Pancreatology 12 (2012) 1–7

Contents lists available at SciVerse ScienceDirect

Pancreatology

journal homepage: www.elsevier.com/locate/pan

Review article

Chemotherapy and autophagy-mediated cell death in pancreatic cancer cells

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Keywords:
Autophagy
Pancreatic cancer
Chemotherapeutic drugs

A B S T R A C T

Autophagy is an evolutionarily preserved degradation process of cytoplasmic cellular constituents and plays important physiological roles in human health and disease. It has been proposed that autophagy plays an important role both in tumor progression and in promotion of cancer cell death, although the molecular mechanisms responsible for this dual action of autophagy in cancer have not been elucidated. Pancreatic ductal adenocarcinoma is one of the most aggressive human malignancies with 2–3% five-year survival rate. Its poor prognosis has been attributed to the lack of specific symptoms and early detection tools, and its relatively refractory to traditional cytotoxic agents and radiotherapy. Experimental evidence pointed at autophagy as a pancreatic cancer cell mechanism to survive under adverse environmental conditions, or as a defective programmed cell death mechanism that favors pancreatic cancer cell resistance to treatment. Here, we consider several phenotypical alterations that have been related to increase or decrease the autophagic process in pancreatic tumor cells. We specially review autophagy as a cell death mechanism in response to chemotherapeutic drugs.

Autophagy is an evolutionarily preserved degradation process of cytoplasmic cellular constituents, which serves as a survival mechanism in starving cells. Autophagy is characterized by the sequestration of bulk cytoplasm and organelles in double-membrane vesicles called autophagosomes, which eventually acquire lysosomal-like features completing the autophagic flow with the degradation of the sequester material. Autophagy is mediated by a set of evolutionarily conserved gene products (termed the Atg proteins) originally discovered in yeast [1]. Among these Atg proteins, one subset is essential for autophagosome formation, and is referred to as the core molecular machinery [2]. These core Atg proteins are composed of four subgroups: first, the Atg1/unc-51-like kinase (ULK) complex; second, the class III phosphatidylinositol 3-kinase (PtdIns3K)/Vps34 complex I; third, two ubiquitin-like protein (Atg12 and Atg8/LC3) conjugation systems; and fourth, two transmembrane proteins, Atg9/mAtg9 (and associated proteins involved in its movement such as Atg18/WIPI-1) and VMP1 (whose expression triggers autophagy). Basal autophagy in unstressed cells is kept down by the action of the mammalian target of rapamycin complex 1 (mTORC1). Key upstream regulators of mTORC1 include the class I phosphoinositide 3-kinase (PI3K)-Akt pathway, which keeps mTORC1 active in cells with sufficient growth factors, and the AMP-activated protein kinase (AMPK) pathway that inhibits mTORC1 upon starvation and calcium signals [2].

Autophagy plays important physiological roles in human health and disease. This catabolic process is involved in the turnover of long-lived proteins and the elimination of damage/old organelles, thus maintains quality control of essential cellular components. In addition to its role in cellular homeostasis, a cytoprotective role is playing when cells encounter environmental stresses such as nutrient starvation, hypoxia, oxidative stress, pathogen infection, radiation or anticancer drug treatment, the level of autophagy can be dramatically augmented resulting in adaptation and survival [1,2]. However, downregulated and excessive autophagy has been implicated in the pathogenesis of diverse diseases, such as certain types of neuronal degeneration and cancer [3]. Autophagy has also been implicated in cell death called autophagic or type II programmed cell death, which was originally described on the basis of morphological studies detecting autophagic vesicles during tissue involution [4].

Cancer cells in general tend to undergo less autophagy than their normal counterparts [5,6]. The beclin1 autophagy gene is monoallelically deleted in 40–75% of cases of human sporadic breast, ovarian, and prostate cancers. Heterozygous disruption of beclin1 increases the frequency of spontaneous malignancies, and accelerates the development of virus-induced premalignant lesions [6],

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1424-3903/$ – see front matter
doi:10.1016/j.pan.2011.11.003

suggesting that defective regulation of autophagy promotes tumorigenesis. It has been proposed that autophagy suppresses tumorigenesis by a cell-autonomous mechanisms involving protection of genome integrity and stability, and a non-autonomous mechanisms involving suppression of necrosis and inflammation [7]. On the other hand, autophagy may support the survival of rapidly growing cancer cells that have outgrown their vascular supply and are exposed to an inadequate oxygen supply or metabolic stress. By contrast, excessive levels of autophagy promote cell death [8]. Accordingly, it has been proposed that autophagy plays an important role both in tumor progression and in promotion of cancer cell death [9], although the molecular mechanisms responsible for this dual action of autophagy in cancer have not been elucidated.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive human malignancies with 2–3% five-year survival rate [10]. It remains a devastating and poorly understood malignancy. Its poor prognosis has been attributed to the inability to make a diagnosis while the tumor is still resectable and a propensity toward early vascular dissemination and spread to regional lymph nodes. Up to 60% of patients have advanced pancreatic cancer at the time of diagnosis, and their median survival time is a dismal 3–6 months [11]. This is due to both the aggressive nature of the disease, the lack of specific symptoms and early detection tools, and its relatively refractory to traditional cytotoxic agents and radiotherapy. Moreover, pancreatic cancer cells become more malignant or survive with an extremely poor blood supply [10,11]. So far, little and contradictory data are available regarding the activity of autophagy and its regulation in pancreatic cancer cells. Experimental evidence pointed at autophagy as a pancreatic cancer cell mechanism to survive under adverse environmental conditions, or as a defective programmed cell death mechanism that favors pancreatic cancer cell resistance to treatment. Here, we review several phenotypical alterations that have been related to increase or decrease the autophagic process in pancreatic tumor cells. We specially review the role of autophagy in chemotherapy-induced cell death in pancreatic cancer cells.

1. Autophagy as a pancreatic cancer cell response to environment insult

1.1. Autophagy as a cancer cell survival response to tumor-associated hypoxia

Hypoxia in pancreatic cancer has been reported to increase its malignant potential. Tumor hypoxia has been used as a marker of poor prognosis [12]. Proliferating cancer cells require more nutrients than surrounding non-cancerous cells do, though nutrition is supplied via functional structurally immature neo-vessels, but mostly this is insufficient to provide adequate nutrients for the aggressive proliferating cancer cells. Because of this, how pancreatic cancer cells become more malignant or survive with an extremely poor blood supply is poorly understood. When cancer cells are exposed to hypoxia, anaerobic glycolysis increases and provides energy for cell survival, but as the glucose supply is also insufficient because of the poor blood supply, there must be an alternative metabolic pathway that provides energy when both oxygen and glucose are depleted [13,14]. Because autophagy-specific genes promote the survival of normal cells during nutrient starvation in all eukaryotic organisms, autophagy may react to the cancer microenvironment to favor the survival of rapidly growing cancer cells. LC3 expression in surgically resected pancreatic cancer tissue shows activated autophagy, where the peripheral intensity level of LC3 staining is stronger than central area of the tumor. The peripheral area, which includes the invasive border with a high rate of proliferation and therefore a greater need for oxygen and nutrients, concomitantly shows enhanced expression of carbonic anhidrase [15]. This observation suggests that autophagy may promote cell viability in hipovascular pancreatic cancer tissue.

1.2. Autophagy as a survival cancer cell response to tumor-associated inflammation

Cancer-associated inflammation results in promotion of carcinogenesis and resistance to therapy. Several phenotypic alterations observed in cancer cells are a result of inflammatory signals found within the tumor microenvironment [16]. The receptor for advanced glycation end products (RAGE) is an induced inflammatory receptor constitutively expressed on many murine and human epithelial tumor cell lines [17,18] and the highest levels of RAGE expression were observed in murine and human pancreatic adenocarcinoma tumors. Genotoxic and/or metabolic stress lead to modest but reproducible increases in overall expression of RAGE on pancreatic cell lines. RAGE expression correlates directly with the ability of both murine and human pancreatic tumor cell lines to survive cytotoxic insult. Targeted knockdown of RAGE significantly increases cell death, whereas forced overexpression promotes survival. Recently was reported that the enhanced sensitivity to cell death in the setting of RAGE-knockdown is associated with increased apoptosis and decreased autophagy. In contrast, overexpression of RAGE is associated with enhanced autophagy, diminished apoptosis and greater pancreatic cancer cell viability. Knockdown of RAGE enhances mTOR phosphorylation in response to chemotherapy, thus preventing induction of a survival response. Inhibition of autophagy by means of silencing beclin1 expression in pancreatic cancer cells enhances apoptosis and cell death [19]. These observations suggest that RAGE expression enhances autophagy in response to environment stress in pancreatic cancer cells. However, increased sensitivity to chemotherapeutic agents in RAGE-knockdown pancreatic cancer cells is dependent of Atg5 expression but independent of beclin1 expression [19]. These last findings suggest that the role of autophagy in the resistance to microenvironment insult or in the sensitivity to chemotherapeutic agent is the result of complex molecular pathways in the pancreatic tumor cell.

1.3. Repression of autophagy as a pancreatic cancer cell response to prolonged hypoxic conditions

Pancreatic cancer cell response to prolonged hypoxia may consist in inhibition of autophagic cell death. The short isoform of single-minded 2 (SIM2s) is a member of the basic helix-loop-helix family of transcriptional regulators [20] and is up regulated in pancreatic cancer. Microarray studies identified the pro cell death gene BNIP3 as a target of SIM2s repression. Prolonged hypoxia induces cell death via an autophagic pathway involving the HIF1alpha-mediated upregulation of BNIP3 [21,22]. Decreased BNIP3 levels and poor prognosis clearly correlate with elevated SIM2s expression in pancreatic cancer [23]. The lost of BNIP3 in pancreatic tumors, either by hypermethylation or by transcriptional repression, is correlated with inhibition of cell death [24,25] whereas upregulation of BNIP3 sensitizes pancreatic carcinoma cells to hypoxia-induced cell death [26]. SIM2s expression, concomitant with its repression of BNIP3, enhances tumor cell survival under prolonger hypoxic conditions. Recent data link increased SIM2s expression with enhanced cell survival during hypoxia-stress concomitantly with BNIP3 repression and the attenuation of hypoxia-induced autophagic processes [27]. Thus,

inhibition of autophagic cell death by BNIP3 repression enhances pancreatic tumor cell survival under prolonged hypoxic conditions.

1.4. Repression of autophagy is a pancreatic cancer cell strategy for resistance to chemotherapeutic agent

Changes in pancreatic tumor cell phenotype may explain reduced autophagy in pancreatic cancer. Upregulation of genes involved in autophagy-inhibition signaling pathways as well as downregulation of autophagy-related genes have been reported in pancreatic tumor cells. The phosphatidylinositol 3-kinase-Akt-mTOR pathway, which is activated in many cancer types, has been shown to suppress autophagy in cancer cells. Rapamycin, an inhibitor of mTOR, induces autophagy [27]. The basal expression of TG2 (Tissue transglutaminase) has been reported elevated in tumor cell lines resistant to chemotherapeutic drugs [28]. Elevated expression of TG2 is detected in pancreatic cancer cells [29,30] and inhibition of TG2 expression results in autophagy in pancreatic cancer cells [31]. In addition, downregulation of PKCδ (Protein kinase C, delta) by either rottlerin or siRNA is accompanied by a parallel decrease in TG2 expression and the induction of autophagy. Moreover, knockdown of beclin1 inhibits TG2 siRNA-induced autophagy, indicating that beclin1 mediates the autophagy observed in MDA-Panc28 cancer cells. Finally, inhibition of TG2 by stable transfection with antisense or transient transfection with siRNA has been shown to restore sensitivity of cancer cells to chemotherapeutic drugs [32]. These observations suggest that downregulation of autophagy by the pancreatic cancer cell contributes to cellular resistance to chemotherapy.

2. Both decreased and increased autophagy show to be related to pancreatic cancer

Tumor cells with a high prevalence of activating mutations in *K-Ras*, like pancreatic cancer, have the distinction of a particularly poor prognosis. *K-Ras* is a member of the Ras family of GTP-binding proteins that mediate a wide variety of cellular functions including proliferation, differentiation and survival. *K-Ras* mutation is one of the earliest genetic events see in human PDAC. Recently, it has been demonstrated that many human cancer cell lines with activating mutations in Ras have high basal autophagy [33,34]. Yang et al. have showed that pancreatic cancer cells exhibit constitutive autophagy under basal conditions and is highly activated in the later stages of PDAC transformation being required for continued malignant growth [33]. Minimal cytoplasmic expression of cleaved LC3 in the normal pancreatic ductal epithelium or in low-grade PanIn-1 and most PanIn-2 samples were found by immunohistochemistry. In contrast, all high-grade PanIn-3 and PDAC showed elevated cleaved LC3 staining, with moderate to strong staining intensity in 81% of lesions [35]. The capacity of autophagy to sustain the growth of tumor cells in the later stages of PDAC may be associated with its capacity to prevent DNA damage, remove dysfunctional organelles produced by oversynthesis of reactive oxygen species (ROS) observed in RAS-transformed cells [36]. Despite this function of autophagic process in PDAC, a role of autophagy in the early stages of pancreatic cancer should not be dismissed. Elgendy et al. have demonstrated that expression of mutated *H-Ras* in the absent of cotransforming oncogenes leads to extensive autophagy which culminates in cell death. In human ovarian surface epithelial cell line, the Ras-induced cell death is associated with features characteristic of autophagy, is dependent on beclin1 and Atg5 expression, and is not associated with caspase activation or other features of apoptosis [34]. In the context where an oncogenic mutation in Ras has just been acquired and drives oncogenic stress, acutely induced Ras expression may stimulate intrinsic cellular pathways that abort malignant transformation by cell autophagy-mediated cell death.

In contrast to the activation of autophagy, in some studies have been reported a decreased of this process in PDAC. Studies of carcinogen-induced pancreatic cancer in animal models have shown that pancreatic adenocarcinoma cells have lower autophagic capacity than premalignant cells [5]. The WIPI protein family, which includes Atg18, the WIPI-1 homolog in *S. cerevisiae*, was genetically identified as a gene contributing to autophagy [37]. Human WIPI-1a is a member of a highly conserved WD- repeats protein family and is linked to starvation-induced autophagy in the mammalian system [38]. Moreover, immunolabelling reveals hWIPI-1 and hWIPI-2 as membrane components of autophagosomes [39]. Interestingly, WIPI proteins are linked pathologically to cellular transformation because all human WIPI genes are reported aberrantly expressed in a variety of matched human cancer samples. Strikingly, hWIPI-2 mRNA expression is substantially decreased in 70% of matched kidney (10 patients) and 100% of pancreatic (seven patients) tumor samples. The majority of these samples were derived from advanced stage tumors, such as pancreatic adenocarcinomas stages I–IV [38]. Hence, pancreatic cancer-associated downregulation of hWIPI-2 supports the possibility that decreased autophagic activity is related to the malignant stages of pancreatic cancer.

The role of autophagy in PDAC is complex. Although opposing functions have been reported for the autophagic process in PDAC, likely all these opposing functions could be possible depending on cellular context. Therefore, tumor cells receiving signal from the surrounding environment, react according to them. Moreover, PDAC is characterized by abundant dense stroma, including activated fibroblasts, stellate cells and inflammatory cells. This microenvironment has direct effects on tumor cells. The activated stroma and particularly chronic inflammation have been implicated in carcinogenesis of pancreatic cancer. Interactions between tumor cells and stroma promote tumor growth and resistance to therapy, even autophagy in stroma cells has been implicated in these actions [40–44]. Thus, the Ras-activated autophagy may promote cell death in early stages of pancreatic cancer and may be necessary for cell survival in advances stages of this cancer. Therefore, autophagy in pancreatic cancer may have different roles depending on the cancer stage and on the signals that cancer cells receive from the environment.

3. Autophagy as a cell death mechanism in response to chemotherapeutic drugs

3.1. Cardiac glycosides

The use of cardiac glycosides in the treatment of human malignant diseases may provide an interesting as well as novel form of therapy [45]. For example, oleandrin the principal cytotoxic component of *Nerium oleander*, has been shown to mediate cell death in human but not murine cell lines [46]. The mechanisms by which oleandrin controls malignant cell proliferation may be related to a preferential decreased activation of transcription factors such as nuclear transcription factor-κB (NF-κB) and activator protein-1, alteration of membrane potential and fluidity, activation of MAPK and JNK pathways, increased calcineurin content with subsequent FasL expression, upregulation of death receptors [46] and induction of ROS and oxidative stress [47] in tumor cells. Collectively, these mechanisms have been associated with induction of apoptosis and cell death in a wide variety of human tumor cell lines (eg, Jurkat, U-937, HL-60, HeLa, PC3, and MCF-7). Oleandrin is a potent inhibitor of the phosphorylation of Akt, a well-established biomarker responsible for activation of tumor cell

proliferation, cell migration, invasion, and prevention of apoptosis [48]. It thus appears that oleandrin is a multimechanistic mediator of tumor cell death capable of selective killing of tumor but perhaps not normal cell types. Exposure of PANC-1 cells to oleandrin results in cell death associated with subcellular changes such as formation of autophagosomes that are abundant even at low nanomolar concentrations of this cardiac glycoside. Mechanistically, both Akt and ERK pathways appear to be involved in cell death induced by oleandrin [48,49]. However, it is possible that oleandrin treatment overwhelms an autophagic defense response of pancreatic tumor cells and eventually leads to cancer cell death.

3.2. Gemcitabine

Gemcitabine monotherapy (2′,2′-difluorodeoxycytidine), a deoxycytidine analog, or its combination with other agents has become the standard chemotherapy for the treatment of advanced pancreatic cancer. Gemcitabine is a relatively effective chemotherapeutic treatment of pancreatic cancer. Two mechanisms of action that contribute to gemcitabine citotoxicity have been described. Firstly, gemcitabine competes with dCTP for the incorporation into DNA causing chain termination, and secondly, gemcitabine serves as an inhibitory alternative substrate for ribonucleotide reductase and leads to a reduction of deoxynucleotide pools [47,50]. This molecule inhibits growth of human pancreatic cancer cells that are insensitive to classic anticancer drugs, including other nucleoside analogs with similar structures. Differences in drug metabolism and mechanisms of action probably explain contrasts in clinical activity. Although gemcitabine seems to exert its toxicity through activation of apoptosis [47], recently we showed that gemcitabine also induces autophagy in pancreatic cancer cells and that this process mediates the cell death–promoting activity of this compound [51]. Early induction of autophagy by gemcitabine leads to cancer cell death and this process is mediated by the activation of VMP1 expression. VMP1 is a transmembrane protein, member of the molecular core machinery of autophagy [2], which triggers autophagy in mammalian cells through the interaction with beclin1 [52,53]. Experiments performed in PANC-1 and MIAPaCa-2 cells showed that gemcitabine treatment is able to induce autophagy. The inhibition of autophagy by 3-methyladenine, a widely used autophagy inhibitor, and VMP1-shRNA significantly reduces the percentage of dead cells in response to gemcitabine. In addition, gemcitabine promotes early VMP1 expression, and downregulation of VMP1 expression significantly reduces cell death. Thus, the VMP1-autophagy pathway promotes cell death in gemcitabine-treated human pancreatic cancer cells. The fact that inhibition of the autophagic pathway reduces pancreatic cancer cell death supports the hypothesis that the autophagic process enhances antitumor potency of the chemotherapeutic drug.

3.3. Capecitabine

It is a pyrimidine analog, an antimetabolite agent given orally to treat different types of malignancies. Capecitabine is a prodrug of the pyrimidine analog 5-fluoruracil. It is relatively efficacious in locally advanced pancreatic cancer when associated with limited field radiotherapy [54]. In one study, preoperative capecitabine-based chemoradiation resulted effective and well-tolerated treatment for patients with borderline resectable PDAC [55]. In a phase III study, combination of capecitabine plus gemcitabine improved objective response rate and progression free survival when compared with gemcitabine alone, associated with a trend toward to improve overall survival ($p = 0.08$) [56]. Capecitabine induces apoptosis in HTB-43 cells (a human larynx cancer cell line) in a dose dependent manner, not affecting human lymphocyte control cells [57]. The combination of capecitabine with vorinostat (a histone deacetylase inhibitor) seems to synergistically increase the apoptotic rate in colorectal cancer cells, probably through up-regulation of thymidine phosphorilase [58]. The activity of capecitabine seems to involve the modulation of Src kinase family, NFkB suppression and a reduction in c-Flip expression [59]. A very well known mechanism for capecitabine resistance involves Src kinase activation. Src kinases play a role in regulating autophagy [60]. Some pyrrolopyrimidine and pyrazolopyrimidine analogs have demonstrated inhibitory effect on Src kinases. Src inhibitors are associated with a high level of autophagy in prostatic cancer cells; when deregulated, excessive autophagy may lead to type II programmed cell death [60]. Nevertheless, in a prostatic cancer model, autophagy blockade enhanced the apoptosis-inducing effect of Src kinase inhibitors. However, although it is conceivable, potential effects of capecitabine in modulating autophagy on pancreatic cancer cells are still unknown.

3.4. Irinotecan

Irinotecan is a topoisomerase I inhibitor which prevents DNA from unwinding. In a randomized phase III trial, the combination of 5-fluoracil, leucovorin, oxaliplatin and irinotecan resulted in better responses, progression free survival and overall survival when compared with the standard single drug therapy with gemcitabine for metastatic pancreatic adenocarcinoma [61]. In small cell prostatic carcinoma, irinotecan demonstrated to increase in autophagy of the treated tumors as indicated by an increase in LC3B marker expression [62]. Even though, authors state that the role of autophagy in cancer is complex, since there is evidence that autophagy supports both promotion and suppression of cancer growth. This study represents the more relevant evidence of the effect of irinotecan on autophagy, and no information on the effect of this drug on pancreatic cancer is currently available.

3.5. Platinum coordination complexes

As mentioned before, a combination treatment including 5-fluoruracil, leucovorin, irinotecan and platinum showed promissory results in a phase III trial on patients with metastatic pancreatic cancer. In cisplatin-resistant SKOV3/DDP human ovarian cancer cells, knockdown of p62 or inhibition of autophagy using 3-methyladenine resensitizes these cells to cisplatin treatment [63]. Some studies carried out on esophageal cancer cell lines showed that cell populations that respond with autophagy are more resistant and will recover more easily following withdrawal of chemotherapeutic agents as cisplatin [64]. No information on oxaliplatin effects on pancreatic cancer cells autophagy is currently available.

3.6. Sorafenib

The multikinase inhibitor sorafenib and histone deacetylase inhibitors (HDACI) interact to kill pancreatic carcinoma cells. Sorafenib and multiple HDACIs interact in a highly synergistic manner to kill pancreatic tumor cells in vitro via activation of CD95 [65]. These effects are magnified when BCL-2 family protein activity is inhibited and restored in a CD95-null/knocked down environment. Both, Sorafenib + HDACI and GX15-070 treatments increase LC3 recruitment, indicative of autophagy; combined exposure to all three drugs further increase LC3 recruitment. Interestingly, knockdown of beclin1 suppresses the lethality of the BCL-2 family inhibitor GX15-070 to enhance sorafenib + HDACI-induced pancreatic tumor cell killing, regardless of CD95 expression [66]. Thus, autophagy induction potentiates antitumor effect because the inhibition of BCL-2 family enhances tumor cell killing by autophagy,

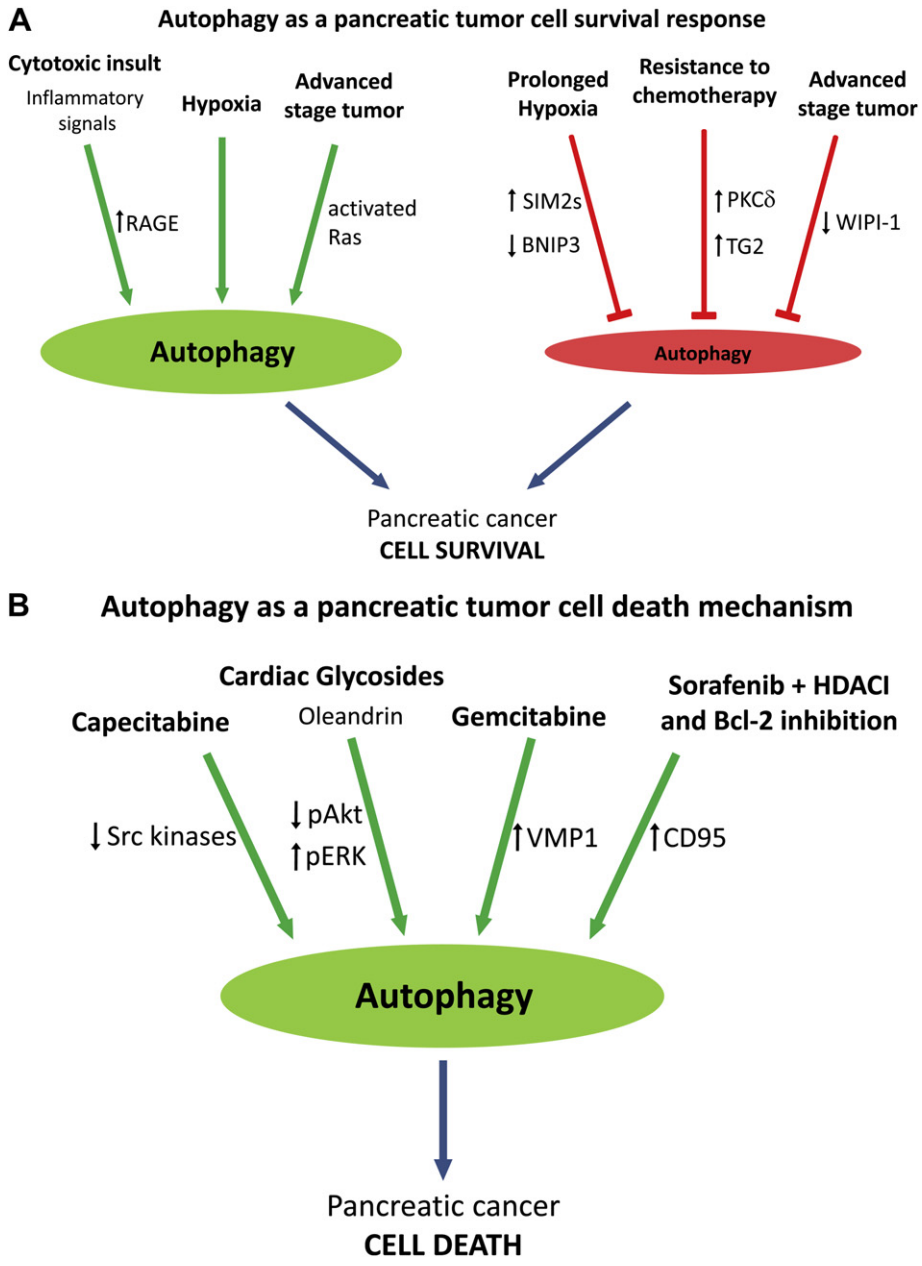

Fig. 1. *A: Autophagy as a pancreatic tumor cell survival response.* Regulation of autophagy in pancreatic cancer cell response to environment insult. This cartoon summarizes phenotypical changes in pancreatic tumor cells that ultimately activate or inhibit autophagy leading to pancreatic cancer cell survival. *B: Autophagy as a pancreatic tumor cell death mechanism.* Autophagy as a cell death mechanism in response to chemotherapeutic drugs. This cartoon shows examples of phenotypical changes of pancreatic tumor cells in response to chemotherapeutic drugs that activate autophagy leading to pancreatic cancer cell death. HDACI: histone deacetylase inhibitors; pAkt: phospho-Akt; pERK: phospho-ERK.

restoring the potency of antitumor drug in cells lacking the death receptor. This is of potential clinical importance because CD95 expression and functional signaling by this death receptor is often lost in the more advanced metastatic tumors.

4. Conclusions

Little is known about the role of autophagy in pancreatic cancer, and genetic/epigenetic modifications related to the disease are not well documented so far. However, autophagy-related phenotypic changes are described in pancreatic tumor cells. Pancreatic tumor cell activates autophagy in response to hypoxia and low nutrient supply. Autophagic features are reported in peripheral area of surgically rejected pancreatic cancer tissue [15]. Moreover, upregulation of autophagy favors survival of pancreatic cancer cell in tumor-associated inflammation. Overexpression of RAGE is associated with enhanced autophagy, diminished apoptosis and greater pancreatic cancer cell viability [19]. On the other hand, repression of autophagy-mediated cell death enhances tumor cell survival and explains in part pancreatic cancer resistance to treatment. Upregulation of SIM2s expression enhances tumor cell survival under prolonger hypoxic conditions by repression of BNIP3-mediated autophagic cell death. TG2 has been reported elevated in tumor cell lines resistant to chemotherapeutic drugs. Inhibition of TG2 expression results in beclin1-mediated autophagy and restores sensitivity of cancer cells to chemotherapeutic drugs [30].

Finally, both decreased and increased autophagic activity is associated to malignant stages of pancreatic cancer. Autophagy-related genes WIPI/Atg18 are substantially decreased in samples derived from advanced stage tumors, such as PDAC stages I–IV [39]. By contrast, pancreatic cancer cells with activating mutations in RAS have high basal autophagy and depend on autophagy for normal growth in PDAC, and conversely, depend on autophagy to cell death in early stages of pancreatic cancer [33–35]. These changes in gene expression suggest that different molecular mechanisms might be involved in autophagy-mediated survival or death. Therefore, phenotypical modifications in pancreatic tumor cells at different stages of the disease may explain the dual role of autophagy in pancreatic cancer and certainly revel that the autophagic process in cancer cells is much more complex than we actually know. Here we have briefly reviewed autophagy as a cell death mechanism in response to chemotherapeutic drugs. Several chemotherapeutic drugs exert their effect at least in part through promotion of autophagy-mediated cell death. Gemcitabine induced cell death is reduced when VMP1-beclin1-mediated autophagic pathway is downregulated at initial steps [51] and knockdown of beclin1 suppresses the lethality of the BCL-2 family inhibitor GX15-070 to enhance sorafenib + HDACI-induced pancreatic tumor cell killing [66]. Fig. 1 summarizes autophagic cell responses leading to pancreatic cancer cell survival (A) and death (B). It is important to emphasize that although the autophagic flow has not been studied in pancreatic cancer, the increase in the number of autophagosomes may be due to increased autophagic activity and also accumulation of autophagosomes may be due to a disruption of the autophagic flux at the lysosomal level. Therefore, results obtained from clinical studies that use chloroquine -a lysosomal hydrolase inhibitor- to test the effect of autophagy-inhibition during chemotherapy, must be careful interpreted [67]. However, it is very interesting to note that no chemotherapeutic drug has been described to kill pancreatic cancer cells by means of the inhibition of autophagosome formation. Furthermore, the effect of chemotherapy drugs such as gemcitabine or BCL-2 inhibitors, which promote autophagy-mediated cell death point at autophagy induction as a promising tool for pancreatic cancer therapy.

Acknowledgments

Our work is supported by the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET-PIP2527), the University of Buenos Aires (UBA-UBACyTM072) and the Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT-PICT01627 and ANPCyT-PICT0411). MIM is an ANPCyT Fellow and and ALR is a CONICET Fellow.

References

[1] Klionsky DJ, Cregg JM, Dunn Emr WA, Emr Jr SD, Sakai Y, Sandoval IV, et al. A unified nomenclature for yeast autophagy-related genes. Dev Cell 2003;5:539–45.
[2] Yang Z, Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. Curr Opin Cell Biol 2009;22:1–8.
[3] Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature 2008;451:1069–75.
[4] Høyer-Hansen M, Jäättelä M. Autophagy: an emerging target for cancer therapy. Autophagy 2008;4:574–80.
[5] Toth S, Nagy K, Palfia Z, Rez G. Cellular autophagic capacity changes during azaserine-induced tumour progression in the rat pancreas: up-regulation in all premalignant stages and down-regulation with loss of cycloheximide sensitivity of segregation along with malignant transformation. Cell Tissue Res 2002;309:409–16.
[6] Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, et al. Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003;112:1809–20.
[7] Chen N, Karantza-Wadsworth V. Role and regulation of autophagy in cancer. Biochim Biophys Acta 2009;1793:1516–23.
[8] Levine B. Cell biology: autophagy and cancer. Nature 2007;446:745–7.
[9] Mathew R, Karantza-Wadsworth V, White E. Role of autophagy in cancer. Nat Rev Cancer 2007;7:961–7.
[10] Warshaw AL, Fernandez-del Castillo C. Pancreatic carcinoma. N Engl J Med 1992;326:455–65.
[11] Jaffee EM, Hruban RH, Canto M, Kern SE. Focus on pancreas cancer. Cancer Cell 2002;2:25–8.
[12] Buchler P, Reber HA, Lavey RS, Tomlinson J, Büchler MW, Friess H, et al. Tumor hypoxia correlates with metastatic tumor growth of pancreatic cancer in an orthotopic murine model. J Surg Res 2004;120:295–303.
[13] Izuishi K, Kato K, Ogura T, Kinoshita T, Esumi H. Remarkable tolerance of tumor cells to nutrient deprivation: possible new biochemical target for cancer therapy. Cancer Res 2000;60:6201–7.
[14] Esumi H, Izuishi K, Kato K, Hashimoto K, Kurashima Y, Kishimoto A, et al. Hypoxia and nitric oxide treatment confer tolerance to glucose starvation in a 5′-AMP-activated protein kinase-dependent manner. J Biol Chem 2002;277:32791–8.
[15] Fujii S, Mitsunaga S, Yamazaki M, Hasebe T, Ishii G, Kojima M, et al. A autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome. Cancer Sci 2008;99:1813–9.
[16] DeNardo DG, Johansson M, Coussens LM. Inflaming gastrointestinal oncogenic programming. Cancer Cell 2008;14:7–9.
[17] Abe R, Yamagishi S. AGE-RAGE system and carcinogenesis. Curr Pharm Des 2008;14:940–5.
[18] Arumugam T, Simeone DM, Van GK, Logsdon CD. S100P promotes pancreatic cancer growth, survival, and invasion. Clin Cancer Res 2005;11:5356–64.
[19] Kang R, Tang D, Schapiro NE, Livesey KM, Farkas A, Loughran P, et al. The receptor for advanced glycation end products (RAGE) sustains autophagy and limits apoptosis, promoting pancreatic tumor cell survival. Cell Death Differ 2009;16:1–11.
[20] Kewley RJ, Whitelaw ML, Chapman-Smith A. The mammalian basic helix-loop-helix/PAS family of transcriptional regulators. Int J Biochem Cell Biol 2004;36:189–204.
[21] Tracy K, Dibling BC, Spike BT, Knabb JR, Schumacker P, Macleod KF. BNIP3 is an RB/E2F target gene required for hypoxia-induced autophagy. Mol Cell Biol 2007;27:6229–42.
[22] Azad MB, Chen Y, Henson ES, Cizeau J, McMillan-Ward E, Israels SJ, et al. Hypoxia induces autophagic cell death in apoptosis-competent cells through a mechanism involving BNIP3. Autophagy 2008;4:195–204.
[23] Burton TR, Gibson SB. The role of Bcl-2 family member BNIP3 in cell death and disease: NIPping at the heels of cell death. Cell Death Differ 2009;16:515–23.
[24] Okami J, Simeone DM, Logsdon CD. Silencing of the hypoxia-inducible cell death protein BNIP3 in pancreatic cancer. Cancer Res 2004;64:5338–46.
[25] Mahon PC, Baril P, Bhakta V, Chelala C, Caulee K, Harada T, et al. S100A4 contributes to the suppression of BNIP3 expression, chemoresistance, and inhibition of apoptosis in pancreatic cancer. Cancer Res 2007;67:6786–95.
[26] Abe T, Toyota M, Suzuki H, Murai M, Akino K, Ueno M, et al. Upregulation of BNIP3 by 5-aza-2'-deoxycytidine sensitizes pancreatic cancer cells to hypoxia-mediated cell death. J Gastroenterol 2005;40:504–10.
[27] Farrall AL, Whitelaw ML. The HIF1alpha-inducible pro-cell death gene BNIP3 is a novel target of SIM2s repression through cross-talk on the hypoxia response element. Oncogene 2009;28:3671–80.
[28] Mehta K, Fok J, Miller FR, Koul D, Sahin AA. Prognostic significance of tissue transglutaminase in drug resistant and metastatic breast cancer. Clin Cancer Res 2004;10:8068–76.
[29] Elsasser HP, MacDonald R, Dienst M, Kern HF. Characterization of a transglutaminase expressed in human pancreatic adenocarcinoma cells. Eur J Cell Biol 1993;61:321–8.
[30] Iacobuzio-Donahue CA, Ashfaq R, Maitra A, Adsay NV, Shen-Ong GL, Berg K, et al. Highly expressed genes in pancreatic ductal adenocarcinomas: a comprehensive characterization and comparison of the transcription profiles obtained from three major technologies. Cancer Res 2003;63:8614–22.
[31] Akar U, Ozpolat B, Mehta K, Fok J, Kondo Y, Lopez-Berestein G. Tissue transglutaminase inhibits autophagy in pancreatic cancer cells. Mol Cancer Res 2007;5:241–9.
[32] Gozuacik D, Kimchi A. Autophagy as a cell death and tumor suppressor mechanism. Oncogene 2004;23:2891–906.
[33] Guo JY, Chen HY, Mathew R, Fan J, Strohecker AM, Karsli-Uzunbas G, et al. Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. Genes Dev 2011;25:460–70.
[34] Elgendy M, Sheridan C, Brumatti G, Martin SJ. Oncogenic Ras-induced expression of Noxa and Beclin-1 promotes autophagic cell death and limits clonogenic survival. Mol Cell 2011;42:23–35.
[35] Yang S, Wang X, Contino G, Liesa M, Sahin E, Ying H, et al. Pancreatic cancers require autophagy for tumor growth. Genes Dev 2011;25:717–29.
[36] Mariño G, Martins I, Kroemer G. Autophagy in Ras-induced malignant transformation: fatal or vital? Mol Cell 2011;42:1–3.
[37] Guan J, Stromhaug PE, George MD, Habibzadegah-Tari P, Bevan A, Dunn Jr WA, et al. Cvt18/Gsa12 is required for cytoplasm-to-vacuole transport, pexophagy, and autophagy in Saccharomyces cerevisiae and Pichia pastoris. Mol Biol Cell 2001;12:3821–38.
[38] Proikas-Cezanne T, Waddell S, Gaugel A, Frickey T, Lupas A, Nordheim A. WIPI-1alpha (WIPI49), a member of the novel 7-bladed WIPI protein family, is aberrantly expressed in human cancer and is linked to starvation-induced autophagy. Oncogene 2004;23:9314–25.

[39] Proikas-Cezanne T, Robenek H. Freeze-fracture replica immunolabelling reveals human WIPI-1 and WIPI-2 as membrane proteins of autophagosomes. J Cell Mol Med 2011;15:2007–10.
[40] Martinez-Outschoorn UE, Trimmer C, Lin Z, Whitaker-Menezes D, Chiavarina B, Zhou J, et al. Autophagy in cancer associated fibroblasts promotes tumor cell survival: role of hypoxia, HIF1 induction and NFκB activation in the tumor stromal microenvironment. Cell Cycle 2010;9: 3515–33.
[41] Lisanti MP, Martinez-Outschoorn UE, Chiavarina B, Pavlides S, Whitaker-Menezes D, Tsirigos A, et al. Understanding the "lethal" drivers of tumor-stroma co-evolution: emerging role(s) for hypoxia, oxidative stress and autophagy/mitophagy in the tumor micro-environment. Cancer Biol Ther 2010;10:537–42.
[42] Kim EJ, Simeone DM. Advances in pancreatic cancer. Curr Opin Gastroenterol 2011;27:460–6.
[43] Von Hoff DD, Ramanathan RK, Borad MJ, Laheru DA, Smith LS, Wood TE, et al. Gemcitabine Plus nab-Paclitaxel is an active regimen in patients with advanced pancreatic cancer: a phase I/II trial. J Clin Oncol; 2011 Oct 3 [Epub ahead of print].
[44] Neesse A, Michl P, Frese KK, Feig C, Cook N, Jacobetz MA, et al. Stromal biology and therapy in pancreatic cancer. Gut 2011;60:861–8.
[45] Chen JQ, Contreras RG, Wang R, Fernandez SV, Shoshani L, Russo IH, et al. Sodium/potassium ATPase (Na+, K+-ATPase) and ouabain related cardiac glycosides: a new paradigm for development of anti-breast cancer drugs? Breast Cancer Res Treat 2005;96:1–15.
[46] Raghavendia PB, Sreenivasan Y, Manna SK. Oleandrin induces apoptosis in human but not murine cells: dephosphorylation of Akt, expression of FasL and alteration of membrane fluidity. Mol Immunol 2007;44:2292–302.
[47] Ewald B, Sampath D, Plunkett W. Nucleoside analogs: molecular mechanisms signaling cell death. Oncogene 2008;27:6522–37.
[48] Vivanco I, Sawyers CL. The phosphatidylinositol 3-kinase Akt pathway in human cancer. Nat Rev Cancer 2002;2:489–501.
[49] Newman RA, Keondo Y, Yokoyama T, Dixon S, Cartwright C, Chan D, et al. Autophagic cell death of human pancreatic tumor cells mediated by oleandrin, a lipid-soluble cardiac glycoside. Integr Cancer Ther 2007;6:354–64.
[50] Wang J, Lohman GJ, Stubbe J. Enhanced subunit interactions with gemcitabine-5'-diphosphate inhibit ribonucleotide reductases. Proc Natl Acad Sci USA 2007;104:14324–9.
[51] Pardo R, Lo Ré A, Archange C, Ropolo A, Papademetrio DL, Gonzalez CD, et al. Gemcitabine induces the VMP1-mediated autophagy pathway to promote apoptotic death in human pancreatic cancer cells. Pancreatology 2010;10: 19–26.
[52] Ropolo A, Grasso D, Pardo R, Sacchetti ML, Archange C, Lo Re A, et al. The pancreatitis-induced vacuole membrane protein 1 triggers autophagy in mammalian cells. J Biol Chem 2007;282:37124–33.
[53] Vaccaro MI. Autophagy and pancreas disease. Pancreatology 2008;8:425–9.
[54] Jackson AS, Jain P, Watkins GR, Whitfield GA, Green MM, Valle J, et al. Efficacy and tolerability of limited field radiotherapy with concurrent capecitabine in locally advanced pancreatic cancer. Clin Oncol (R Coll Radiol) 2010;22:570–7.
[55] Stokes JB, Nolan NJ, Stelow EB, Walters DM, Weiss GR, de Lange EE, et al. Preoperative capecitabine and concurrent radiation for borderline resectable pancreatic cancer. Ann Surg Oncol 2011;18:619–27.
[56] Cunningham D, Chau I, Stocken DD, Valle JW, Smith D, Steward W, et al. Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. J Clin Oncol 2009;27: 5513–8.
[57] Wisniewska-Jarosinska M, Sliwinski T, Kasznicki J, Kaczmarczyk D, Krupa R, Bloch K, et al. Cytotoxicity and genotoxicity of capecitabine in head and neck cancer and normal cells. Mol Biol Rep 2011;38:3679–88.
[58] Di Gennaro E, Piro G, Chianese MI, Franco R, Di Cintio A, Moccia T, et al. Vorinostat synergises with capecitabine through upregulation of thymidine phosphorylase. Br J Cancer 2010;103:1680–91.
[59] Sheikh R, Walsh N, Clynes M, O'Connor RO, McDermott R. Challenges of drug resistance in the management of pancreatic cancer. Expert Rev Anticancer Ther 2010;10:1647–61.
[60] Kung HJ. Targeting tyrosine kinases and autophagy in prostate cancer. Horm Cancer 2011;2:38–46.
[61] Conroy T, Desseigne F, Ychou M, Ducreux M, Bouche O, Guimbaud R, et al. Randomized phase III trial comparing FOLFIRINOX (F: 5FU/leucovorin [LV], irinotecan [I], and oxaliplatin [O]) versus gemcitabine (G) as first-line treatment for metastatic pancreatic adenocarcinoma (MPA): preplanned interim analysis results of the PRODIGE 4/ACOORD 11 trial. J Clin Oncol 2010;28(May 20 Supplement):4010.
[62] Tung WL, Wang Y, Gout PW, Liu DM, Gleave M, Wang Y. Use of irinotecan for treatment of small cell carcinoma of the prostate. Prostate 2011;71:675–81.
[63] Yu H, Su J, Xu Y, Kang J, Li H, Zhang L, et al. p62/SQSTM1 involved in cisplatin resistance in human ovarian cancer cells by clearing ubiquitinated proteins. Eur J Cancer 2011;47:1585–94.
[64] O'Donovan TR, O'Sullivan GC, McKenna S. Induction of autophagy by drug-resistant esophageal cancer cells promotes their survival and recovery following treatment with chemotherapeutics. Autophagy 2011;7:509–24.
[65] Zhang G, Park MA, Mitchell C, Hamed H, Rahmani M, Martin AP, et al. Vorinostat and sorafenib synergistically kill tumor cells via FLIP suppression and CD95 activation. Clin Cancer Res 2008;14:5385–99.
[66] Martin AP, Park MA, Mitchell C, Walker T, Rahmani M, Thorburn A, et al. BCL-2 family inhibitors enhance histone deacetylase inhibitor and sorafenib lethality via autophagy and overcome blockade of the extrinsic pathway to facilitate killing. Mol Pharmacol 2009;76:327–41.
[67] Naumann P, Fortunato F, Zentgraf H, Büchler MW, Herr I, Werner J. Autophagy and cell death signaling following dietary sulforaphane act independently of each other and require oxidative stress in pancreatic cancer. Int J Oncol 2011; 39:101–9.