Noncoding RNAs as key modulators of autophagy in pancreatic cancer (Review)

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Abstract. Inability of early detection as well as lack of proper therapeutic intervention, both add to the complexity of pancreatic cancer. Understanding of the basic cellular processes is of the utmost importance and autophagy is one of these processes. Considering the importance of this process in normal cellular functions as well as in pathological states, elaboration of the updated information on the mechanism of autophagy was initially carried out. Autophagy is a process for degradation of damaged cellular organelles, abnormal proteins and even nutrients which happen via formation of autophagosomes. Incidentally, autophagy has been shown to play both oncogenic and tumour-suppressive functions in cancer and has also been shown to modulate stemness of cancer cells, recurrence and resistance to chemotherapeutic agents. The contribution of autophagy genes and pathways in pancreatic tumorigenesis was also evaluated. Regulation is the key step in any such cellular phenomenon and noncoding RNA-mediated regulation is an emerging field. While miRNAs participate mainly in post-transcriptional regulation, long noncoding RNAs and circular RNAs have more diverse regulatory functions. Noncoding RNAs are also shown to modulate both the tumour-promoting and tumour-suppressing functions of autophagy in pancreatic cancer. The implication of noncoding RNA-mediated regulation with respect to radio-resistance and chemo-resistance of pancreatic cancer cells was also assessed. To the best of our knowledge, this is the first ever attempt trying to decipher the cross-talk between autophagy-noncoding RNAs and genes involved in the development and progression of pancreatic cancer.

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Abbreviations: HDI, human development index; K-RAS, Kirsten rat sarcoma viral proto-oncogene; CDKN2A, cyclin-dependent kinase inhibitor 2A; TP53, tumour protein P53; SMAD4, mothers against decapentaplegic homolog 4; miRNA, micro-RNA/ribonucleic acid; lncRNA, long-noncoding ribonucleic acid; MRE, miRNA response element; ULK1, Unc-51-like autophagy activating kinase 1; ATGs, autophagy-related genes; mTOR, mechanistic target of rapamycin kinase; FIP200, 200 kDa; FAK, family kinase-interacting protein; RB1CC1, RB1-inducible coiled-coil protein 1; PI3K, phosphatidylinositol 3-kinase; USP22, ubiquitin-specific peptidase (USP)-22; UVRAG, ultraviolet radiation resistance-associated gene protein; LC3, light chain 3; SQSTM1, sequestosome 1; NBR1, neighbor of BRCA1 gene 1 protein; TIA-1, T-cell intracellular antigen-1; PTBP1, polypyrimidine tract-binding protein 1; PKM1, pyruvate kinase muscles 1; SNARE, synaptosome associated protein receptor; VAMP8, vesicle associated membrane protein 8; EMT, epithelial mesenchymal transition; STAT3, signal transducer and activator of transcription 3; VEGFR2, vascular endothelial growth factor receptor 2; ROS, reactive oxygen species; YAP/TAZ, yes associated protein/tafazzin; BCL2, B-cell CLL/lymphoma 2; TFEB, transcription factor EB; LKB1, liver kinase B1; HMGB1, high mobility group box 1; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; eIF5A2, eukaryotic translation initiation factor 5A2; YY1: Yin and yang 1; LAMP2, lysosomal associated membrane protein 2; HOTAIR, HOX transcript antisense RNA; AGR2, anterior gradient 2; ceRNA, competing endogenous RNA; MACC1, metastasis-associated in colon cancer protein 1; MET, mesenchymal epithelial transition protooncogene; ERK, extracellular-signal-regulated kinase; SNHG14, small nucleolar RNA host gene 14; linc-ROR, long intergenic non-protein coding RNA, regulator of reprogramming

Key words: pancreatic ductal adenocarcinoma, autophagy, regulation, noncoding RNA

Contents

1. Introduction

Pancreatic cancer is one of the most aggressive cancer types with a high mortality rate and low 5-year survival rate despite all the advances in treatment (1,2). Patients with resectable tumours generally have more prolonged survival than patients with unresectable tumours, but only 10-20% of the patients are

diagnosed at a stage amenable to resection for lack of atypical symptoms and early diagnostic biomarkers (3,4). Even after curative resection, cancer recurs in the majority of patients and about 90% of the cases develop distant metastasis. Distinct somatic mutations in *KRAS, CDKN2A, TP53* and *SMAD4* genes are most prevalent in pancreatic cancer building up the fundamental premise for the genetic alterations underlying pancreatic tumorigenesis (5).

Autophagy is a highly regulated cellular pathway involved in degradation and recycling of cellular elements. Autophagy enables the cell to dissipate the redundant intracellular components of the cell via lysosomal degradation and also recycle the primary elements in order to combat any stress condition and maintain cell viability (6). Salient cargos for autophagy are damaged DNA, non-functional organelles, protein aggregates, reactive oxygen species (ROS) and other biochemical contents which otherwise can affect normal cellular machinery (6). Thus, autophagy has a crucial role to play in normal cellular physiology and thus its aberration is highly correlated to diseases such as cancer. Notably, it has been shown to play both oncogenic and tumour-suppressive roles in modulating cancers of different organs in different cellular conditions. Pancreatic ductal adenocarcinoma (PDAC) is no exception and available evidence suggests an important role of autophagy mainly towards the development of high-grade pancreatic intraepithelial neoplasia (PanIN) and promotion of PDAC (6). Therefore, considering the importance of autophagy in pancreatic cancer, another aspect to be focused in greater detail is the regulation of the phenomenon. This particular area has also observed advancement and multiple studies have coordinated between them to draw an informative picture of cross talk between autophagy pathway genes and cancer-related genes showing their regulation in pancreatic cancer. Key players in this regulatory axis are the noncoding RNAs (ncRNAs), albeit the noncoding module of autophagy regulators is almost a grey zone in the context of pancreatic tumorigenesis. miRNAs are the major ncRNAs playing a dual role acting as both pro- and anti-autophagic during cancer initiation and development (7). Similarly, oncogenic or tumour-suppressing roles of lncRNA in cancer have been identified (8). Findings of previous studies have also revealed an emerging role of lncRNAs in the regulation of autophagy, further contributing to cancer development and progression (9,10). Various types of RNA including the transcripts of pseudogenes, lncRNAs, and circRNAs, can act as competing endogenous RNA (ceRNA) by competing with mRNA for the binding of miRNA and affect the gene expression at the post-transcriptional level. Any changes in these biological processes as a result of noncoding RNA dysfunction leads to changes in the cellular homeostasis, which further affects pancreatic tumorigenesis. In the present review, the mechanism of autophagy was examined, evaluating the involvement of individual genes in PDAC and elaborating existing information on the contribution of miRNA, lncRNA and circular RNAs to the regulatory network controlling the effect of autophagy on PDAC. The aim was to address an important part of basic mechanistic aspects of pancreatic cancer and help the scientific community to have an idea on how autophagy-related genes and pathways could be targeted for therapeutic purposes against pancreatic cancer.

2. Mechanism of autophagy

Autophagy, the self-eating mechanism of the body is an evolutionarily conserved adaptive process in response to various cellular stress conditions including nutrient deprivation, hypoxia, oxidative stress, protein aggregates, toxic metabolites and infection (11). This cytoprotective machinery intends to degrade and recycle the damaged intracellular constituents by means of lysosomal degradation. Following induction, the process of autophagy begins with the formation of ULK1 or ATG1 complex at the phagophore assembly site (PAS) on endoplasmic reticulum. As a consequence of the stress conditions, major cell growth regulator serine/threonine kinase mTOR (mTORC1 subtype only) is inhibited, which in turn results in autophosphorylation and dissociation of ULK1 from mTOR, thereby activating ULK1. This event is followed by several phosphorylation cascades and eventually leads to the formation of ULK1 complex consisting of ULK1, ULK2, ATG13, FIP200 (also known as RB1CC1), and ATG101, which in turn activates a class III PI3K complex of VPS15, VPS34 (PIK3C3), ATG14, Beclin-1 (Atg6), UVRAG (p63), and activating molecule in *BECN1*-regulated autophagy protein 1 (AMBRA1) (11). This nucleation of proteins on the PAS site of an isolation membrane form a cup-shaped structure and is termed phagophore. This contributing intracellular membrane can be endoplasmic reticulum (ER)-exit sites (ERES), Golgi complex, mitochondria, contact membrane of ER with Golgi body and mitochondria, plasma membrane or recycling endosomes (12-18). Whether preference between the membrane sources is based on any specific criteria remains obscure; however, it is assumed to vary depending on the cell type, stimulation for autophagy induction, type of cargo to be carried and other substantial conditions. The isolation membrane then elongates gradually to engulf the cargo or the damaged cellular material and then fuses to form a double-membrane bound autophagic vesicle, known as autophagosome. The elongation and maturation steps are driven mainly by two ubiquitin-like conjugation systems, the Atg12 and Atg8/LC3 (lipidation) conjugation systems. The Atg5-Atg12 conjugation system forms a multimeric complex of Atg5-Atg7-Atg10-Atg12-Atg16L1 which is critical to LC3 lipidation exhibiting as an E3-like ligase (19). The second conjugating system Atg8/LC3B-PE consists of Atg4B-atg7-Atg3 and the activated Atg8 is then conjugated to a phospholipid, phosphatidylethanolamine (PE) and thus the membrane-bound LC3B-IPE conjugate (LC3II) is formed on both the autophagic membranes which is a prime feature for autophagic vesicle formation. Microtubule-associated protein light chain 3 (LC3/LC3B) and GABAA receptor-associated protein (GABARAP) are the two conventional mammalian homologs of Atg8 (20). In addition to elongation and fusion of the phagophore, Atg8/LCB-II act as a receptor for selective uptake and degradation of poly-ubiquitinated protein aggregates. LC3B-II interacts with ubiquitin-binding receptors p62/SQSTM1 and NBR1 and other LC3-interacting regions (ILR domain) on the surface of the cargo that promotes turnover of sequestered proteins (21). Intracellular membrane trafficking proteins, Rab-GTPases, membrane-tethering fusion proteins such as HOPS and SNARE complex of VAMP8, Syntaxin 17 and SNAP29, are in charge of the motility and fusion of the autophagosome to lysosome, forming autolysosome (22). After

the formation of autolysosome, LC3-II on the outer surface of autophagosome is degraded by ATG4B to recycle it for further autophagosome formation. Ultimately the cytosomal cargo is degraded by the lysosomal proteases including cathepsins and other acid hydrolases. Degraded products are then recycled through nutrient transporters and used for cell growth. Fig. 1 shows the mechanism of autophagy.

3. Autophagy and cancer

Autophagy has a dual role in cancer because autophagic genes are both oncogenic and tumour suppressive depending on type and stage of cancer. In the early stages of tumorigenesis, autophagy acts in an onco-suppressive mode that is vital for anticancer immunosurveillance by eradicating endogenous ROS, oncogenic p62 protein aggregates, inflammatory response, damaged cell and mount adequate measures against genotoxic stress (23). Heterozygous disruption of autophagy-execution gene Beclin-1 (*BECN1*) promotes spontaneous malignancy. Monoallelic deletion of *BECN1* gene is evident in 75% of ovarian cancers, 50% of breast cancers and 40% of prostate cancers (24). Thus, the gene is demonstrated as a haplo-insufficient autophagic tumour-suppressor gene (24). Frameshift mutation in core autophagic proteins ATG2B, ATG5, ATG9B, ATG12 and UVRAG is prevalent in gastric and colorectal cancer patients with microsatellite instability (25). Loss-of-function mutations of these genes restrain the genome-stabilizing effects of autophagy and make the cells susceptible to tumorigenesis.

Autophagy also has a different role in cancer progression by providing cellular metabolites for tumour growth and energy requirement and perpetuating redox homeostasis for promoting their survival. Substantial evidence demonstrates that autophagy may cause resistance to cancer cells against therapeutic agents by helping them to withstand the stress (26). Due to the high glucose demand of cancer cells, glycolytic enzyme pyruvate kinase M1 (*PKM1*)-induced autophagy promotes malignancy in a *KRAS*-G12D mouse model. Furthermore, embryonic fibroblast cells from *PKM1-ATG7* knockout mice have been shown to limit tumour growth compared to the corresponding wild-type *ATG7* cells (27). Autophagic paradox is also apprehended in metastatic cascade. In early metastatic episode, autophagy regulates EMT, tumor cell migration and invasion. High LC3B expression has been correlated with metastasis in hepatocellular carcinoma, and melanoma. An increased autophagy gene signature expression is also associated with an aggressive and invasive type of glioblastoma (28). Emerging evidence also indicates the ability of autophagy to maintain the stemness of cancer stem cells (CSCs). Increased expression of stem cell marker CD44, mesenchymal marker vimentin, other core stemness factors such as Forkhead box 3A (*FOXO3A*), Sex determining region Y-box (*SOX2*), Nanog Homeobox (*NANOG*), and *STAT3* upon induction of autophagy demonstrates the critical role of autophagy in maintaining CSCs. Thus, autophagy maintains the pluripotency of the stem cells and promotes their survival in a hypoxic and low nutrient environment (29). Accumulation of ROS in tumor endothelial cells (TECs) enhanced TEC migration and upregulated angiogenic gene expression such as *VEGFR2*. Autophagy is a prime mechanism of modulating redox homeostasis of the endothelial cells and thus the process of tumor angiogenesis is also influenced by this (30). Epigenetic modification of autophagy regulators has been shown to impact the cancer progression. Promoter hypermethylation of autophagy genes including *ATG2B, ATG4D, ATG9, ATG5*, *BECN1* and *ULK2* appears to induce tumour progression in several cancer types (31). Thus, the role of autophagy is a double-edged sword in the framework of carcinogenesis (Fig. 2).

4. Autophagy and pancreatic cancer

The link between autophagy and development of pancreatic ductal adenocarcinoma is also an enigma to the researchers. Findings suggest the autophagic response facilitates the survival of pancreatic tumor cells. Primary pancreatic malignant tumours and cell lines exhibit elevated LC3-II expression and autophagosome count per cell, which suggests constitutive activation of autophagy at the basal level (32). Additionally, genetic or pharmacologic inhibition of autophagy led to increased ROS level, DNA damage and metabolic deformity that ultimately resulted in robust tumour regression prolonging their survival (6). Heterozygous disruption of *ATG5* has been shown to promote tumour development and metastasis but homozygous loss of *ATG5* blocked tumorigenesis in oncogenic *KRAS* expressing primary pancreatic cancer cell as well as human PDAC samples (33). The finding suggests a possible relationship between *ATG5* dosage and its function. Elevated precursor of nerve growth factor (proNGF) expression provides anoikis resistance to pancreatic cancer cells by promoting autophagic genes *ATG1* and *BECN1*; giving survival advantage to them (34). However, the context-dependent aspect of autophagy has also been portrayed in case of PDAC development. Status of p53 is considered a cogent determinant of tumour-suppressive or tumour-promoting outcome of autophagy. Humanized genetically modified mouse models of PDAC lacking autophagy genes *ATG5* or *ATG7* impede the progression of low-grade pre-malignant pancreatic lesions into high grade, suggesting the protective role of autophagy in early stages of tumorigenesis. By contrast, in mice having oncogenic *KRAS* and lacking p53, deficient autophagy exerts a pro-tumorigenic role (35). Therapeutic resistance is also a notable feature of PDAC due to autophagy. PDAC cells display culminated autophagic flux which is pro-survival to the cancer cells. Constitutive activation and nuclear transport of MiT/TFE family of transcription factors drives the coherent gene network of autophagy and lysosomal catabolism in PDAC cells (36). Sustained anchorage-independent growth of PDAC cell largely depends on concurrent mTORC1 inactivation and activated phosphatase for ULK1 (PP2A-B55α complex) (37). Ablation of autophagy in PDAC cells affects the mitochondrial function that dampens oxidative phosphorylation and ATP levels (38). ROS-induced autophagy promotes cytosolic translocation of high-mobility group box 1 protein (HMGB1) and its binding to beclin-1, which is a positive feedback regulation of autophagy in PDAC cells, giving protection against oxidative stress (39). Tumour-stroma-associated pancreatic stellate cells (PSCs) produce alanine through an autophagy-dependent

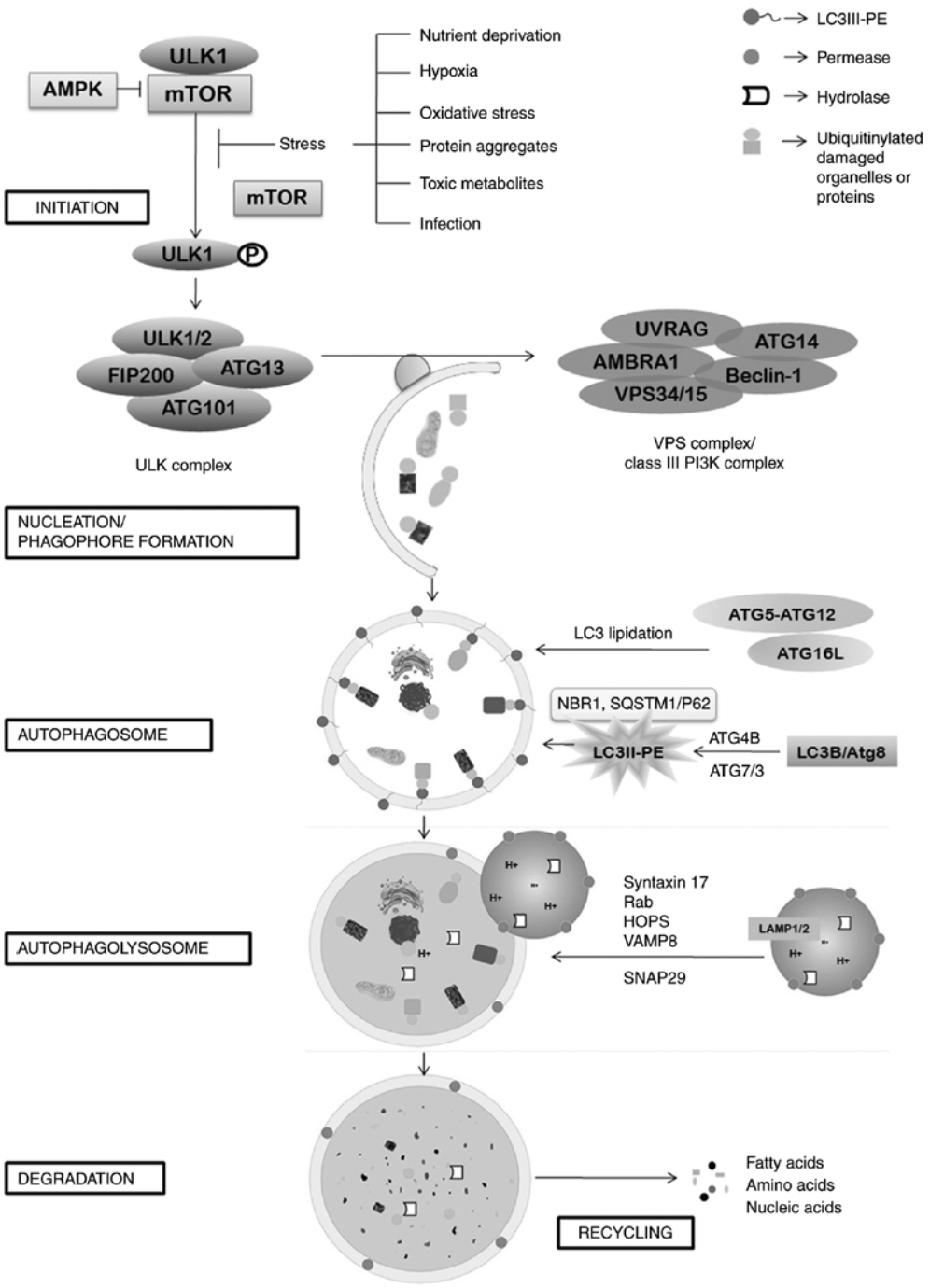

Figure 1. Overview of molecular mechanism of autophagy regulation. Autophagy is a cellular process induced by several stress conditions and growth factors. It initiates with the dissociation of ULK1 from mTOR and formation of ULK complex followed by VPS complex formation. The process next sequesters ubiquitinylated damaged proteins and organelles into a phagophore. The phagophore then starts elongating into an autophagosome and ultimately fuses with lysosome to form autophagolysosome. Finally, the damaged cargo is degraded due to the lysosomal hydrolases and the essential nutrients are recycled for further cellular utilities.

mechanism that serves as an alternative carbon source to support tumour growth in nutrient-deprived condition (40). YAP/TAZ signaling aids the process of autophagosome turnover and also advocates the process of dedifferentiation into stem cell population. Thus, the signaling pathway is found to be relevant in linking autophagy and PDAC cancer stem cells (32). Considering the aforementioned studies, it can be concluded that although pro- and anti-tumorigenic function of autophagy in PDAC has been reported; mostly autophagy has been seen to promote pancreatic carcinogenesis.

5. Noncoding RNAs: Key regulatory module of autophagy

Noncoding RNAs (ncRNA) are functional molecules lacking protein-coding regions and accounts for 98% of the transcriptome (41). MicroRNAs (miRNAs) and circular RNAs (circRNAs) come under the group of highly conserved ncRNAs whereas long noncoding RNAs (lncRNAs) lack general conservation across species (42). ncRNAs function as key regulators of various biological and cellular processes including gene expression at transcriptional and post-transcriptional level,

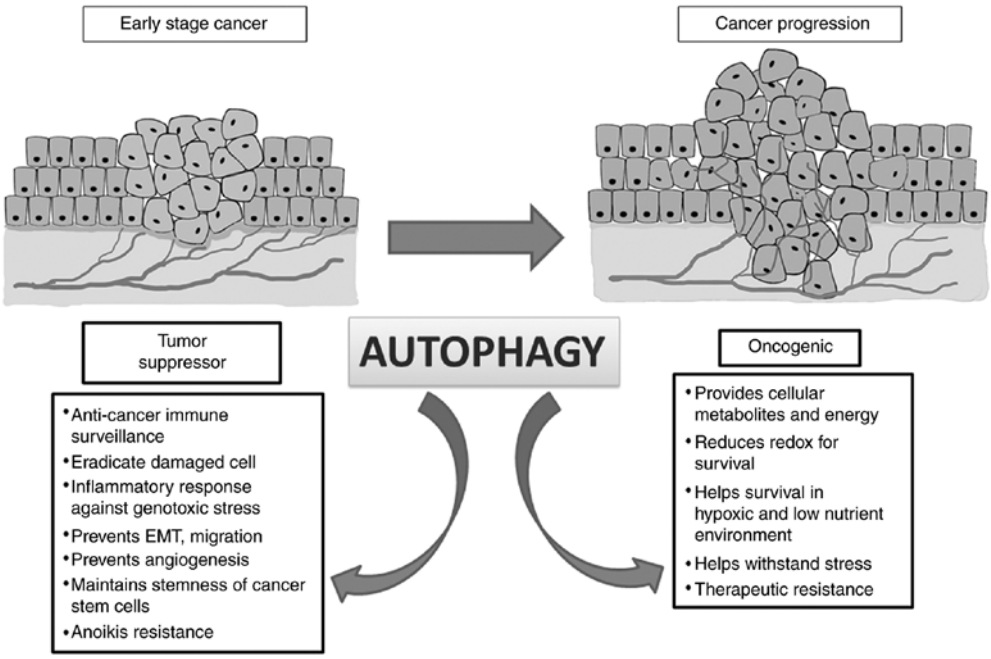

Figure 2. Dual role of autophagy in balancing tumour progression. At early stage of tumorigenesis, autophagy modulates the molecular pathways that are involved in tumour suppression. Once the cancer cells overcome the initial stress, autophagy acts in their favour to conquer further stress and impart survival advantage to the cancer cells.

DNA synthesis or genome rearrangement and protection of genomes from foreign nucleic acids (43). In the current review, the important aspects of autophagy regulation by noncoding RNAs and their impact on the mechanisms of pancreatic cancer are discussed.

miRNA-mediated regulation. miRNAs are highly conserved, endogenous small non-coding RNAs approximately 22 nt in length that bind 3'-UTR of the target mRNA and regulate post-transcriptional gene expression (44). Therefore, the underlying mechanism of miRNA functioning is translational repression or degradation of the target mRNA. Thus, miRNAs play a crucial role in biological events including cell proliferation, differentiation, metabolism and development, signal transduction, apoptotic cell death, host-virus interaction, tumorigenesis and tumor progression via miRNA-RNA-induced silencing complex (miRNA-RISC) (44). Along with mRNAs, miRNAs interact with other noncoding RNAs such as lncRNAs and circRNAs to trigger their decay and this forms a crosstalk and regulatory networks linking the associated target genes (45). miRNAs also participate in the regulation of autophagy genes at the transcriptional and post-transcriptional level and modulate different stages of autophagy (46). miR-30a is identified as one of the first autophagy-related ncRNAs targeting *BECN1* in a way that affects cellular processes in various cancer cells (47). Several studies have indicated that dysregulation of autophagy-related miRNAs may be associated with the tumorigenesis of PC.

Induction of autophagy by miRNAs in pancreatic cancer. Several microRNAs can induce autophagy by targeting anti-autophagic genes which subsequently affect the tumorigenesis of pancreatic cancer. The tumour-suppressor role of miR-506 in PDAC has been suggested in a study that triggered autophagic flux and autophagy-related cell death through targeting the *STAT3-BCL2-BECN1* axis (48). miR-506 has been reported to downregulate the expression of *STAT3* which ultimately led to inhibition of *BCL2* and induction of *BECN1*. Another microRNA, miR-221, also served as a tumour suppressor in pancreatic cancer as it was significantly downregulated in highly invasive pancreatic cancer cells and involved in autophagy-related regulation in tumour cells of PDAC (49). Another interesting study demonstrated that *HDAC6* may serve as a target of miR-221 and miR-221 may induce autophagy by suppressing HDAC6 expression and promoting apoptosis in pancreatic cancer cells (50). Histone deacetylase-6 (*HDAC6*) participates in the clearance of aggresomes by helping in the retrograde transport of autophagosomes and lysosomes. Cells need both HDAC6 and microtubule cytoskeleton for recruitment of the Atg-group of proteins, damaged aggregates and lysosomes for incorporation into aggresomes and use this transport mechanism to enhance autophagic degradation of aggregated proteins (51). miR-23b can directly target an important component of autophagy, *ATG12*, and promote autophagy in pancreatic cancer cells (52). Table I shows the list of miRNAs regulating autophagy in pancreatic cancer.

Suppression of autophagy by miRNAs in pancreatic cancer. MicroRNA-mediated translational repression of autophagy genes impedes the process of autophagy which can further modulate pancreatic malignancy. miR-29a can act as a potent autophagy inhibitor in pancreatic cancer. miR-29a has been reported to be significantly downregulated in pancreatic cancer cells and it inhibits autophagy when overexpressed (53). Increased accumulation of autophagosomes/autophagolysosomes and autophagy markers LC3B and p62 and decreased autophagosome-lysosome fusion constitutes the manifestation

Table I. A summary of micro-RNAs targeting different autophagy-related genes and their effect in pancreatic cancer.

microRNA	Effect on autophagy	Effect on PC	Related genes or molecules	PMID
miR-506	Induction	Suppression	*STAT3-BCL2-BECN1*	28121485
miR-221	Induction	Suppression	*HDAC6*	30546469
miR-23b	Induction	Promote radioresistance	*ATG12*	23916944
miR-30a	Induction	Increases chemosensitivity	*YY1, ATG5* and *BECN1*	29052509 31602254
miR-29a	Inhibition	Sensitizes PC cells to gemcitabine	*TFEB, ATG9A*	27626694
miR-137	Inhibition	Chemosensitizes PC cells to Dox	*ATG5*	30710750
miR-7	Inhibition	Suppression	*LKB1, ULK2, ATG4A* and *ATG7*	28450156
miR-372	Inhibition	Suppression	*ULK1*	28677209
miR-138-5p	Inhibition	Suppression	*SIRT1*	28052003
miR-410-3p	Inhibition	Attenuates gemcitabine resistance in PDAC	*HMGB1*	29296182
miR-216a	Inhibition	Enhances radiosensitivity	*BECN1*	26134156
miR-29c	Inhibition	Increases chemosensitivity	*USP22*	29807360

of the blockade of autophagy flux by miR-29a in pancreatic cancer cells. miR-29a acts as a late-stage autophagy inhibitor and restricts autophagosome-lysosome fusion by reducing the expression of autophagy proteins, TFEB and ATG9A, essential for lysosomal function and vesicular trafficking (53). Evidence has shown the prevention of autophagy by miR-137 via targeting the 3'-UTR of *ATG5* and negatively regulating *ATG5* expression in pancreatic cancer cells (54). miR-7 targets several autophagy-related genes, including *LKB1*, *ULK2*, *ATG4A* and *ATG7* and upregulates the LKB1-AMPK-mTOR signaling pathway to reduce the supply of intracellular glucose to glycolysis in pancreatic cancer. Thus, miR-7 can suppress pancreatic cancer progression by inhibiting autophagy steps and vesicle elongation to impair the activity of aerobic glycolysis (55). A tumor-suppressor role of miR-372 has been reported in human pancreatic adenocarcinoma by regulating autophagy, where miR-372 causes the downregulation of ULK1 expression in pancreatic cancer cell lines. Thus, the miR-372/ULK1 axis is involved in pancreatic cancer development by suppressing cancer cell proliferation, migration, invasion and autophagy (56). miR-138-5p can inhibit autophagy and tumour cell growth in pancreatic cancer cells by targeting serum starvation-induced autophagic flux. This particular miRNA directly targets the 3'-untranslated region of autophagy-related gene *SIRT1* and suppresses its expression level (57). Another miRNA miR-410-3p targets 3'-UTR sequences of *HMGB1*, a primary regulator of autophagy that binds to Beclin-1 and modulates Beclin1-PI3KC3 complex formation and is known to be involved in cancer development via interfering with signaling pathways. In the gemcitabine-treated PDAC cells, silencing of miR-410-3p promotes autophagic activation and cell growth and suppresses cell apoptosis. miR-410-3p can also attenuate chemoresistance to gemcitabine by inhibiting HMGB1-induced autophagy in PDAC (58). In addition to these, miR-216a inhibits beclin-1-mediated autophagy in pancreatic cancer and promotes apoptosis of pancreatic cancer cells in response to radiation, thus enhancing the radiosensitivity of pancreatic cancer cells (59). By contrast, miR-29c increases the chemosensitivity of pancreatic cancer cells by inhibiting USP22-mediated autophagy and cell survival by downregulating USP22 (60). There is evidence that upregulated miR-375 suppresses autophagy and promotes apoptosis of acinar cells by negatively regulating ATG7 in pancreatitis (61). However, evidence also suggests downregulation of miR-375 in several types of cancer, including pancreatic cancer, having a role in cancer cell proliferation. Thus, the tumor-suppressive role of miR-375 in pancreatic cancer in the context of autophagy remains to be investigated (62). Similarly, miR-155 affects the PI3K/AKT/mTOR signaling pathway and impairs pancreatic autophagy by targeting Rictor (RPTOR-independent companion of MTOR complex 2) in pancreatitis (63). miR-9 has been reported to be significantly downregulated in PDAC cells and overexpression of miR-9 sensitized PDAC cells to doxorubicin via inhibition of autophagy by directly targeting 3'-UTR of eIF5A2 transcript. eIF5A2 is known to be involved in the proliferation of some cancer cells (64). The microRNA miR-30a directly targets the autophagy genes *ATG5* and *BECN1* and negatively regulates their expression to suppress autophagy. However, miR-30a expression is suppressed in pancreatic cancer cells by a transcriptional modulator protein YY1 (65,66). Overall, a number of miRNAs function by suppression of autophagy. However, in most of the cases expression of these miRNAs is decreased in pancreatic cancer cells so that they obtain the survival advantage.

Long noncoding RNA mediated regulation. lncRNAs are transcripts longer than 200 nucleotides which do not encode proteins. There are over 15,000 lncRNAs present across different species (67). The lncRNA category includes antisense, intronic, intergenic molecules as well as pseudogenes and retrotransposons. Gene regulatory mode of function of lncRNAs is implemented by several mechanisms such as epigenetic modification, aiding the assembly of transcriptional modulators, sponging miRNAs and post-transcriptional modification by interfering RNA-binding proteins to the target genes (68). There are reports that lncRNAs also participate in the regulation of autophagy.

Table II. A summary of lncRNAs and circ-RNAs modulating genes involved in autophagy regulation and their effect in pancreatic cancer.

Non-coding RNA	microRNA	Effect on autophagy	Effect on PC	Related genes or molecules	PMID
MALAT1		Induction	Promotes proliferation and metastasis	*LC3, P62, LAMP-2*	27371730
PVT1	miR-20a-5p	Induction	Promotes PDA development	*ULK1*	30001707
linc-ROR	miR-124	Induction	Gemcitabine resistance	*PTBP1/PKM2*	27785603
HCP5	miR-214-3p		Regulates gemcitabine resistance	*HDGF*	31632071
HOTAIR		Induction	Enhances radiosensitivity	*LC3-II/LC3-I* and *ATG7*	30464623
LINC01207	miR-143-5p	Induction	Prevents progression	*AGR2, LC3II, BECN1, P62*	30991076
SNHG14	miR-101	Induction	Promotes PDAC cell progression	*RAB5A* and *ATG4D*	30737032
circ-PED8A	miR-338	Inhibition	Promotes metastasis	*MACC/MET/ERK* or *AKT* pathways	29709702; 31610988
hsa_circ_103076 and hsa_circ_100435	miR-15a	Induction	Inhibits PC cell proliferation	Rictor	29620241; 25945419
hsa_circ_101717 and hsa_circ_10408	miR-506	Induction	Tumor suppression	*STAT3-BCL2-BECN1*	29620241; 28121485
ciRS-7	miR-7	Inhibition	Suppress PC progression; inhibit glycolysis	*LKB1-AMPK-mTOR*	24014594; 23446346; 30898507

Metastasis-associated lung adenocarcinoma transcript 1 (*MALAT1*) functions as a pro-tumorigenic lncRNA that can promote the proliferation and metastasis of pancreatic cancer cells via inducing autophagy. Elevated expression of MALAT1 is associated with poor prognosis of PDAC and modulates several cellular autophagic flux genes including *LC3*, *P62* and *LAMP-2*. Mechanistically, MALAT1 regulates tumorigenesis through HuR-TIA-1-mediated autophagic activation, both of which are potent regulators of mRNA translation and stability. Silencing of MALAT1 reduces the degradation of LC3 in pancreatic cancer cells and it also limits the formation of autophagosomes *in vitro* and *in vivo*. It is found that P62 level increases while LAMP-2 level decreases when MALAT1 is silenced. Therefore, MALAT1 can be said to influence only the degradation of autophagosomes but not the formation of autophagosome or the autophagosome-lysosome fusion (69). Similarly, overexpression of HOTAIR increases the ratio of LC3-II/-I and expression of ATG7 thereby enhancing the formation of autophagosome which further promotes autophagy in pancreatic cancer (70). lncRNAs regulating autophagy in pancreatic cancer are listed in Table II.

lncRNA acts as a sponge for miRNAs and regulates miRNA at the transcriptional level as bioinformatics analysis has identified miRNA recognition elements (MREs) on lncRNA sequences (71). It is demonstrated that PVT1 can act as a ceRNA to sponge miR-20a-5p to upregulate ULK1 at the post-trancriptional level and promote cytoprotective autophagy and cell growth of PDAC cells with increased levels of LC3b-II. Thus, overexpression of oncogenic PVT1 in PDAC is often associated with poor prognosis and provides survival advantage to the chemoresistant pancreatic cancer cells (72). Another oncogenic lncRNA, lincRNA-ROR (linc-ROR) has been identified to be upregulated in pancreatic cancer which acts as a ceRNA by sponging miR-124 and inducing autophagy. Linc-ROR has been reported to be negatively correlated with miR-124 expression in PDAC tissues and miR-124 directly targets PTBP1, a splicing factor that switches the isoform expression of PKM to PKM1 following a higher expression of LC3-II (73). The lncRNA HLA complex P5 (*HCP5*) can also act as a ceRNA by sponging miR-214-3p to target hepatoma-derived growth factor (HDGF) which leads to the regulation of GEM-resistant pancreatic cancer cell proliferation, invasion, migration, apoptosis, and autophagy. Previous findings showed that the expression of *HCP5* is upregulated in pancreatic cancer tissues and negatively modulates miR-214-3p expression. In addition, sh-HCP5 induces Beclin1, LC3-I/-II and a decreased p62 expression whereas the opposite occurred in the case of miR-214-3p inhibitor (74). Similarly, overexpression of HOTAIR increased the ratio of LC3-II/-I and the expression of ATG7, thereby enhancing the formation of autophagosome which further promotes autophagy in pancreatic cancer (70).

The lncRNAs are also involved in the crosstalk between autophagy and apoptosis in pancreatic cancer. For example, long intergenic non-protein coding RNA 1207 (*LINC01207*) has been reported to be involved in autophagy and apoptosis via the *LINC01207*/miR-143-5p/*AGR2* axis in pancreatic cancer cells (75). Previous findings have shown upregulated expression of *LINC01207* and *AGR2*, while miR-143-5p was downregulated. *AGR2* acts as a target gene of miR-143-5p and binding of *LINC01207* to miR-143-5p upregulates *AGR2* expression. Elevated *AGR2* expression also inhibits apoptosis in pancreatic cells by increasing the Bcl-2 expression. Thus, results of that study suggest that silencing of *LINC01207* can promote autophagy and apoptosis by sponging miR-143-5p through an increase in LC3-II and Beclin-1 protein expression

while reducing the p62, AGR2 and ratio of Bcl-2/Bax expression in pancreatic cancer (75). The aforementioned findings clearly show the importance of lncRNAs in modulating autophagy in pancreatic cancer.

Circular RNA-mediated regulation. Circular RNAs are an important member of the noncoding RNA family. Circular RNAs (circRNAs) are a group of abundant, conservative and highly stable novel type of endogenous non-coding RNAs that are produced by back-splicing event and form a three-dimensional covalently closed loop structure by linking 3'- and 5'-ends (76). circRNAs can be divided into three types such as exonic circRNAs, intronic circRNAs and exon-intronic circRNAs (77). Previous findings suggest that circRNAs have many biological functions including miRNA sponges, protein sponges, enhancer of protein function, protein scaffolding, protein recruiter and template for translation, and can regulate several biological processes related to tumour development, proliferation, apoptosis, and invasion often through competitive binding (78,79). Recent findings suggest potential ceRNA networks of circRNA and miRNA are involved in the autophagy of PDAC which has been predicted using bioinformatics analysis (80). High expression level of exosomal circ-PED8A was reported to be associated with poor survival rate, lymphatic invasion and TNM stage in pancreatic ductal adenocarcinoma (81). circ-PDE8A inhibits autophagy by acting as a ceRNA for miR-338 to promote invasive metastasis through the MACC/MET/ERK or AKT pathways in PDAC. circ-PDE8A also induces the invasive growth of PDAC cells by upregulating MET and sponging miR-338 to regulate MACC1 (77,81). MACC1 has been shown to induce autophagy via the AMPK-ULK1 signaling pathway (82). Similarly, hsa_circ_103076 and hsa_circ_100435 were upregulated and associated with miR-15a in PC. miR-15a can inhibit pancreatic cancer cell proliferation and also induces autophagy by directly targeting Rictor, a component of mTORC2 (83). Therefore, hsa_circ_103076 and hsa_circ_100435 can induce autophagy via functioning as miR-15a sponge (84). It has also been found that hsa_circ_101717 and hsa_circ_10408 are upregulated in pancreatic cancer tissues and both of them can exert a tumour suppression function by sponging miR-506 which triggers autophagy-related cell death via the STAT3-BCL2-BECN1 axis in PDAC (48). ciRS-7 has been reported to be one of the few oncogenic circular RNAs which can inhibit tumour suppressor miR-7 (85). ciRS-7 has been found to be upregulated in PDAC. In addition, ciRS-7 could inhibit miR-7 activity which affects the proliferation and invasion of PDAC. As mentioned earlier miR-7 can also suppress pancreatic cancer progression via inhibiting the LKB1-AMPK-mTOR autophagy axis. We can assume ciRS-7 may be partly associated in the regulation of autophagy via miR-7 in PDAC (86) (Table II). The field of circular RNA is rapidly developing and the roles of circular RNAs in the regulation of cancer are currently under investigation. Thus, the correlation between circRNAs and their sponge effect on autophagy-related miRNAs can provide new insight into the treatment and prognosis of pancreatic cancer. The regulation of autophagy in pancreatic cancer by ncRNAs is shown in Fig. 3.

Therapeutic and diagnostic implication of autophagy-related ncRNAs in pancreatic cancer. Several miRNAs have the ability to modulate autophagy-related proteins and thus regulate different stages of autophagy in cancer. Some miRNAs which participate in autophagy regulation are known to act as marker for tumor diagnosis (77). Several studies have demonstrated that miRNA can regulate radiosensitivity in cancer cells by modulating autophagy. For example, miR-214 increases radiosensitivity by inhibiting *ATG12*-mediated autophagy (87), while miR-183-5p enhances radio-resistance by targeting *ATG5* in colorectal cancer (88). *ATG5* is also known to be targeted by miR-137 to inhibit autophagy and chemo-sensitize PC cells to doxorubicin (Dox) (54). *BECN1*-mediated autophagy inhibition by miR-216a has been reported to increase the radiosensitivity of pancreatic cancer cells, where radiation therapy is a significant approach for patients with unresectable malignancy (59). It has been demonstrated that the miR-9/eIF5A2 axis regulates autophagy in PDAC to increase the anti-cancer effect of doxorubicin in tumor cells (89). Furthermore, miR-29a can function as a novel therapeutic agent as it sensitizes chemo-resistant cancer cells to gemcitabine and decreases the invasive potential of pancreatic cancer cells (53), while miR-410-3p can attenuate chemoresistance to gemcitabine by inhibiting HMGB1-induced autophagy in PDAC (58).

Several lncRNAs are also known to influence radiosensitivity in cancer cells. lncRNA *HOTAIR* was found to be highly expressed in pancreatic tumour tissues after radiotherapy and knockdown of HOTAIR can increase radiosensitivity of pancreatic cancer cells by regulating autophagy (70). Blockade of autophagy in pancreatic cancer cells can sensitize it to gemcitabine and reduce the activity of pancreatic cancer stem cells (90). lncRNA *SNHG14* can enhance gemcitabine resistance in PC by inhibiting cell apoptosis via the *SNHG14*/miR-101/autophagy axis. *SNHG14* has been reported to sponge miR-101 where *SNHG14* is upregulated while miR-101 was downregulated in the PDAC tissues. Overexpression of *SNHG14* can increase autophagy-related proteins RAB5A and ATG4D, thus enhancing PDAC cell progression (91). Understanding of these interactions between noncoding RNAs and autophagy genes in cancer cells may be helpful to design a potential therapeutic approach for pancreatic cancer patients. There is, however, no reported study on the specific aspects of circular RNAs and their therapeutic implications involving autophagy pathways in pancreatic cancer; mainly due to the fact that not much work has been performed using such pathways.

Additionally, neoadjuvant-based systemic chemotherapy has also been an important mode of treatment for solid tumours and also for pancreatic cancer, promoting patient survival (92,93). Evidence in breast cancer and osteosarcoma shows that neoadjuvants suppress autophagy and increase drug sensitivity of the malignant cells. Consequently, well-known autophagy inhibitory drugs are being used as neoadjuvants to increase the cytotoxic effect of anti-cancer drugs or radiotherapy (94,95). We have seen thus far that noncoding RNAs emerged as key regulators of autophagy and it is imperative that there be cross-talk between neo-adjuvant chemotherapy, ncRNAs and autophagy in cancer (96-98). However, to the best of our knowledge, there has not been a single report for similar interaction studies in pancreatic cancer. Therefore, the field holds true promise to have these interactions explored in order to have meaningful explanation of prognosis and treatment of pancreatic cancer. There could be two possibilities:

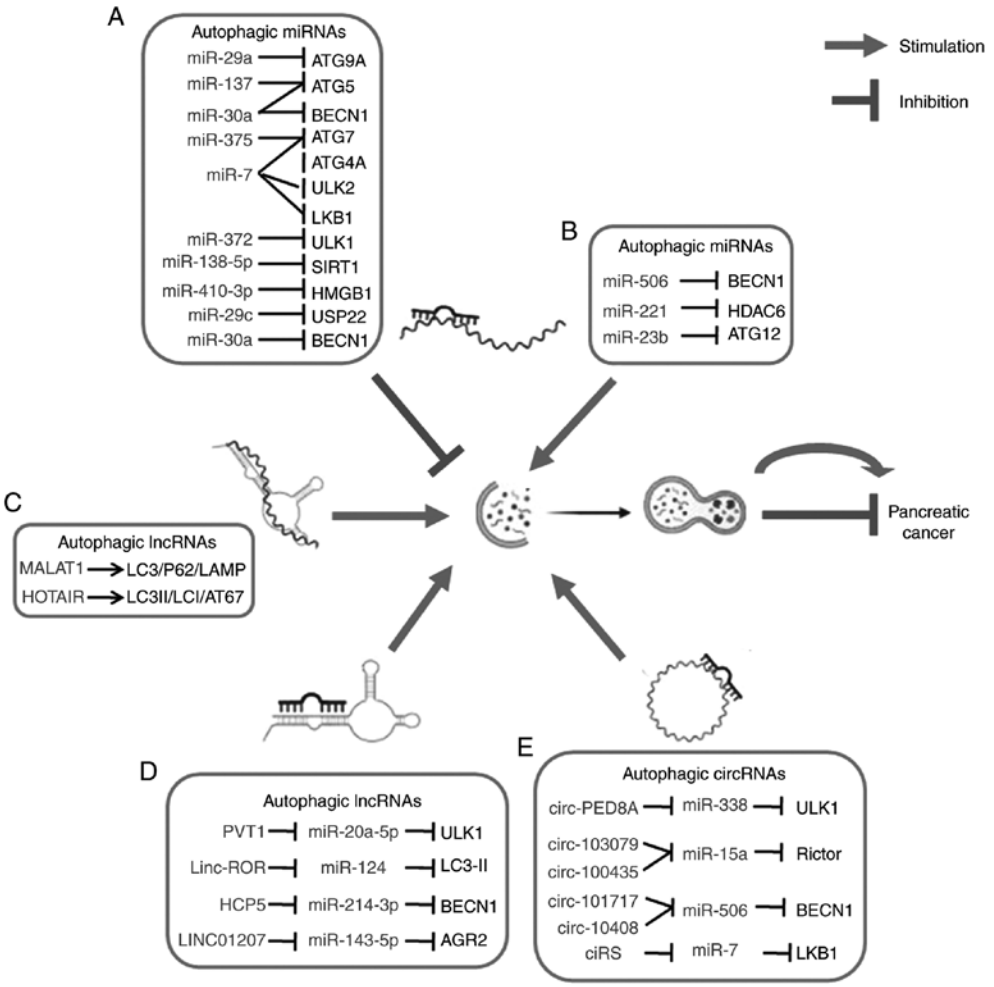

Figure 3. Schematic diagram showing regulation of the autophagy pathways by different types of ncRNAs leading to PDAC. microRNAs target specific autophagy pathway genes either to (A) suppress or (B) activate the process in pancreatic cancer, while lncRNAs are known to influence the process either (C) directly or through (D) miRNA sponging. Circular-RNAs mostly contribute to the process as (E) miRNA-sponges.

i) noncoding RNAs can be a predictive biomarker of neoadjuvant therapy response. For example, lncRNAs, microRNAs or circRNAs that promote autophagy can be a marker for resistance to neoadjuvant therapy or ii) noncoding RNAs that are known as important regulators of autophagy could serve as novel therapeutic targets for systematic treatment with neoadjuvant therapy molecules.

6. Conclusion and future direction

Autophagy is considered an important cellular process having a significant role in the development of various diseases, including cancer. In the current review we examined the contribution of autophagy genes and key autophagic pathways in the development and progression of pancreatic cancer. Moreover, we have discussed in detail the regulatory role of ncRNAs in the process. However, the field is expanding rapidly, especially, with the identification of newer and newer lncRNAs and circRNAs, and the demand to understand the mechanistic aspects is on the increase as well. Thus, a significant part of our future effort should help delineating the role of newly discovered lncRNAs and circRNAs, in the factors they are interacting with, whether they are sponging miRNAs or RBPs or whether their mechanism of action is through modulation of transcription of autophagy genes or through their post-transcriptional regulation. Another important aspect is linking the basic mechanistic studies to the clinically relevant ones where the diagnostic or therapeutic significance of these molecules should be tested with much attention. Lastly, future studies should utilize the recent advancement in technologies addressing the global changes in gene expression pattern upon alteration of key autophagy genes or pathways and then correlate them with pancreatic cancer pathogenesis. Similarly, studies aiming to determine autophagy-related coding and noncoding RNAs are altered in different stages of pancreatic cancer or between precursor lesions and malignancy could also open up new avenues contributing to both enhancement of basic knowledge and translation.

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Availability of data and materials

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Authors' contributions

MM and SP read the papers, interpreted the results, wrote the review and drafted the manuscript with help from BS and BC. SG conceptualized the study and developed the structure and overall objectives. All the authors read, edited and approved the final manuscript, revised it critically for important intellectual content.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information

Not applicable.

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