Review

Possible Role of Autophagy in the Treatment of Pancreatic Cancer with Histone Deacetylase Inhibitors

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Abstract: Pancreatic cancer is a lethal disease and notoriously difficult to treat. Only a small proportion is curative by surgical resection, whilst standard chemotherapy for patients with advanced disease has only a modest effect with substantial toxicity. Clearly there is a need for the continual development of novel therapeutic agents to improve the current situation. Currently, there is a bulk of data indicating the important function of autophagy in cancer. While genetic evidence indicates that autophagy functions as a tumor suppressor, it is also apparent that autophagy can promote the survival of established tumors under stress conditions and in response to chemotherapy. This review provides a spectrum of potential pharmacological agents and autophagic approaches to enhance cell killing in pancreatic cancer.

Keywords: pancreatic cancer; autophagy; apoptosis; epigenetics; histone deacetylase; ER stress

1. Introduction

Pancreatic cancer lacks specific symptoms and early detection tools and thus, has the worst mortality rate and the lowest overall survival of all cancers. In particular, pancreatic ductal adenocarcinoma is an extremely aggressive and devastating neoplasm, which often invades and destroys surrounding stromal components, including lymphatic, vascular, and perineural systems, ultimately metastasizing to distant organs. Another reason for the poor prognosis of pancreatic cancer
is the insensitivity to most therapies, such as chemotherapy, radiotherapy and immunotherapy. Thus, novel strategies to treat this deadly disease are urgently needed.

Chemotherapy is still the only option in metastatic pancreatic cancer treatment. In particular, gemcitabine has represented the standard chemotherapy for all stages of pancreatic adenocarcinoma in the last decade; however, neither gemcitabine alone nor gemcitabine-based combinational chemotherapy achieves a favorable outcome in advanced cases. New adjuvant therapy targeting specific markers in pancreatic cancer using the molecular approach may represent a promising strategy in the diagnosis and treatment of pancreatic cancer. Indeed, these molecular approaches have rapidly developed in recent years, and include anti-sense oligonucleotides, RNA interference, gene restoration, suicide gene therapy, small molecule inhibitors, oncolytic viral therapy, and antibody therapy. However, many of those approaches have yet to be tested in clinical applications, and most of the treatments need to be combined with standard chemotherapy or radiotherapy for maximum benefits [1].

2. New Molecular Approaches to Pancreatic Cancer Treatment

Gemcitabine (2′-2′-difluorodeoxycytidine) is the most active chemotherapy agent used for the treatment of pancreatic cancer. It is an analog of deoxycytidine that is incorporated into double-stranded DNA during the S (synthesis) phase, resulting in inhibition of DNA synthesis, arrest of cell cycle progression, and induction of apoptosis. In general, resistance to chemotherapy, whether intrinsic or acquired, is believed to be a major cause of treatment failure in pancreatic cancer [2]. It is certainly considered that resistance to gemcitabine treatment is mainly attributed to an altered apoptotic threshold in pancreatic cancer cells [3]. Gemcitabine is also incorporated into RNA, depending on the cell line, at similar levels as in DNA, resulting in RNA synthesis inhibition in human cell lines. Sensitivity to gemcitabine seems to be related to differences in RNA incorporation. However, collateral sensitivity to gemcitabine in doxorubicin-resistant cells was related to an increased incorporation into DNA but not to RNA incorporation. Attempts to better understand the molecular basis for these characteristics of pancreatic cancer have focused on studying the gene and protein expression profiles of both resected tumors and pancreatic cancer cell lines. Accordingly, tumor suppressor genes related to anti-oxidant activity, apoptosis, the cell cycle, signal transduction and intracellular adhesion, are often mutated or silenced by epigenetic modifiers, such as histone deacetylases (HDACs) and DNA methyltransferases (DNMTs). Recent studies have shown that clinically relevant HDAC inhibitors, such as suberoylanilide hydroxamic acid (SAHA), can restore sensitivity to gemcitabine and other agents [4]. Thus, it may be possible to use HDAC inhibitors to restore drug sensitivity to pancreatic cancers. Eventually, a strategy for sensitizer (lowering the apoptotic threshold)/inducer (activation of the apoptotic machinery), could be a potential approach for a rational molecular-based tumor therapy for pancreatic cancer.

In addition, a recent study showed that blocking sphingosine kinase-1 (SphK1) activity using small interfering RNA (siRNA) can sensitize pancreatic cancer cells to gemcitabine treatment, indicating that development of combinational therapy of siRNA with gemcitabine may represent a promising approach in pancreatic cancer treatment [5]. siRNA-mediated silencing of anti-apoptotic Bcl-2 enhances chemotherapy sensitivity in human pancreatic cancer cells in vitro. Moreover, replacement of p53 in pancreatic cancer cells previously treated with gemcitabine increased cytotoxicity both in vitro
and in vivo [6]. HSV-TK (herpes simplex virus-thymidine kinase) gene delivery followed by gancyclovir was found to be effective in inhibiting the tumor growth and metastasis of pancreatic cancer [7]. Tissue-specific promoters are preferred in suicide gene therapy in order to achieve maximal efficacy and minimal toxicity. Erlotinib, a small molecule tyrosine kinase inhibitor, targets the intracellular domain of the epidermal growth factor receptor (EGFR), and has been shown to improve survival when used in combination with gemcitabine to treat metastatic pancreatic cancers [8]. In addition, a patient with stage IV pancreatic cancer showed a response to chemotherapy with the addition of bevacizumab, a recombinant humanized monoclonal antibody targeting vascular endothelial growth factor (VEGF), while it was initially unresponsive to gemcitabine, 5-fluorouracil, irinotecan, and cisplatin treatment [9]. These therapeutic agents will provide a new perspective on pancreatic cancer treatment using molecular approaches.

3. Genetic Background of Pancreatic Cancer Cells

Cancer cells express the successive accumulation of gene mutations (oncogenes, tumor-suppressor genes, and stability genes), which increase aggressiveness and confer resistance to conventional chemotherapy and radiotherapy [10]. In particular, pancreatic cancer cells seem to differ from cervical, prostate, colorectal, and small bowel cancer cells in their expression of Bcl-xL, Bcl-2, cyclin D1, and TRAIL decoy receptor 2 (DcR2) [11]. Moreover, the p53 mutation occurs in 50–75% of infiltrating pancreatic adenocarcinomas, being rare in chronic pancreatitis [12]. p53 plays a central role in modulating cellular responses to cytotoxic stresses by contributing to both cell-cycle arrest and apoptosis. Mutations in cyclin-dependent kinase inhibitor p16 and Smad4, a downstream target of transforming growth factor β, also exhibit high mutation frequencies in pancreatic tumors [13]. Activation of the K-Ras oncogene has been implicated in more than 90% of pancreatic carcinogenesis, and K-Ras mutation represents one of the earliest genetic alterations in pancreatic cancer development [14]. Chemotherapeutic resistance is often associated with mutations in codon 12 of the K-Ras gene [15]. Oncogenic K-Ras promotes pancreatic tumorigenesis through the activation of multiple downstream pathways, including PI3K/Akt, ERK, Bad, and NF-κB [16]. Moreover, overexpression of p21WAF1/CIP1 (wild type p53 activated fragment-1/poly(c)-binding protein 1) has been reported to be an early event in the development of pancreatic intraepithelial neoplasia [17]. Resistance to apoptosis is also a hallmark of pancreatic cancers, and therefore lowering the apoptotic threshold is a therapeutic goal [18]. NF-κB is constitutively active in pancreatic cancers, which protects the cells from apoptosis and, in some cases, stimulates their growth. TNF-α-induced apoptosis is inhibited by the concomitant overexpression of NF-κB signaling induction, in which free active NF-κB migrates into the nucleus, inducing the transcription of different anti-apoptotic genes, such as Cox-2, IAPs, XIAP, Survivin, Bcl-xL, Bcl-2, and FLIP (Flice-like inhibitory protein) [19].

4. Epigenetic Alterations in Pancreatic Cancer

Such genetic backgrounds originate from missense mutations at residues that are essential for activity, from mutations that result in a truncated protein, from deletions or insertions of various sizes, or from epigenetic silencing. Epigenetic alterations are defined as heritable changes in gene expression that are not accompanied by changes in DNA sequence. The past several years have provided an
explosive increase in our knowledge of epigenetic features in human cancers. Along with genetic events, tumor-associated epigenetic alterations are important determinants in the initiation and progression of pancreatic cancer [20]. Indeed, pancreatic cancers harbor numerous epigenetic alterations, these alterations can be observed in neoplastic precursor lesions, and their prevalence increases as lesions become more advanced [21]. Specific events driving changes that lead to cancer development and progression are interconnected complex molecular modifications, including DNA methylation, histone acetylation, phosphorylation, ubiquitylation and ADP ribosylation.

4.1. Aberrant DNA Methylation

Epigenetic alterations in transformed cells involve changes in DNA methylation, including global hypomethylation and locus-specific hypermethylation. DNA methylation is an enzyme-driven chemical change to the DNA sequence that most commonly occurs at CpG dinucleotides in mammals. The global methylated cytosine content is often reduced in cancer, including pancreatic cancers. One consequence of genome-wide hypomethylation may be genomic instability, a characteristic of most pancreatic cancers. By contrast, hypermethylation of $p16^{INK4A}$ in a subset of pancreatic cancers was one of the early reports of aberrant methylation in pancreatic cancers. The $p16^{INK4A}$ is inactivated in approximately 95% of pancreatic adenocarcinomas, approximately 15% of which were attributable to aberrant promoter methylation. The $p16^{INK4A}$ gene is aberrantly methylated in 27% of pancreatic cancer cell lines [22]. The reversal of methylation by a DNMT inhibitor, 5-aza-2’-deoxycytidine (5-Aza-dC), results in increased mRNA expression of epigenetically-relevant genes. It has been shown that 5-Aza-dC inhibits the growth of pancreatic cancer cell lines. In addition, pretreatment with 5-Aza-dC increased the sensitivity of pancreatic cancer cells to other chemotherapy agents, including TNF-α, cisplatin, and gemcitabine [23]. DNMT inhibition by zebularine showed a promising anti-cancerous effect in pancreatic cancer cells in vitro and in vivo, leading to apoptosis induction, growth suppression and phenotypic stabilization. This effect could be augmented by co-incubation with the HDAC inhibitor SAHA in vitro, but was not observed in the xenograft model [24].

4.2. Histone Modifications

Histone acetylation/deacetylation alters the status of open chromatin domains and thus affects gene transcription. This process is modulated by histone acetyltransferases (HATs) and HDACs. Acetylation correlates with the remodeling of nucleosomes, resulting in relaxation of the chromatin structure which facilitates the accessibility of a variety of factors to DNA causing transcriptional activation. In contrast, deacetylation of the histone tails induces transcriptional repression through chromatin condensation. Locus-specific changes in histone acetylation have been linked to the altered expression of several critical genes in pancreatic cancer, whereas widespread changes in gene expression after the treatment of cell lines with HDAC inhibitors suggest that histone modifications may play a much broader role in regulating gene expression in pancreatic cancer [25]. HDAC inhibitors seem to be specifically selective against tumor cells and show very low toxicity [26]. For example, HDAC inhibitors trichostatin A (TSA) [27,28], SAHA [4] and FK228 [29] have exerted growth-inhibitory and pro-apoptotic effects on pancreatic cancer cell models. Combined treatment with TSA and gemcitabine synergistically inhibited the growth of four pancreatic adenocarcinoma cell
lines and induced apoptosis. This effect was associated with the induction of reactive oxygen species (ROS) by gemcitabine, and increased the expression of the pro-apoptotic Bim gene by both TSA and gemcitabine. In vivo studies on xenografts of pancreatic adenocarcinoma cells in nude mice showed that the association of TSA and gemcitabine reduced the tumor mass by 50% and did not cause any apparent toxicity, while treatments with TSA or gemcitabine alone were ineffective [27].

5. HDAC Expression in Pancreatic Cancer

Histone acetylation of the ε-amino site of lysines located at the N-terminal tail of histones by HAT plays a key role in the regulation of transcription by modulating the structure of chromatin, and is precisely regulated by the balance between the activities of HAT and HDAC. HDACs deacetylate and lead to repressive chromatin formation (heterochromatin) and the suppression of gene expression [30]. In addition to the condensation of chromatin, non-histone proteins have been identified as acetylation targets, and reversible lysine acetylation in these proteins plays an important role(s) in the regulation of mRNA stability, protein localization and degradation, and protein-protein and protein-DNA interactions. Many of these proteins are transcription factors, such as p53, C/EBPβ, NF-κB and STATs. A common finding in several cancer cells is high HDAC expression levels and, consequently, a low level of histone acetylation when compared with normal tissues (Table 1). The majority of reports has focused on class I HDACs and suggests the clinical manifestations of aberrant HDAC expression. For example, in cancers of the gastrointestinal system, high HDAC1, HDAC2, and HDAC3 expression correlated with a poor clinical outcome [31], whereas HDAC5 mRNA transcript was shown to be down-regulated in colon cancer [32]. Neuroblastoma, among all HDAC family members investigated, only HDAC8 was associated with advanced-stage disease and poor prognosis [33]. Moreover, SIRT8 was shown to be overexpressed in thyroid carcinoma and cell lines but not in adenomas [34], whereas the SIRT2 gene was shown to be down-regulated in human gliomas [35]. The expression of individual HDAC family members therefore seems to be tumor specific.

In human pancreatic cancers (Table 2), 56% of pancreatic cancers show positive immunohistochemical staining for HDAC1, and the co-expression of HDAC1 and hypoxia-inducible factor-1α (HIF-1α) markedly correlates with poor prognosis [36]. HDAC2, which is highly expressed, controls resistance to TRAIL. Using tissue microarrays, the overexpression of HDAC2 was detected, especially in moderately differentiated (G2) and undifferentiated (G3) pancreatic cancers [37]. At the molecular level, HDAC2 inhibition opens the locus of the epigenetically silenced NOXA gene, a BH3-only protein and apical initiator of apoptosis [37]. In addition, nine of the 11 pancreatic adenocarcinomas (approximately 81%) showed a significant increase of HDAC7 mRNA levels [38]. It has been demonstrated that HDAC7 may regulate the initiation of apoptosis [39]. A recent study has reported that the SAHA, the first HDAC inhibitor approved for clinical use in the treatment of the cancer cutaneous T-cell lymphoma [40], selectively suppresses the expression of HDAC7 [41]. Down-regulation of HDAC7 by SAHA is more pronounced in transformed cells sensitive to inhibitor-induced cell death than in normal cells or cancer cells resistant to induced cell death. These data suggest a role for HDAC isoform overexpression in pancreatic cancers; however, a more detailed investigation of HDAC expression in pancreatic cancers is necessary, especially in larger cohorts and in correlation with clinical and prognostic parameters.
Table 1. HDAC expression in cancer.

| Tumor type       | HDAC isoform                  | Expression | Ref. |
|------------------|-------------------------------|------------|------|
| Breast cancer    | HDAC4, HDAC6                  | Increase   | [66] |
| Colon cancer     | HDAC1, HDAC2, HDAC3, HDAC8    | Increase   | [67] |
| Colorectal cancer| HDAC1, HDAC2, HDAC3, SIRT1    | Increase   | [68] |
| Gastric cancer   | HDAC1, HDAC2, HDAC3           | Increase   | [31] |
| Glioma           | SIRT2                         | Decrease   | [35] |
| Lung cancer      | HDAC1, HDAC3, HDAC5, HDAC10   | Increase   | Decrease | [69] |
| Neuroblastoma    | HDAC8                         | Increase   | [33] |
| Oral cancer      | HDAC2, HDAC6                  | Increase   | [70] |
| Ovarian cancer   | HDAC3                         | Increase   | [71] |
| Prostate cancer  | HDAC1, HDAC2, HDAC3           | Increase   | [72] |
| Thyroid carcinoma| SIRT8                         | Increase   | [34] |

Table 2. HDAC expression in pancreatic cancer.

| HDAC isoform | Function                                                                 | Ref. |
|--------------|--------------------------------------------------------------------------|------|
| HDAC1        | Poor prognosis                                                           | [36] |
|              | Chemotherapy resistance                                                 |      |
|              | Resistance to cell cycle arrest, growth inhibition, and apoptosis       |      |
|              | Autophagy resistance                                                    |      |
|              | Repression of promoters of tumor suppressor genes                       |      |
|              | Induction of proliferation and dedifferentiation                        |      |
| HDAC2        | TRAIL resistance                                                        | [37] |
|              | Etoposide resistance                                                    |      |
|              | NOXA gene silencing                                                     |      |
|              | Resistance to differentiation, apoptosis and p53 independent p21 expression|      |
| HDAC7        | Apoptosis initiation                                                    | [38,39]|
|              | Mitochondrial localization                                              |      |
|              | Malignant progression                                                   |      |
|              | Overexpression of PDGF-B                                                |      |
|              | Control angiogenesis through regulation of angiogenic genes              |      |

6. Induction of Autophagy by HDAC Inhibitors

The ubiquitin–proteasome system and autophagy are two major intracellular pathways for protein degradation. The ubiquitin–proteasome system degrades short-lived proteins whose functions are usually critical to the control of cell proliferation and cell death. Autophagy is an evolutionarily conserved catabolic process where a cell self-digests its cytoplasmic contents. Autophagy is a key mechanism for long-lived protein degradation and organelle turnover, and serves as a critical adaptive response that recycles energy and nutrients during starvation or stress [42]. Autophagy is tightly regulated by a limited number of highly conserved genes called autophagy regulators (Atgs); however, whether Atg genes work through their expected mechanisms of autophagy regulation and/or through as-yet-undefined functions in the development of cancer remains to be further clarified. Autophagy is
activated in response to multiple stresses during cancer progression, such as nutrient starvation, unfolded protein response, endoplasmic reticulum (ER) stress, and hypoxia (Table 3); it is also observed upon treatment of cancers with a wide spectrum of cytotoxic and targeted chemotherapeutic agents [43]. Recent findings indicate that suppression of the ubiquitin–proteasome system by proteasome inhibitors induces autophagy through multiple pathways, including activation of HDAC6, which deacetylates α-tubulin in the cytoplasm [44]. It is thought that the deacetylation of α-tubulin by HDAC6 is necessary for transport of the aggresome by microtubules to the lysosome for degradation. The role of autophagy as a survival mechanism in response to these diverse stressors has been well established. Moreover, it has become increasingly clear that a basal level of autophagy serves housekeeping functions vital for maintaining cellular homeostasis; specifically, the failure to clear protein aggregates or damaged organelles via autophagy has been implicated in multiple pathological conditions, including cancer [45].

Table 3. Autophagy modulators and their mode of action.

| Mode of action          | Modulator                               | Effect on autophagy | Ref.  |
|-------------------------|-----------------------------------------|---------------------|-------|
| mTORC1 inhibition       | Rapamycin, CCI-779, Rottlein, Curcumin  | Activator           | [73]  |
| Akt inhibition          | Perifosine, Curcumin, Resveratrol        | Activator           | [74]  |
| GSK3 βP inhibition      | Lithium                                 | Activator           | [75]  |
| Inositol and IP3 reduction | Sodium valproate                     | Activator           | [76]  |
| Ca²⁺ level reduction    | Verapamil                               | Activator           | [77]  |
| Calpain inhibition      | Calpastatin                             | Activator           | [78]  |
| cAMP level reduction    | Clonidine                               | Activator           | [77]  |
| HDAC inhibition         | SAHA, Butyrate, Sodium valproate        | Activator           | [47]  |
| Tyrosine kinase inhibition | Imatinib                             | Activator           | [79]  |
| PI3 kinase inhibition   | LY294002, Wortmannin, 3-Methyladenine   | Inhibitor           | [80]  |
| p38 MAP kinase inhibition | SB202190                          | Inhibitor           | [81]  |
| Bcl-2, Bcl-xL inhibition | Arsenic trioxide, ABT737               | Activator           | [82]  |
| Lysosomotropic drug     | Chloroquine                             | Inhibitor           | [83]  |
| HSP70 inhibition        | 2-Phenylethanesulfonamide              | Activator           | [84]  |
| Tubulin inhibition      | Vincristine, Paclitaxel                 | Inhibitor           | [85]  |
| Cytochrome c release    | Resveratrol                             | Activator           | [74]  |
| Atg5 function           | Phenethyl isothiocyanate               | Activator           | [86]  |
| Dopamine antagonist     | Fluspirilene                            | Activator           | [87]  |
| ER antagonist           | Tamoxifen                               | Activator           | [88]  |
| DNA damage              | Radiation                               | Activator           | [89]  |

To investigate if a class I HDAC isotype is involved in autophagy, a specific class I HDAC inhibitor and an siRNA of HDAC1 were used to treat HeLa cells. Both inhibition and genetic knock-down of HDAC1 in the cells significantly induced autophagic vacuole formation and lysosome function [46]. Two distinct HDAC inhibitors, butyrate and SAHA, induced caspase-3 activation and cell death in multiple human cancer cell lines; however, Apaf-1 knockout, overexpression of Bcl-xL, and pharmacological inhibition of caspase activity did not prevent SAHA and butyrate-induced cell death. The cells undergoing such caspase-independent death had unambiguous morphological features of
autophagic cell death. Induction of autophagic cell death by HDAC inhibitors has clear clinical implications in treating cancers with apoptotic defects [47]. HDAC inhibitor (SAHA) induced autophagy in cancer cells through inhibition of Akt/mTOR pathway and induction of ER stress response. Inhibition of autophagy reduced SAHA-induced cytotoxicity, indicating that SAHA elicited autophagic cell death. SAHA is an attractive candidate for the treatment of pancreatic cancer and pharmacological targeting of autophagy provides promise for the management of cancer therapy. Autophagy is negatively regulated by the class I PI3K signaling pathway. SAHA diminished mTOR expression and mediated caspase-independent cytotoxicity in endometrial sarcoma cells via autophagic mechanisms [48]. Furthermore, HDAC inhibitor FK228 (depsipeptide) inhibited proliferation and induced apoptosis in the malignant rhabdoid tumors cell lines tested. Preincubation with the pancaspase inhibitor zVAD-fmk did not completely rescue FK228-induced cell death, although it did inhibit apoptosis. Disrupting autophagy with chloroquine treatment enhanced FK228-induced cell death [49]. HDAC inhibitor TSA induced AIF release from the mitochondria in human pancreatic adenocarcinoma cell lines [50], but no study has suggested that AIF translocation into the nucleus mediates autophagy. Autophagy rather than the apoptosis-inducing activity for both H40 and SAHA was observed in prostate cancer PC-3M cells [51]. HDAC inhibitor-induced expression of p21WAF1/CP1, a modulator of apoptosis, was evident in PC-3M and HL-60 cell lines; however, it is not known whether the inability to induce apoptosis by these drugs in PC-3M cells can be attributed to their induction of p21WAF1/CP1 expression (Table 4).

Table 4. Effects of HDAC inhibitors in pancreatic cancer cells.

| HDAC inhibitor     | Pancreatic cell line     | Effects of HDAC inhibitor | Ref. |
|--------------------|--------------------------|---------------------------|------|
| Benzyl isothiocyanate | BxPC-3, Capan-2         | Growth suppression        | [90] |
| Valproic acid     | MiaPaCa2, Panc1          | TRAIL sensitivity         | [91] |
| Valproic acid     | MiaPaCa2, Panc1, BxPc3   | Etoposide sensitivity     | [37] |
| SAHA              | Panc1, BxPC-3            | Gemcitabine sensitivity   | [92] |
| Depsipeptide      | Panc1                    | Heterochromatin-associated protein 1 | [93] |
| Trichostatin A    | MiaPaca2, PaCa3, Panc1   | Gemcitabine sensitivity   | [27] |
| 4-Phenybutyrate    | Panc1, T4M-4, BxPc3      | Gemcitabine sensitivity   | [94] |
| Trichostatin A    | MiaPaca2, PaCa3, Panc1   | Gemcitabine, 5-fluorouracil sensitivity | [95] |
| FR901228          | Capan-1, BxPC-3, Panc-1, MIAPaCa-2 | Apoptosis induction | [29] |

7. Autophagy in Pancreatic Cancer Cells

Autophagy is increased in pancreatic cancer cells in resected tumors and correlates with poor patient outcome [52]. Hypoxia in pancreatic cancer has been reported to increase its malignant potential. Autophagy is thought to be a response to factors in the cancer microenvironment, such as hypoxia and poor nutrient supply. Autophagic capacity is elevated in the earliest premalignant lesions and remains high, although it varies throughout premalignant progression, but drops with the appearance of adenocarcinoma [53]. Both murine and human pancreatic tumor cell lines showed clear evidence of enhanced autophagy following treatment with chemotherapeutic agents. In addition, most pancreatic cancers with mutations in apoptotic pathways illuminate the importance of autophagic cell
death [54]. In contrast to apoptosis, cell death associated with autophagy is caspase-independent and does not involve nuclear fragmentation.

7.1. Triptolide-Induced Autophagy

A diterpene triepoxide, triptolide, was extracted from a Chinese herb that inhibited the proliferation of cancer cells in vitro and reduced the growth and metastases of tumors in vivo. Knock-down of caspase-3 using the siRNA pool in pancreatic cancer cell lines S2-013 and S2-VP10 cells did not affect cell viability after triptolide treatment [55], suggesting the involvement of a non-apoptotic and caspase-independent cell death pathway in these cells. The induction of autophagy was confirmed by the following responses to triptolide: the increase in the LC3-II form is both time- and dose-dependent; triptolide-treated S2-013 tumors also show an increase in LC3-II in vivo; the increase in acridine orange staining in response to triptolide can be reversed by the addition of an autophagy-specific inhibitor, 3-methyladenine. Knock-down of \textit{atg}5 or \textit{beclin1} genes, essential in autophagy, did not prevent triptolide-mediated cell death in pancreatic cancer cell lines but instead triggered apoptosis, whereas dual silencing of \textit{beclin1} and \textit{caspase-3} rescued triptolide-mediated cell death. Evaluating the effect of a stimulator on these regulators of the autophagic and apoptotic pathway will aid in explaining why different pancreatic cancer cells have a differential response to stimulator. In pancreatic adenocarcinoma, \textit{K-ras}, \textit{p53}, \textit{p16}, and \textit{DPC4} genes are altered most frequently. Pancreatic cancer cells harbor intact apoptotic machinery, but preferentially activate the autophagic pathway in response to triptolide [55]. The choice of the autophagic cell death pathway could depend on the metastatic potential of the cell lines being more metastatic than the others, which merits further investigation.

7.2. Signaling Pathways in Autophagy

Although many signaling pathways regulate autophagy, signaling from the cytoplasm to the autophagy machinery is mainly controlled in a negative manner through a serine/threonine kinase, mTOR. Akt, a positive regulator of mTOR, suppresses the formation of autophagosomes and inhibits autophagy [56]. The PI3K/Akt pathway can be deregulated via different mechanisms, as demonstrated for various cancers, including constitutive activation of growth factor receptors, PI3K amplification/mutation, inactivation of PTEN, amplification of Akt, and mutational activation of Akt itself [57]. Interestingly, overexpression of Akt, and the inactivation and loss of PTEN are frequently observed in pancreatic cancers [58,59], indicating that the pathway is a putative autophagic target in pancreatic cancers. Triptolide-induced autophagy is associated with inactivation of the Akt/mTOR/p70S6K pathway and up-regulation of the ERK1/2 pathway [55]. In addition, the role of the Ras/Raf-1/ERK1/2 signaling pathway in autophagy was confirmed in the human colon cancer cell line HT-29 [60]. Although increasing evidence implicates the importance of autophagy in cancer and tumor development, the fundamental question, whether autophagy kills cancer cells or protects them from unfavorable conditions, remains controversial. The threshold between autophagy as a cytoprotective process or programmed cell death is difficult to establish and probably depends on the extent of degradation of cellular components. Pro-apoptotic protein Bid may serve as a molecular switch between apoptosis and autophagy. For example, Bid knock down in MCF-7 cells exposed to
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CPT leads to a shift of cell death from apoptosis to autophagy. Since pro-apoptotic genes undergo mutations in malignant cells, the ability of cancer cells commitment to autophagy may have important therapeutic implications.

7.3. ER Stress in Autophagy

The ER functions to synthesize proteins, allow proper folding of secretory and transmembrane proteins, and stores high intracellular calcium that can activate calcium-dependent chaperone proteins that assist in protein folding. Disruption of any of these processes, such as by glucose depletion, which prevents the proper glycosylation of proteins, or by alterations in calcium homeostasis, such that calcium-dependent chaperones cannot function properly, leads to the accumulation of misfolded proteins that triggers ER stress [61]. Often seen as a protective mechanism, this build-up of misfolded proteins and subsequent stress leads to the unfolded protein response (UPR) mediated by three ER-localized transmembrane proteins that act as sensors: protein kinase RNA-like ER kinase (PERK), inositol requiring-1α (IRE1α), and activating transcription factor-6 (ATF6) [62]. Recently, it has been shown that prolonged ER stress can also lead to cell death mediated by autophagy [63]; therefore, while typically believed to be protective, excessive ER stress can instead result in autophagic cell death. Pancreatic epithelial cells have a highly developed ER due to heavy engagement in insulin and digestive enzyme secretion, and it has been suggested that they may be particularly sensitive to ER stress-induced apoptosis. By targeting the ER, a proteasome inhibitor, bortezomib, may be an effective therapy for pancreatic cancer, which may be hypersensitive to protein aggregation and subsequent ER stress-mediated apoptosis [64]. Cannabinol induces human pancreatic cancer cell death through the stimulation of autophagy. Cannabinol induced ceramide accumulation and eIF2α (eukaryotic initiation factor 2α) phosphorylation and thereby activated an ER stress response that promoted autophagy via inhibition of the Akt/mTORC1 axis [65].

8. Concluding Remarks

Pancreatic cancer remains one of the most lethal malignancies, and patients with metastatic pancreatic cancer have a bleak prognosis. The high mortality can be attributed to late diagnosis, rapid disease progression, and poor response to chemotherapy or radiotherapy. Traditional cancer therapy evokes cell death by inducing apoptosis; however, the apoptotic resistance inherent in cancer cells has been a significant barrier to effective chemotherapy. Autophagy is defined as a survival mechanism during stress conditions and a cell death pathway when apoptotic cell death mechanisms are deficient. As a result of the survival effects of autophagy, which were initially observed in vitro, the suggestion is that autophagy inhibition, in combination with standard chemotherapy, would be beneficial for tumor therapy. Better understanding of the genetic and epigenetic alterations, cellular and molecular signaling mechanisms, and apoptotic and autophagic mechanisms of pancreatic cancer would offer opportunities to develop novel therapeutics. HDAC inhibitors can induce both mitochondria-mediated apoptosis and caspase-independent autophagic cell death. Induction of autophagic cell death by HDAC inhibitors has clear clinical implications in treating cancers with apoptotic defects.
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References

1. Wong, H.H.; Lemoine, N.R. Biological approaches to therapy of pancreatic cancer. *Panreatology* 2008, 8, 431–461.
2. Mimeault, M.; Hauke, R.; Batra, S.K. Recent advances on the molecular mechanisms involved in the drug resistance of cancer cells and novel targeting therapies. *Clin. Pharmacol. Ther.* 2008, 83, 673–691.
3. Schniewind, B.; Christgen, M.; Kurdow, R.; Haye, S.; Kremer, B.; Kalthoff, H.; Ungefroren, H. Resistance of pancreatic cancer to gemcitabine treatment is dependent on mitochondria-mediated apoptosis. *Int. J. Cancer* 2004, 109, 182–188.
4. Kumagai, T.; Wakimoto, N.; Yin, D.; Gery, S.; Kawamata, N.; Takai, N.; Komatsu, N.; Chumakov, A.; Imai, Y.; Koeffler, H.P. Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (vorinostat, SAHA) profoundly inhibits the growth of human pancreatic cancer cells. *Int. J. Cancer* 2007, 121, 656–665.
5. Guillermet-Guibert, J.; Davenne, L.; Pchejetski, D.; Saint-Laurent, N.; Brizuela, L.; Guilbeau-Frugier, C.; Delisle, M.B.; Cuvillier, O.; Susini, C.; Bousquet, C. Targeting the sphingolipid metabolism to defeat pancreatic cancer cell resistance to the chemotherapeutic gemcitabine drug. *Mol. Cancer Ther.* 2009, 8, 809–820.
6. Cascalló, M.; Calbó, J.; Gelpí, J.L.; Mazo, A. Modulation of drug cytotoxicity by reintroduction of wild-type p53 gene (Ad5CMV-p53) in human pancreatic cancer. *Cancer Gene Ther.* 2000, 7, 545–556.
7. Liu, S.H.; Davis, A.; Li, Z.; Ballian, N.; Davis, E.; Wang, X.P.; Fisher, W.; Brunicardi, F.C. Effective ablation of pancreatic cancer cells in SCID mice using systemic adenoviral RIP-TK/GCV gene therapy. *J. Surg. Res.* 2007, 141, 45–52.
8. Morgan, M.A.; Parsels, L.A.; Kollar, L.E.; Normolle, D.P.; Maybaum, J.; Lawrence, T.S. The combination of epidermal growth factor receptor inhibitors with gemcitabine and radiation in pancreatic cancer. *Clin. Cancer Res.* 2008, 14, 5142–5149.
9. Bruckner, H.W.; Hrehorovich, V.R.; Sawhney, H.S. Bevacizumab as treatment for chemotherapy-resistant pancreatic cancer. *Anticancer Res.* 2005, 25, 3637–3639.
10. Vogelstein, B.; Kinzler, K.W. Cancer genes and the pathways they control. *Nat. Med.* 2004, 10, 789–799.
11. Bai, J.; Sui, J.; Demirjian, A.; Vollmer, C.M., Jr.; Marasco, W.; Callery, M.P. Predominant Bcl-XL knockdown disables antiapoptotic mechanisms: Tumor necrosis factor-related apoptosis-inducing ligand-based triple chemotherapy overcomes chemoresistance in pancreatic cancer cells *in vitro*. *Cancer Res.* 2005, 65, 2344–2352.
12. Morton, J.P.; Timpson, P.; Karim, S.A.; Ridgway, R.A.; Athineos, D.; Doyle, B.; Jamieson, N.B.; Oien, K.A.; Lowy, A.M.; Brunton, V.G.; Frame, M.C.; Evans, T.R.; Sansom, O.J. Mutant p53 drives metastasis and overcomes growth arrest/senescence in pancreatic cancer. Proc. Natl. Acad. Sci. USA 2010, 107, 246–251.

13. Moore, P.S.; Orlandini, S.; Zamboni, G.; Capelli, P.; Rigaud, G.; Falconi, M.; Bassi, C.; Lemoine, N.R.; Scarpa, A. Pancreatic tumours: Molecular pathways implicated in ductal cancer are involved in ampullary but not in exocrine nonductal or endocrine tumorigenesis. Br. J. Cancer 2001, 84, 253–262.

14. Marchese, R.; Muleti, A.; Pasqualetti, P.; Bucci, B.; Stigliano, A.; Brunetti, E.; De Angelis, M.; Mazzoni, G.; Tocchi, A.; Brozzetti, S. Low correspondence between K-ras mutations in pancreatic cancer tissue and detection of K-ras mutations in circulating DNA. Pancreas 2006, 32, 171–177.

15. Bardeesy, N.; DePinho, R.A. Pancreatic cancer biology and genetics. Nat. Rev. Cancer 2002, 2, 897–909.

16. Campbell, P.M.; Groehler, A.L.; Lee, K.M.; Ouellette, M.M.; Khazak, V.; Der, C.J. K-Ras promotes growth transformation and invasion of immortalized human pancreatic cells by Raf and phosphatidylinositol 3-kinase signaling. Cancer Res. 2007, 67, 2098–106.

17. Biankin, A.V.; Kench, J.G.; Morey, A.L.; Lee, C.S.; Biankin, S.A.; Head, D.R.; Hugh, T.B.; Henshall, S.M.; Sutherland, R.L. Overexpression of p21WAF1/CIP1 is an early event in the development of pancreatic intraepithelial neoplasia. Cancer Res. 2001, 61, 8830–8837.

18. Hamacher, R.; Schmid, R.M.; Saur, D.; Schneider, G. Apoptotic pathways in pancreatic ductal adenocarcinoma. Mol. Cancer 2008, 7, 64–74.

19. El-Rayes, B.F.; Ali, S.; Ali, I.F.; Philip, P.A.; Abbruzzese, J.; Sarkar, F.H. Potentiation of the effect of erlotinib by genistein in pancreatic cancer: The role of Akt and NF-κB. Cancer Res. 2006, 66, 10553–10559.

20. Maitra, A.; Hruban, R.H. Pancreatic cancer. Annu. Rev. Pathol. 2008, 3, 157–188.

21. Fukushima, N.; Sato, N.; Ueki, T.; Rosty, C.; Walter, K.M.; Wilentz, R.E.; Yeo, C.J.; Hruban, R.H.; Goggins, M. Aberrant methylation of preproenkephalin and p16 genes in pancreatic intraepithelial neoplasia and pancreatic ductal adenocarcinoma. Am. J. Pathol. 2002, 160, 1573–1581.

22. Moore, P.S.; Sipos, B.; Orlandini, S.; Sorio, C.; Real, F.X.; Lemoine, N.R.; Gess, T.; Bassi, C.; Klöppel, G.; Kalthoff, H.; Ungefroren, H.; Löhr, M.; Scarpa, A. Genetic profile of 22 pancreatic carcinoma cell lines. Analysis of K-ras, p53, p16 and DPC4/Smad4. Virchows Arch. 2001, 439, 798–802.

23. Missiaglia, E.; Donadelli, M.; Palmieri, M.; Cmogorac-Jurcevic, T.; Scarpa, A.; Lemoine, N.R. Growth delay of human pancreatic cancer cells by methylase inhibitor 5-aza-2’-deoxycytidine treatment is associated with activation of the interferon signaling pathway. Oncogene 2005, 24, 199–211.

24. Neureiter, D.; Zopf, S.; Leu, T.; Dietze, O.; Hauser-Kronberger, C.; Hahn, E.G.; Herold, C.; Ocker, M. Apoptosis, proliferation and differentiation patterns are influenced by Zebularine and SAHA in pancreatic cancer models. Scand. J. Gastroenterol. 2007, 42, 103–116.

25. Kumagai, T.; Akagi, T.; Desmond, J.C.; Kawamata, N.; Gery, S.; Imai, Y.; Song, J.H.; Gui, D.; Said, J.; Koeffler, H.P. Epigenetic regulation and molecular characterization of C/EBPα in pancreatic cancer cells. Int. J. Cancer 2009, 124, 827–833.
26. Suzuki, M.; Endo, M.; Shinohara, F.; Echigo, S.; Rikiishi, H. Enhancement of cisplatin cytotoxicity by SAHA involves endoplasmic reticulum stress-mediated apoptosis in oral squamous cell carcinoma cells. *Cancer Chemother. Pharmacol.* 2009, 64, 1115–1122.

27. Donadelli, M.; Costanzo, C.; Beghelli, S.; Scupoli, M.T.; Dandrea, M.; Bonora, A.; Piacentini, P.; Budillon, A.; Caraglia, M.; Scarpa, A.; Palmieri, M. Synergistic inhibition of pancreatic adenocarcinoma cell growth by trichostatin A and gemcitabine. *Biochim. Biophys. Acta* 2007, 1773, 1095–1106.

28. Bai, J.; Demirjian, A.; Sui, J.; Marasco, W.; Callery, M.P. Histone deacetylase inhibitor trichostatin A and proteasome inhibitor PS-341 synergistically induce apoptosis in pancreatic cancer cells. *Biochem. Biophys. Res. Commun.* 2006, 348, 1245–1253.

29. Sato, N.; Ohta, T.; Kitagawa, H.; Kayahara, M.; Ninomiya, I.; Fushida, S.; Fujimura, T.; Nishimura, G.; Shimizu, K.; Miwa, K. FR901228, a novel histone deacetylase inhibitor, induces cell cycle arrest and subsequent apoptosis in refractory human pancreatic cancer cells. *Int. J. Oncol.* 2004, 24, 679–685.

30. Yang, X.J.; Seto, E. The Rpd3/Hda1 family of lysine deacetylases: From bacteria and yeast to mice and men. *Nat. Rev. Mol. Cell Biol.* 2008, 9, 206–218.

31. Weichert, W.; Roske, A.; Niesporek, S.; Noske, A.; Buckendahl, A.C.; Dietel, M.; Gekeler, V.; Boehm, M.; Beckers, T.; Denkert, C. Class I histone deacetylase expression has independent prognostic impact in human colorectal cancer: Specific role of class I histone deacetylases *in vitro* and *in vivo*. *Clin. Cancer Res.* 2008, 14, 1669–1677.

32. Scanlan, M.J.; Welt, S.; Gordon, C.M.; Chen, Y.T.; Gure, A.O.; Stockert, E.; Junghluth, A.A.; Ritter, G.; Jäger, D.; Jäger, E.; Knuth, A.; Old, L.J. Cancer-related serological recognition of human colon cancer: Identification of potential diagnostic and immunotherapeutic targets. *Cancer Res.* 2002, 62, 4041–4047.

33. Oehme, I.; Deubzer, H.E.; Wegener, D.; Pickert, D.; Linke, J.P.; Hero, B.; Kopp-Schneider, A.; Westermann, F.; Ulrich, S.M.; von Deimling, A.; Fischer, M.; Witt, O. Histone deacetylase 8 in neuroblastoma tumorigenesis. *Clin. Cancer Res.* 2009, 15, 91–99.

34. Nigris, F. De.; Cerutti, J.; Morelli, C.; Califano, D.; Chiarotti, L.; Viglietto, G.; Santelli, G.; Fusco, A. Isolation of a SIR-like gene, SIR-T8, that is overexpressed in thyroid carcinoma cell lines and tissues. *Br. J. Cancer* 2002, 86, 917–923.

35. Hiratsuka, M.; Inoue, T.; Toda, T.; Kimura, N.; Shirayoshi, Y.; Kamitani, H.; Watanabe, T.; Ohama, E.; Tahimic, C.G.; Kurimasa, A.; Oshimura, M. Proteomics-based identification of differentially expressed genes in human gliomas: Down-regulation of SIRT2 gene. *Biochem. Biophys. Res. Commun.* 2003, 309, 558–566.

36. Miyake, K.; Yoshizumi, T.; Imura, S.; Sugimoto, K.; Batmunkh, E.; Kanemura, H.; Morine, Y.; Shimada, M. Expression of hypoxia-inducible factor-1alpha, histone deacetylase 1, and metastasis-associated protein 1 in pancreatic carcinoma: Correlation with poor prognosis with possible regulation. *Pancreas* 2008, 36, e1–9.

37. Fritsche, P.; Seidler, B.; Schüler, S.; Schnieke, A.; Göttlicher, M.; Schmid, R.M.; Saur, D.; Schneider, G. HDAC2 mediates therapeutic resistance of pancreatic cancer cells via the BH3-only protein NOXA. *Gut* 2009, 58, 1399–1409.
Cancers 2010, 2

38. Ouaissi, M.; Sielezneff, I.; Silvestre, R.; Sastre, B.; Bernard, J.P.; Lafontaine, J.S.; Payan, M.J.; Dahan, L.; Pirrò, N.; Seitz, J.F.; Mas, E.; Lombardo, D.; Ouaissi, A. High histone deacetylase 7 (HDAC7) expression is significantly associated with adenocarcinomas of the pancreas. *Ann. Surg. Oncol.* 2008, 15, 2318–2328.

39. Bakin, R.E.; Jung, M.O. Cytoplasmic sequestration of HDAC7 from mitochondrial and nuclear compartments upon initiation of apoptosis. *J. Biol. Chem.* 2004, 279, 51218–51225.

40. Marks, P.A.; Breslow, R. Dimethyl sulfoxide to vorinostat: Development of the histone deacetylase inhibitor as an anticancer drug. *Nat. Biotechnol.* 2007, 25, 84–90.

41. Dokmanovic, M.; Perez, G.; Xu, W.; Ngo, L.; Clarke, C.; Parmigiani, R.B.; Marks, P.A. Histone deacetylase inhibitors selectively suppress expression of HDAC7. *Mol. Cancer Ther.* 2007, 6, 2525–2534.

42. Mizushima, N.; Klionsky, D.J. Protein turnover via autophagy: Implications for metabolism. *Annu. Rev. Nutr.* 2007, 27, 19–40.

43. Kondo, Y.; Kanzawa, T.; Sawaya, R.; Kondo, S. The role of autophagy in cancer development and response to therapy. *Nat. Rev. Cancer* 2005, 5, 726–734.

44. Rodriguez-Gonzalez, A.; Lin, T.; Ikeda, A.K.; Simms-Waldrip, T.; Fu, C.; Sakamoto, K.M. Role of the aggresome pathway in cancer: Targeting histone deacetylase 6-dependent protein degradation. *Cancer Res.* 2008, 68, 2557–2560.

45. Levine, B.; Kroemer, G. Autophagy in the pathogenesis of disease. *Cell* 2008, 132, 27–42.

46. Oh, M.; Choi, I.K.; Kwon, H.J. Inhibition of histone deacetylase1 induces autophagy. *Biochem. Biophys. Res. Commun.* 2008, 369, 1179–1183.

47. Shao, Y.; Gao, Z.; Marks, P.A.; Jiang, X. Apoptotic and autophagic cell death induced by histone deacetylase inhibitors. *Proc. Natl. Acad. Sci. USA* 2004, 101, 18030–18035.

48. Hrzenjak, A.; Kremser, M.L.; Strohmeier, B.; Moinfar, F.; Zatloukal, K.; Denk, H. SAHA induces caspase-independent, autophagic cell death of endometrial stromal sarcoma cells by influencing the mTOR pathway. *J. Pathol.* 2008, 216, 495–504.

49. Watanabe, M.; Adachi, S.; Matsubara, H.; Imai, T.; Yui, Y.; Mizushima, Y.; Hiraumi, Y.; Watanabe, K.; Kamitsuji, Y.; Toyokuni, S.Y.; Hosoi, H.; Sugimoto, T.; Toguchida, J.; Nakahata, T. Induction of autophagy in malignant rhabdoid tumor cells by the histone deacetylase inhibitor FK228 through AIF translocation. *Int. J. Cancer* 2009, 124, 55–67.

50. García-Morales, P.; Gómez-Martínez, A.; Carrato, A.; Martínez-Lacaci, I.; Barberá, V.M.; Soto, J.L.; Carrasco-García, E.; Menéndez-Gutierrez, M.P.; Castro-Galache, M.D.; Ferragut, J.A.; Saceda, M. Histone deacetylase inhibitors induced caspase-independent apoptosis in human pancreatic adenocarcinoma cell lines. *Mol. Cancer Ther.* 2005, 4, 1222–1230.

51. Long, J.; Zhao, J.; Yan, Z.; Liu, Z.; Wang, N. Antitumor effects of a novel sulfur-containing hydroxamate histone deacetylase inhibitor H40. *Int. J. Cancer* 2009, 124, 1235–1244.

52. Fujii, S.; Mitsunaga, S.; Yamazaki, M.; Hasebe, T.; Ishii, G.; Kojima, M.; Kinoshita, T.; Ueno, T.; Esumi, H, Ochiai, A. Autophagy is activated in pancreatic cancer cells and correlates with poor patient outcome. *Cancer Sci.* 2008, 99, 1813–1819.
53. 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, 409–416.

54. Jones, S.; Zhang, X.S.; Parsons, W.; Lin, J.C.H.; Leary, R.J.; Angenendt, P.; Mankoo, P.; Carter, H.; Kamiyama, H.; Jimeno, A.; et al. Core signaling pathways in human pancreatic cancers revealed by global genomic analyses. Science 2008, 321, 1801–1806.

55. Mujumdar, N.; Mackenzie, T.; Dudeja, V.; Chugh, R.; Antonoff, M.; Borja-Cacho, D.; Sangwan, V.; Dawra, R.; Vickers, S.M.; Saluja, A.K. Triptolide induces cell death in pancreatic cancer cells by apoptotic and autophagic pathways. Gastroenterology 2010, 139, 598–608.

56. Ferté, C.; André, F.; Soria, J.C. Molecular circuits of solid tumors: Prognostic and predictive tools for bedside use. Nat. Rev. Clin. Oncol. 2010, 7, 367–380.

57. Hennessy, B.T.; Smith, D.L.; Ram, P.T.; Lu, Y.; Mills, G.B. Exploiting the PI3K/AKT pathway for cancer drug discovery. Nat. Rev. Drug Discov. 2005, 4, 988–1004.

58. Altomare, D.A.; Tanno, S.; De Rienzo, A.; Klein-Szanto, A.J.; Tanno, S.; Skele, K.L.; Hoffman, J.P.; Testa, J.R. Frequent activation of AKT2 kinase in human pancreatic carcinomas. J. Cell. Biochem. 2002, 87, 470–476.

59. Schlieman, M.G.; Fahy, B.N.; Ramsamooj, R.; Beckett, L.; Bold, R.J. Incidence, mechanism and prognostic value of activated AKT in pancreas cancer. Br. J. Cancer 2003, 89, 2110–2115.

60. Pattingre, S.; Bauvy, C.; Codogno, P. Amino acids interfere with the ERK1/2-dependent control of macroautophagy by controlling the activation of Raf-1 in human colon cancer HT-29 cells. J. Biol. Chem. 2003, 278, 16667–16674.

61. Schroder, M. Endoplasmic reticulum stress responses. Cell. Mol. Life Sci. 2008, 65, 862–894.

62. Kim, I.; Xu, W.; Reed, J.C. Cell death and endoplasmic reticulum stress: Disease relevance and therapeutic opportunities. Nat. Rev. Drug Discov. 2008, 7, 1013–1030.

63. Yorimitsu, T.; Klionsky, D.J. Endoplasmic reticulum stress: A new pathway to induce autophagy. Autophagy 2007, 3, 160–162.

64. Nawrocki, S.T.; Carew, J.S.; Dunner, K., Jr.; Boise, L.H.; Chiao, P.J.; Huang, P.; Abbruzzese, J.L.; McConkey, D.J. Bortezomib inhibits PKR-like endoplasmic reticulum (ER) kinase and induces apoptosis via ER stress in human pancreatic cancer cells. Cancer Res. 2005, 65, 11510–11519.

65. Salazar, M.; Carracedo, A.; Salanueva, I.J.; Hernández-Tiedra, S.; Lorente, M.; Egia, A.; Vázquez, P.; Blázquez, C.; Torres, S.; García, S.; Nowak, J.; Fimia, G.M.; Piacentini, M.; Cecconi, F.; Pandolfi, P.P.; González-Feria, L.; Iovanna, J.L.; Guzmán, M.; Boya, P.; Velasco, G. Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. J. Clin. Invest. 2009, 119, 1359–1372.

66. Duong, V.; Bret, C.; Altucci, L.; Mai, A.; Duraffourd, C.; Loubersac, J.; Harmand, P.O.; Bonnet, S.; Valente, S.; Maudelonde, T.; Cavailles, V.; Boulle, N. Specific activity of class II histone deacetylases in human breast cancer cells. Mol. Cancer Res. 2008, 6, 1908–1919.

67. Wilson, A.J.; Byun, D.S.; Popova, N.; Murray, L.B.; L’Italien, K.; Sowa, Y.; Arango, D.; Velcich, A.; Augenlicht, L.H.; Mariadason, J.M. Histone deacetylase 3 (HDAC3) and other class I HDACs regulate colon cell maturation and p21 expression and are deregulated in human colon cancer. J. Biol. Chem. 2006, 281, 13548–1358.
68. Nosho, K.; Shima, K.; Irahara, N.; Kure, S.; Firestein, R.; Baba, Y.; Toyoda, S.; Chen, L.; Hazra, A.; Giovannucci, E.L.; Fuchs, C.S.; Ogino, S. SIRT1 histone deacetylase expression is associated with microsatellite instability and CpG island methylator phenotype in colorectal cancer. *Mod. Pathol.* **2009**, *22*, 922–932.

69. Osada, H.; Tatenuma, Y.; Saito, H.; Yatabe, Y.; Mitsudomi, T.; Takahashi, T. Reduced expression of class II histone deacetylase genes is associated with poor prognosis in lung cancer patients. *Int. J. Cancer* **2004**, *112*, 26–32.

70. Sakuma, T.; Uzawa, K.; Onda, T.; Shiiba, M.; Yokoe, H.; Shibahara, T.; Tanzawa, H. Aberrant expression of histone deacetylase 6 in oral squamous cell carcinoma. *Int. J. Oncol.* **2006**, *29*, 117–124.

71. Hayashi, A.; Horiuchi, A.; Kikuchi, N.; Hayashi, T.; Fuseya, C.; Suzuki, A.; Konishi, I.; Shiozawa, T. Type-specific roles of histone deacetylase (HDAC) overexpression in ovarian carcinoma: HDAC1 enhances cell proliferation and HDAC3 stimulates cell migration with downregulation of E-cadherin. *Int. J. Cancer* **2010**, *127*, 1332–1346.

72. Weichert, W.; Röske, A.; Gekeler, V.; Beckers, T.; Stephan, C.; Jung, K.; Fritzche, F.R.; Niesporek, S.; Denkert, C.; Dietel, M.; Kristiansen, G. Histone deacetylases 1, 2 and 3 are highly expressed in prostate cancer and HDAC2 expression is associated with shorter PSA relapse time after radical prostatectomy. *Br. J. Cancer* **2008**, *98*, 604–610.

73. Takeuchi, H.; Kondo, Y.; Fujiwara, K.; Kanzawa, T.; Aoki, H.; Mills, G.B.; Kondo, S. Synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by phosphatidylinositol 3-kinase/protein kinase B inhibitors. *Cancer Res.* **2005**, *65*, 3336–3346.

74. Opipari, A.W., Jr.; Tan, L.; Boitano, A.E.; Sorenson, D.R.; Aurora, A.; Liu, J.R. Resveratrol-induced autophagocytosis in ovarian cancer cells. *Cancer Res.* **2004**, *64*, 696–703.

75. Heiseke, A.; Aguib, Y.; Riemer, C.; Baier, M.; Schätzl, H.M. Lithium induces clearance of protease resistant prion protein in prion-infected cells by induction of autophagy. *J. Neurochem.* **2009**, *109*, 25–34.

76. Fu, J.; Shao, C.J.; Chen, F.R.; Ng, H.K.; Chen, Z.P. Autophagy induced by valproic acid is associated with oxidative stress in glioma cell lines. *Neuro. Oncol.* **2010**, *12*, 328–340.

77. Williams, A.; Sarkar, S.; Cuddon, P.; Tiofi, E.K.; Saiki, S.; Siddiqi, F.H.; Jahreiss, L.; Fleming, A.; Pask, D.; Goldsmith, P.; O’Kane, C.J.; Floto, R.A.; Rubinsztein, D.C. Novel targets for Huntington's disease in an mTOR-independent autophagy pathway. *Nat. Chem. Biol.* **2008**, *4*, 295–305.

78. Pedrozo, Z.; Sánchez, G.; Torrealba, N.; Valenzuela, R.; Fernández, C.; Hidalgo, C.; Lavandero, S.; Donoso, P. Calpains and proteasomes mediate degradation of ryanodine receptors in a model of cardiac ischemic reperfusion. *Biochim. Biophys. Acta* **2010**, *1802*, 356–362.

79. Ohtomo, T.; Miyazawa, K.; Naito, M.; Moriya, S.; Kuroda, M.; Itoh, M.; Tomoda, A. Cytoprotective effect of imatinib mesylate in non-BCR-ABL-expressing cells along with autophagosome formation. *Biochem. Biophys. Res. Commun.* **2010**, *391*, 310–315.

80. Li, J.; Hou, N.; Faried, A.; Tsutsumi, S.; Kuwano, H. Inhibition of autophagy augments 5-fluorouracil chemotherapy in human colon cancer in vitro and in vivo model. *Eur. J. Cancer* **2010**, *46*, 1900–1909.
81. Thyagarajan, A.; Jedinak, A.; Nguyen, H.; Terry, C.; Baldridge, L.A.; Jiang, J.; Sliva, D. Triterpenes from Ganoderma Lucidum induce autophagy in colon cancer through the inhibition of p38 mitogen-activated kinase (p38 MAPK). *Nutr. Cancer* **2010**, *62*, 630–640.

82. Huang, S.; Sinicrope, F.A. Celecoxib-induced apoptosis is enhanced by ABT-737 and by inhibition of autophagy in human colorectal cancer cells. *Autophagy* **2010**, *6*, 256–269.

83. Carew, J.S.; Medina, E.C.; Esquivel, J.A. II; Mahalingam, D.; Swords, R.; Kelly, K.; Zhang, H.; Huang, P.; Mita, A.C.; Mita, M.M.; Giles, F.J.; Nawrocki, S.T. Autophagy inhibition enhances vorinostat-induced apoptosis via ubiquitinated protein accumulation. *J. Cell. Mol. Med.* **2010**, *14*, 2448–2459.

84. Leu, J.I.; Pimkina, J.; Frank, A.; Murphy, M.E.; George, D.L. A small molecule inhibitor of inducible heat shock protein 70. *Mol. Cell* **2009**, *36*, 15–27.

85. Tang, D.; Kang, R.; Cheh, C.W.; Livesey, K.M.; Liang, X.; Schapiro, N.E.; Benschop, R.; Sparvero, L.J.; Amoscasto, A.A.; Tracey, K.J.; Zeh, H.J.; Lotze, M.T. HMGB1 release and redox regulates autophagy and apoptosis in cancer cells. *Oncogene* **2010**, *29*, 5299–5310.

86. Bommeredy, A.; Hahm, E.R.; Xiao, D.; Powolny, A.A.; Fisher, A.L.; Jiang, Y.; Singh, S.V. Atg5 regulates phenethyl isothiocyanate-induced autophagic and apoptotic cell death in human prostate cancer cells. *Cancer Res.* **2009**, *69*, 3704–3712.

87. Xia, H.G.; Zhang, L.; Chen, G.; Zhang, T.; Liu, J.; Jin, M.; Ma, X.; Ma, D.; Yuan, J. Control of basal autophagy by calpain1 mediated cleavage of ATG5. *Autophagy* **2010**, *6*, 61–66.

88. Scarlatti, F.; Bauvy, C.; Ventrutti, A.; Sala, G.; Cluzeaud, F.; Vandewalle, A.; Ghidoni, R.; Codogno, P. Ceramide-mediated macroautophagy involves inhibition of protein kinase B and upregulation of beclin 1. *J. Biol. Chem.* **2004**, *279*, 18384–18391.

89. Zois, C.E.; Koukourakis, M.I. Radiation-induced autophagy in normal and cancer cells: Towards novel cytoprotection and radio-sensitization policies? *Autophagy* **2009**, *5*, 442–450.

90. Batra, S.; Sahu, R.P.; Kandala, P.K.; Srivastava, S.K. Benzyl isothiocyanate-mediated inhibition of histone deacetylase leads to NF-kappaB turnoff in human pancreatic carcinoma cells. *Mol. Cancer Ther.* **2010**, *9*, 1596–1608.

91. Schüler, S.; Fritsche, P.; Diersch, S.; Arlt, A.; Schmid, R.M.; Saur, D.; Schneider, G. HDAC2 attenuates TRAIL-induced apoptosis of pancreatic cancer cells. *Mol. Cancer Ther.* **2010**, *9*, 80–89.

92. Chun, S.G.; Zhou, W.; Yee, N.S. Combined targeting of histone deacetylases and hedgehog signaling enhances cytotoxicity in pancreatic cancer. *Cancer Biol. Ther.* **2009**, *8*, 1328–1339.

93. Wu, L.P.; Wang, X.; Li, L.; Zhao, Y.; Lu, S.; Yu, Y.; Zhou, W.; Liu, X.; Yang, J.; Zheng, Z.; Zhang, H.; Feng, J.; Yang, Y.; Wang, H.; Zhu, W.G. Histone deacetylase inhibitor depsipeptide activates silenced genes through decreasing both CpG and H3K9 methylation on the promoter. *Mol. Cell Biol.* **2008**, *28*, 3219–3235.

94. Ammerpohl, O.; Trauzold, A.; Schniewind, B.; Griep, U.; Pilarsky, C.; Grutzmann, R.; Saeger, H.D.; Janssen, O.; Sipos, B.; Kloppel, G.; Kalthoff, H. Complementary effects of HDAC inhibitor 4-PB on gap junction communication and cellular export mechanisms support restoration of chemosensitivity of PDAC cells. *Br. J. Cancer* **2007**, *96*, 73–81.
95. Piacentini, P.; Donadelli, M.; Costanzo, C.; Moore, P.S.; Palmieri, M.; Scarpa, A. Trichostatin A enhances the response of chemotherapeutic agents in inhibiting pancreatic cancer cell proliferation. *Virchows Arch.* 2006, *448*, 797–804.

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