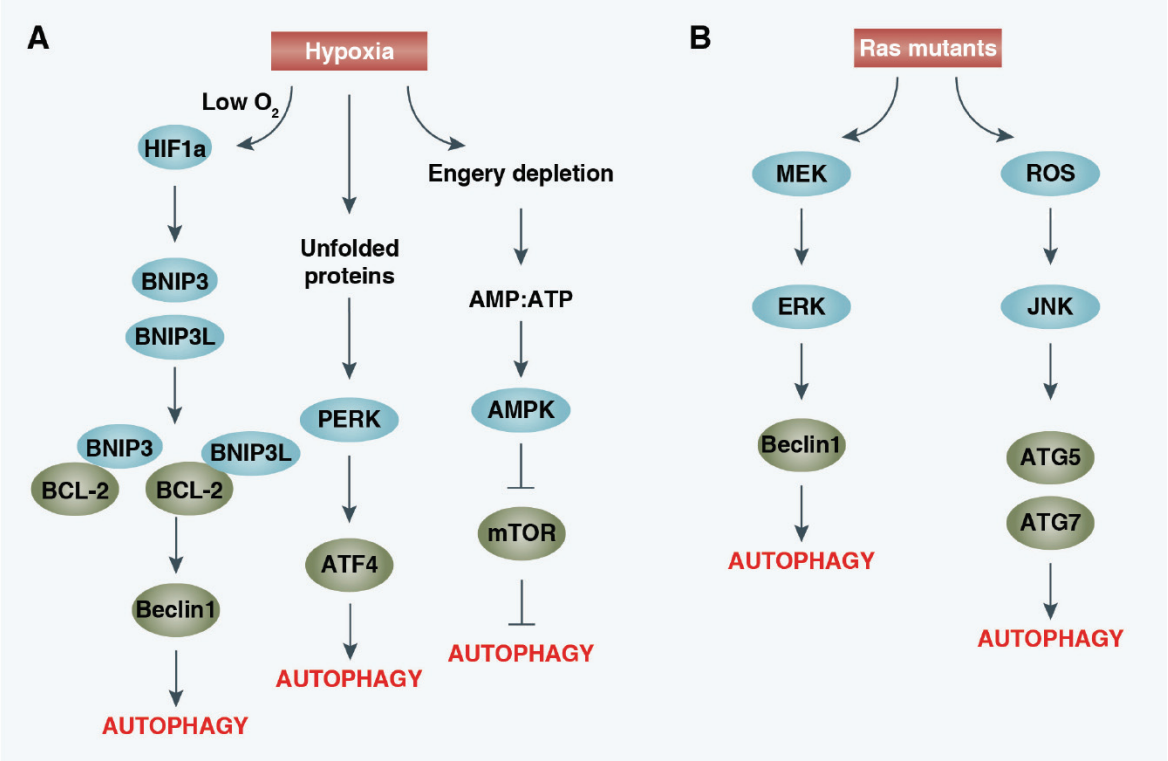

Figure 3. Pathways of autophagy induction by hypoxia and mutant RAS. (A) During hypoxia, autophagy is activated by sensors that detect low oxygen, unfolded proteins, and energy depletion. Low oxygen induces autophagy involving the HIF1α-mediated up-regulation of BNIP3 and BNIP3L. They compete with Bcl-2 for interaction with Beclin 1, and orient Beclin 1 to autophagosomes. AMPK is a major regulator of energy homeostasis. The kinase is sensitive to the AMP/ATP ratio: binding of AMP activates AMPK and induces phosphorylation by the tumor suppressor LKB1. Activation of AMPK inhibits mTOR-dependent signaling. Inhibition of mTOR induces autophagy. In addition, autophagy may be induced during hypoxia as a result of signals generated by the PERK-ATF4 mediated unfolded protein response in the endoplasmic reticulum. (B) The induction of autophagy by oncogenes such as mutant Ras is importance in tumorigenesis. Ras mutant activates the ROS-dependent JNK signaling pathway and upregulates the Atg5 gene to induce autophagy. Under certain circumstances, Ras mutant activates the MEK/ERK signaling pathway and upregulates the Beclin1 gene to induce autophagic cell death.

hanced levels of autophagy render pancreatic cancer stem-like cells resistant to induction of apoptosis by hypoxia and starvation [92]. In contrast, inhibition of autophagy induces death of pancreatic cancer stem-like cells and inhibits self-renewal potential [92].

Stromal cells have recently gained much attention as a critical determinant of tumor progression and clinical outcome in pancreatic cancer [93]. Several studies have indicated that autophagy in cancer associated fibroblasts promotes tumor cell survival in the tumor stromal microenvironment [94, 95]. Caveolae, as a versatile signaling molecule, can bud from the plasma membrane and function in cellular processes such as endocytosis and the transcytosis of molecules. Loss of caveolae-1 expression in cancer-associated fibroblasts results in an activated tumor microenvironment, thereby driving early tumor recurrence, metastasis, and poor clinical outcome in breast and prostate cancers [96, 97]. However, overexpression of caveolin-1 correlates with tumor progression markers in PDAC [98]. Hypoxia-induced autophagy and activation of the NF-κB pathway is indicated to drive caveolae-1 degradation in cancer-associated fibroblasts [94]. It is unknown whether stromal cells directly induce cell-mediated autophagy (C-MA) in pancreatic cancer, although lymphocyte-induced C-MA promotes several cancer cell, including pancreatic cancer cell, survival [99].

Autophagy and molecular biology of pancreatic cancer

Since initial recognition of the importance of K-Ras mutations in pancreatic cancer during the late 1980s, understanding of the biologic mechanisms underlying the behavior of this cancer has been growing at an increasingly rapid pace [100]. K-Ras is a member of a highly homologous group of approximately 21 kDa monomeric, membrane-localized GTPases. K-Ras as well as H- and N-Ras is important for cell growth, differentiation, and survival by through a complex interplay of extracellular signals and intracellular signaling cascades. Of them, the Raf/MEK/ERK pathway is the best characterized Ras regulated signaling pathway. Somatic K-Ras mutations, as a transforming protein in human tumors, are found at high rates in pancreatic cancer, lung cancer, and colon cancer. Of note, mutations of the K-Ras gene occur in over 90% of pancreatic carcinomas. Recent studies suggest that autophagy and mitophagy are required for the optimal growth of tumor cells transformed with oncogenic H-Ras^{V12} or K-Ras^{V12} [101-104]. Expression of constitutively active Ras mutants induced autophagy in an ATG5 and Beclin 1 dependent manner [103, 105]. Autophagy-defective Ras-transformed cells are not able to proliferate in nutrient-deficient conditions and do not form tumors efficiently *in vivo*, suggesting a critical role for autophagy in Ras-mediated tumor maintenance [103, 104]. However, one study demonstrated that Ras-induced autophagy may cause cell death in breast tumor cells on the verge of oncogenic transformation [105], suggesting a complex relationship between Ras-mediated autophagy and cell death depending on the context [106]. Most solid tumor cells are characterized by energy dependence on aerobic glycolysis accompanied by impairment of the mitochondrial respiratory capacity; these characteristics are consistent with the proposed Warburg effect [107, 108]. Ras-transformed cells require autophagy and mitophagy to maintain mitochondrial function [103]. Biochemical analysis demonstrates that autophagy defects result in decreased production of tricarboxylic acid cycle intermediates and impaired oxygen consumption [103] and glycolysis [104].

p53, the most commonly mutated gene in human cancers including pancreatic cancer, plays multiple roles in apoptosis, autophagy, metabolism, cell cycle progression, and many other processes [109]. p53 also regulates autophagy through both transcription-dependent and independent mechanisms. Cytosolic p53 inhibits autophagy through a transcription-independent mechanism [110]. Damage-regulated autophagy modulator (DRAM) and unc-51-like kinase 1 (ULK1) have recently been identified as p53 transcriptionally-regulated genes through which p53 promotes autophagy in contrast to its cytosolic role [111, 112]. Interestingly, the endogenous wildtype p53 posttranscriptionally down-regulates LC3 in colon cancer cells under prolonged starvation [113]. Our findings suggest that HMGB1 and p53 form complexes that regulate the cytoplasmic localization of the reciprocal binding protein and subsequent levels of autophagy and apoptosis [114]. Another important area is delineating whether and how autophagy generally impacts oncogene mutations in pancreatic cancer.

Autophagy and pancreatic cancer treatment

There are three primary treatments for patients with exocrine pancreatic cancer: surgery, radiation therapy, and chemotherapy. Autophagy can be activated by chemotherapy and ionizing radiation in the treatment of pancreatic cancer cells [14-17, 115, 116]. The cytoprotective role of autophagy following chemotherapy and radiation therapy has been confirmed by many investigators [117]. Gemcitabine, as first-line therapy for patients with advanced pancreas cancer, triggers autophagy in pancreatic cancer cells. Autophagy suppression promotes apoptotic cell death in response to gemcitabine and other stimuli in pancreatic adenocarcinoma [20, 117]. Other studies suggested that gemcitabine -induced VMP1-mediated autophagy leads apoptotic cell death [18]. However, zymophagy, a novel selective autophagy pathway which is activated by experimental pancreatitis and mediated by the VMP1-USP9x-p62 pathway, prevents pancreatic cell death [118]. In addition, an enhanced cytotoxic effect of gemcitabine, when combined with cannabinoids, induces reactive oxygen species (ROS)-mediated autophagic cell death in pancreatic tumor cells [119]. Indeed, cannabinoids are also an apoptotic inducer by activation of the endoplasmic reticulum stress pathway in pancreatic cancer cells [120]. ROS act as signaling molecules in various intracellular processes, including apoptosis and autophagy [121]. Mitochondria are a

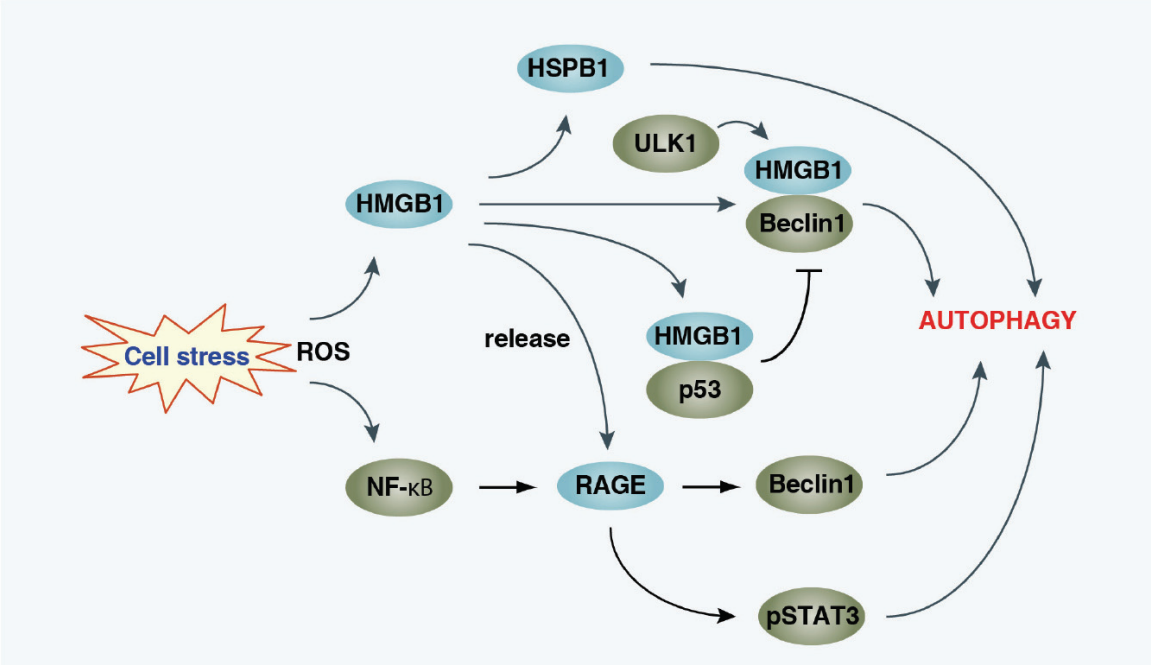

Figure 4. Pathways of autophagy induction by HMGB1 and RAGE. HMGB1 is an autophagy sensor in oxidative stress. HMGB1 plays important intranuclear, cytosolic, and extracellular roles in the regulation of autophagy. Nuclear HMGB1 regulates HSPB1 expression, which is required for the dynamic of mitophagy to control mitochondrial quality. Cytosolic HMGB1 is a Beclin1 binding protein. HMGB1-Beclin1 complex is a downstream signal from ULK1 complex. Interaction between HMGB1 and p53 regulates the level of HMGB1-Beclin1 complex. Loss of p53 promotes HMGB1 cytosolic translocation and increased HMGB1-Beclin1 complex and autophagy. In contrast, loss of HMGB1 promotes p53 cytosolic translocation and inhibits autophagy. Exogenous HMGB1 binds RAGE to active autophagy. ROS such as H_2O_2 increase activity of NF-κB and subsequently result in RAGE overexpression. This RAGE upregulation protects pancreatic tumor cells against oxidative injury and increases drug resistance by increasing Beclin1 dependent autophagy and decreasing apoptosis. In addition, RAGE is required for IL-6-induced phosporation of STAT3 (pSTAT3) and subsequently, autophagy induction.

major source of ROS. Radiation therapy and some forms of chemotherapy rely on ROS toxicity to eradicate tumor cells. Inhibition of autophagy or mitophagy increase ROS production, resulting in apoptotic cell death. The concept of autophagic cell death was first established based on observations of increased autophagic markers in dying cells. An increasing number of studies indicate that autophagic cell death is cell death with autophagy rather than cell death by autophagy [59, 122].

The efficacy of autophagy inhibitors in preclinical models mostly came from using chloroquine and its derivative hydrochloroquine. Chloroquine is a synthetic 4-aminoquinoline that has been used for 60 years in humans to treat and prevent rheumatoid arthritis, HIV, and malaria in humans. Chloroquine inhibits acidification of the lysosome and therefore prevents autophagy by blocking autophagosome fusion and degradation. In ongoing cancer treatment clinical trials, chloroquine is often used in combination with chemotherapeutic drugs, radiation, and immunotherapy [123]. Of note, a recent study indicated that chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy [61]. Chloroquine has been demonstrated to inhibit pancreatic tumor growth in a mice model [60, 124]. Currently, our ongoing trials examine whether chloroquine or hydrochloroquine combined with gemcitabine enhances the efficacy of tumor cell killing in pancreatic cancer patients.

HMGB1-rage pathway regulates autophagy in pancreatic cancer

HMGB1 belongs to a family of highly conserved proteins that contain HMG box domains. In the

nucleus, HMGB1 is important in the regulation of DNA replication, repair, and recombination, as well as gene transcription. During stress, HMGB1 is released to the extracellular environment and functions as a DAMP [125]. The major receptor for HMGB1 is RAGE and TLR. Overexpression of HMGB1 and RAGE have been demonstrated in many tumors, including pancreatic cancer [126, 127]. RAGE is a member of the immunoglobulin superfamily and plays important roles in regulation of ROS generation and inflammatory response [128, 129]. In pancreatic cancer, RAGE is compatible with PanIn formation [8]. Gemcitabine promotes RAGE expression by ROS-mediated NF-κB activation [20]. This RAGE upregulation protects pancreatic tumor cells against oxidative injury and increases drug resistance by increasing autophagy and decreasing apoptosis (**Figure 4**) [117]. Knockdown of RAGE by short hairpin RNA inhibits anticancer agents (e.g., gemcitabine, oxaliplatin, and melphalan) induced autophagy by regulates Beclin 1-Vps34 interaction [117]. ROS also trigger the translocation and release of HMGB1 in pancreatic cancer cells [21]. However, reduced HMGB1 promotes autophagy whereas oxidized HMGB1 induces apoptosis [9]. RAGE is required for extracellular HMGB1-mediated autophagy [9]. As a cellular defense mechanism, cytosolic HMGB1 directly interacts with Beclin 1, which is controlled by ULK1 complex (**Figure.4**) [130, 131]. Antioxidant enzymes (e.g., superoxide dismutases) and small-molecule antioxidants (e.g., N-acetyl-L-cysteine) inhibit HMGB1 activation as well as autophagy induction in pancreatic cancer cells [21]. In the nucleus, HMGB1 regulates expression of heat shock protein beta-1 (HSPB1) (**Figure 4**) [132]. HSPB1 inhibits apoptosis and its phosphorylation promotes autophagy through regulation of the actin cytoskeletal response, which is required to promote the processes of autophagy and mitophagy [132].

Conclusions

Autophagy is a highly conserved mechanism to degrade intracellular components and promote programmed survival of cells in metabolic distress by providing a nutrient source such as ATP and amino acid. Autophagy is associated with pancreatic cancer, a highly fatal cancer, and plays multiple roles in pancreatic cancer pathogenesis and treatment, which may depend on the tumor type, stage, risk factors, and anticancer agents being used in combination with autophagy inhibitor. The K-Ras oncogene and p53 tumor suppressor gene have been demonstrated to regulate autophagy at different levels. In addition, our studies suggest that the HMGB1 -RAGE pathway plays a central role in the regulation of autophagy in pancreatic cancer cells. The expression of RAGE is permissive for early pancreatic neoplasia. It is worth highlighting that hydrochloroquine, a potential autophagy inhibitor, is already approved by the U.S. Food and Drug Administration and may prove to be an effective modulator of autophagy in patients with pancreatic cancer.

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