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Mini-review

Therapeutic potential of histone deacetylase inhibitors in pancreatic cancer

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ABSTRACT

Pancreatic cancer is a devastating disease with a dismal prognosis. Surgical resection is the only curative option but is heavily hampered by delayed diagnosis. Due to few therapeutic treatments available, novel and efficacious therapy is urgently needed. Histone deacetylase inhibitors (HDACIs) are emerging as a prominent class of therapeutic agents for pancreatic cancer and have exhibited significant anticancer potential with negligible toxicity in preclinical studies. Clinical evaluations of HDACIs are currently underway. HDACIs as monotherapy in solid tumors have proven less effective than hematological malignancies, the combination of HDACIs with other anticancer agents have been assessed for advanced pancreatic cancer. In this review, we describe the molecular mechanism underpin the anticancer effect of HDACIs in pancreatic cancer and summarize the recent advances in the rationales of combination strategies incorporating HDACIs. In addition, we discuss the importance of identifying predictors of response to HDACI-based therapy.

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1. Introduction

Pancreatic cancer is one of the most aggressive human cancers. It is the fourth leading cause of cancer-related death in the western world with a 5-year survival lower than 5%. Moreover, with gradually rising incidence and mortality rates [1], pancreatic cancer leads to an estimated 43,920 newly diagnostic cases and 37,930 deaths in the United States in 2012 [2]. Due to rapid progression and lack of typical presenting symptom, pancreatic cancer is often diagnosed at advanced stages when patients miss the opportunity to have surgical resection with curative intention [1]. Present chemotherapeutic agents are marginally efficacious in pancreatic cancer. Gemcitabine-based therapy has been recommended to constitute the first-line treatment for advanced pancreatic cancer despite its poor response rate [3]. The majority of patients are insensitive to the currently available regimens. Recently, targeted therapies have been developed for advanced pancreatic cancer [4]. Erlotinib, a small-molecule inhibitor of the human epidermal growth factor receptor (EGFR), combined with gemcitabine in a phase III clinical trial, demonstrating a statistically survival advantage compared with gemcitabine alone [5]. However, the therapies are only active in a defined subset of patients [6]. The evidence indicates that pancreatic cancer remains a therapeutic challenge with unmet need of effective and well-tolerated therapies.

Pancreatic cancer has long been thought to be a disease of defective genes such as mutations and amplifications as well as chromosomal deletions [1]. It was observed the k-ras oncogene is mutated in nearly all the pancreatic cancer samples [7]. In addition to the well known genetic alterations, uncontrolled growth of pancreatic cancer cells is also regulated by the epigenetic modification such as DNA methylation [8], chromatin remodeling and micro RNA [9]. DNA methylation is one of the most known epigenetic mechanisms, which often correlates with gene silence. To date, methylation-specified PCR analysis has identified aberrant DNA hypo- and hypermethylation in pancreatic cancer, which provide new implications in the biology of disease development [10]. Recent studies also revealed that alterations of methylation occurred in the early stage of pancreatic cancer and increased with progression of diseases, making it a potential candidate for early diagnosis and therapeutic target [11]. Microarray data and large scale analysis of miRNA profiles have observed many aberrantly expressed miRNAs in pancreatic cancer, such as miR-200 [12], miR-107 [13] and miR-132 [14]. They participated in the regulation of genes associated with cellular proliferation and metastasis [15]. There are growing interest in the exploration of histone modifications such as deacetylation, methylation, phosphorylation, which contribute to the dynamic properties of histones [16]. Here, we

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summarize the existing evidence of cellular pharmacology aspects of histone deacetylase inhibition and review studies concerning combination therapies including HDAC inhibitors.

2. Histone deacetylase

Histone deacetylase modify the structure of chromatin through altering the acetylation status of histone and non-histone proteins [17] and regulate the expression of target genes. HDACs catalyze the removal of acetyl moiety from lysine residues of histone tail, leading to compaction of chromatin structure and transcription repression. Conversely, histone acetyltransferases (HATs) involve in the addition of acetyl group to histone, resulting in relaxed chromatin configuration and activation of transcription [18,19]. A disrupted balance between acetylation and deacetylation results in aberrant expression of a wide range of genes affecting the regulation of proliferation, apoptosis, metastasis and angiogenesis [20].

In addition to histones, HDACs control the acetylation status of a variety of non-histone proteins, such as chaperon proteins, cytoskeletal proteins, metabolic enzymes and transcription factors, which involved in cell cycle progression, DNA repair as well as mitotic division [21,22]. These non-histone targets of HDAC play pivotal roles in multiple physiologic and pathological processes beyond the chromatin remodeling and transcription alteration [23].

According to the homology to yeast deacetylases, eighteen HDACs are divided into four groups: class I HDACs (HDAC1-3 and 8) are associated with yeast RPD3; class II HDACs (HDAC4-7 and 9, 10) share homology with the yeast HDA1; class III HDACs (SIRT1-7) are related to yeast Sir2 and class IV HDAC (HDAC11) exhibits features of both class I and class II HDACs [24]. Class I, II, IV HDACs are zinc dependent, whereas class III HDACs require cofactor NAD^+ for their catalytic activity, so this particular class HDACs are non-responsive to compounds that inhibit zinc dependent deacetylases [25].

3. HDAC expression in pancreatic cancer

Aberrant HDAC expression has been found in pancreatic cancer, especially class I HDAC. In Lehmann's cohort (82 cases) 32%, 63% and 79% of pancreatic adenocarcinomas displayed strong nuclear immunoreactions for HDAC1, 2 and 3 proteins [26]. One study observed that elevated level HDAC2 expression was intimately interlinked with poor tumor differentiation. As evaluated by scoring of nuclear HDAC expression, a pronounced 3.1-fold up-regulation of HDAC2 was observed in G3 tumors compared to normal tissues. This was also confirmed by microarray data [27]. In accordance, expression profile of class I HDACs as observed by immunohistochemistry were strong in pancreatic cancer with HDAC1, HDAC2, HDAC3 and HDAC8 being positive in 7(85%), 18(90%), 20(100%) and 18 (90%) of 20 cases [28]. In another study, high HDAC1 protein expression was observed in 56% of pancreatic carcinomas. In this cohort (39 cases), elevated HDAC1 expression exerted a negative prognostic impact on overall survival (OS) in univariate survival analysis [29]. Consistently, Wang and colleagues observed that the expression prevalence of HDAC1 progressively increased from precursor lesion to pancreatic cancer and intimately interlinked with TNM staging and degree of tumor differentiation [30]. For HDAC7, using qPCR-based approach and Western blot, significant increase of both mRNA and protein levels was observed in 9 of 11 pancreatic adenocarcinoma samples compared to normal pancreas and serous cystadenoma (SC), intraductal papillary mucinous tumor of the pancreas (IMPN), chronic pancreatitis (CP) specimens [31]. One recent study has identified that SIRT1, a class III HDAC, is highly expressed in 36 of 129 (27.9%) pancreatic ductal adenocarcinomas. Elevated SIRT1 expression was associated with poorly differentiated carcinomas. Both univariate and multivariate analysis confirmed the prognostic role of SIRT1 [32]. Since the knowledge concerning the patterns of HDAC expression in pancreatic cancer is limited. Further studies should be focused on analyzing the patterns of HDAC expression in a large cohort of pancreatic cancers and on the correlation with clinicopathological features.

4. HDAC inhibitors

Because of its ability to reverse epigenetic abnormalities involved in cancer onset and progression, numerous HDAC inhibitors (HDACIs) have been developed for therapeutic intervention [18]. HDACIs are classified into six groups based on their chemical structures, including short-chain fatty acids (such as valporoic acid, butyric acid); hydroxamic acids (such as TSA, SAHA); cyclic tetrapeptides (depspeptide); benzamides (entinostat); electrophilicketones (trifluoromethylketone); miscellaneous compounds (MGCD0103) [33,34]. These structurally diverse compounds suppress the enzymatic activity of HDACs with varying specificity [35]. Most compounds are pan-HDAC inhibitors, targeting multiple HDACs such as belinostat, TSA, whereas some inhibit the activity of specific class or individual HDAC such as tubacin, identified as HDAC6 inhibitor and entinostat, which selectively inhibit class I HDACs [36]. To date, the information about the role of individual HDAC is sparse. The development of isoform-selective HDAC inhibitors could offer further insights into the function of individual HDAC in cancer formation and progression. It is still under investigation whether isotype-selective HDAC inhibitors could obtain improved therapeutic effect without increasing toxicity compared with pan-HDAC inhibitors.

HDAC inhibitors have been reported to elicit pleiotropic anticancer effects on pancreatic cancer cells including stimulation of cell cycle arrest, induction of apoptosis as well as inhibition of metastasis and angiogenesis (Fig. 1). Vorinostat (SAHA) was the first HDAC inhibitor to be approved by FDA for the treatment of cutaneous T cell lymphoma (CTCL) in 2006 [37]. In the case of pancreatic cancer, the antitumor effect of HDAC inhibitors have been evaluated in cell lines and tumor xenograft models, either alone or combined with other therapeutic agents [38,39]. Consistently, a number of ongoing clinical trials attempt to determine the

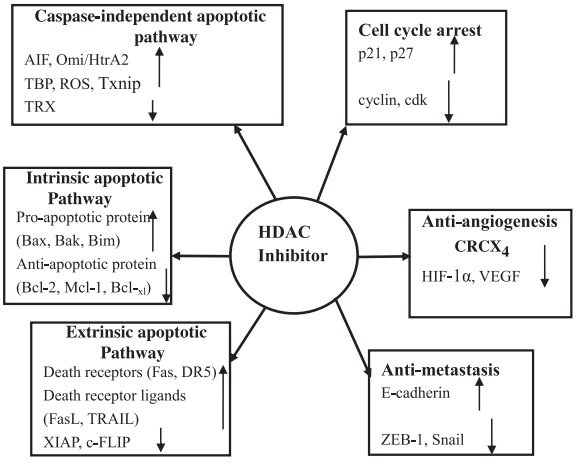

Fig. 1. HDAC inhibitors elicit pleiotropic anti-cancer effects in pancreatic cancer cells including activation of apoptosis pathways, induction of cell cycle arrest and inhibition of metastasis and angiogenesis. HDAC, histone deacetylase; AIF, apoptotic inducing factor; TRX, thioredoxin; TBP, thioredoxin binding protein; ROS, reactive oxygen species; Txnip, thioredoxin interacting protein; HIF-1α, hypoxia-inducible factor-1 alpha; VEGF, vascular endothelial growth factor; TRAIL, tumor-necrosis factor-related apoptosis-inducing ligand. Downregulation ↓ upregulation ↑.

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application of HDAC inhibitors in pancreatic cancer. Some have shown encouraging clinical potential at low doses well tolerated by patients [40].

5. Cellular pharmacology studies in pancreatic cancer

5.1. HDAC inhibitor and cell cycle arrest

HDAC inhibitors induce cell cycle arrest in a p53 independent way in pancreatic cancer cells. Cyclin dependent kinase (CDK) inhibitor p21 is a critical modulator of cell cycle progression in pancreatic cancer. Ocker M and colleagues observed that HDACIs induced expression of p21 through enhancing histone acetylation surrounding the p21 promoter as determined by ChIP assay [41]. Pancreatic cancer cell lines underwent G1/S or/and G2/M cell cycle arrest by induction of p21 after treatment of belinostat [42], MGCD0103 [43] and chidamide [44]. Besides p21, examples of genes which can be commonly altered by HDACIs are Cyclin dependent kinase (cdk), cyclins and cdk inhibitors. For example, treatment of eight pancreatic cancer cell lines with HDAC inhibitor TSA led to up-regulation of cdk inhibitors including p21, p19 and p57 and down-regulation of cyclin A and cdk10 assessed by global gene expression [45]. As assessed by Western blot, TSA or SK-7041 prolonged G2/M cell cycle arrest in PDAC cell lines via increasing the expression of p21 and cyclinD2 and reducing that of cyclinB1 [46]. Collectively, HDACIs promote cell cycle arrest by down regulating the cyclins and cdks such as cyclinD, cdk10 and/or up regulating the cdk inhibitors such as p21, p27.

Recent study further addressed the therapeutic potential of class I and class II selective HDAC inhibitors on cell cycle progression in pancreatic cancer cell lines. Treatment with class I selective HDACI caused cell cycle arrest in G2/M. Conversely, class II selective HDACI showed marginal effects on cell cycle. Interestingly, combination of class I and class II selective inhibitors resulted in synergistic cell cycle arrest through cooperative inhibition of p21 [47], indicating different class-specific HDAC inhibitors have diverse cellular functions on the same cell line and pan-HDAC inhibitors may be more efficient than class-selective inhibitors for stimulating cell cycle arrest in pancreatic cancer cell lines.

5.2. HDAC inhibitor and activation of apoptotic pathway

Pancreatic cancer cells are intrinsically insensitive to apoptosis mainly due to the constitutive up-regulation of the anti-apoptotic proteins such as Mcl-1, Bcl-2, XIAP [48]. HDACIs sensitize pancreatic cancer cells to the extrinsic apoptotic pathway through down-regulation of inhibitors of apoptotic pathway and induction of death-receptors (FAS, DR5) and its corresponding ligands (FASL, TRAIL). HDAC inhibitor LBH589 have been shown to promote ubiquitination and proteasomal degradation of c-FLIP, leading to augment of TRAIL (tumor-necrosis factor-related apoptosis-inducing ligand)-induced apoptosis in Panc-1, Capan-2 and Bxpc cell lines [49]. Another study revealed that depletion of HDAC2 sensitized pancreatic cancer cell lines to TRAIL-mediated apoptosis through increasing expression of death-receptors. VPA, a specific class I selective HDAC inhibitor, can overcome apoptosis resistance of pancreatic cell lines by enhancing the expression of TRAIL receptor 1 (DR5) [50]. Natoni *et al.* showed that HDAC inhibitor NaBt favored Fas-mediated apoptosis through inhibiting the expression of FLIP and sensitized pancreatic cancer cell lines to the extrinsic apoptotic pathway [51].

HDACI-based treatment confers pancreatic cancer cells prone to the intrinsic pathway by increasing the expression of pro-apoptotic molecules such as Bax, Bak, Bim and down-regulating anti-apoptotic molecules such as Bcl-2, Bcl-xl and Mcl-1 [52,53]. Elevated Bcl-2 protein is associated with the resistance to HDACI-based treatment [54]. Recent studies showed that inhibitor of Bcl-2, such as ABT-737 was able to enhance the HDACI-induced apoptosis both *in vitro* and *in vivo* [55]. Donadelli *et al.* found that pro-apoptotic Bim was markedly induced by TSA in pancreatic cancer cells. Silence of endogenous Bim by using RNA interference (RNAi) render cells to resistance to TSA-induced apoptosis, suggesting Bim must play a role in TSA-mediated apoptosis response [56]. In addition to the induction of pro-apoptotic genes like Bim, gene expression profiling identified that the expression of the anti-apoptotic genes such as Bcl-xl and Bcl-w were suppressed by HDAC inhibitors in pancreatic cancer cells [45].

Caspases are required to exert apoptotic effect in both extrinsic and intrinsic pathways. In addition to the well-characterized caspase-dependent apoptosis, HDACIs can induce caspase-independent apoptosis [57,58]. Morales and colleagues observed neither pan-caspase inhibitors nor selective caspase inhibitors were able to block TSA-induced apoptosis in three pancreatic cancer cell lines, indicating that TSA mediated apoptosis through caspase-independent pathway. The study revealed that HDACIs increased Bax protein expression, which promoted apoptotic inducing factor (AIF) and Omi/HtrA2 releasing from the mitochondrial. AIF translocates to the nuclei to stimulate DNA fragmentation and a serine protease activity of Omi/HtrA2. Taken together, TSA is likely inducing apoptosis in pancreatic cancer cells through Bax-AIF-nuclei-serine protease-dependent pathway [58].

Other studies have shown that TBP-2 and Trx appeared to participate in HDACI-mediated caspase-independent apoptosis [59]. Trx is a scavenger of reactive oxygen species (ROS), which protects cells against cellular stress-induced apoptosis. TBP2 interacts with Trx and act as a negative regulator. Researchers have shown that SAHA and belinostat increased expression of Trx1 inhibitor thioredoxin interacting protein (Txnip) and consequently repressed the activity of Trx1, resulting in high level of ROS in transformed cells [42,60]. Notably, normal cells are relatively insensitive to HDACI-mediated cell death. One possible explanation is HDAC inhibitors selectively increase the expression of Trx in normal cells but not in transformed cells, which associated with clearance of ROS. It is proposed that the elevated level of ROS was in part responsible for the HDACI-mediated caspase-independent apoptosis [61]. Wang and colleagues observed that treatment of pancreatic cancer cell lines with belinostat led to an accumulation of intracellular ROS, whereas anti-oxidant NAC was sufficient to blunt the apoptotic activity of belinostat [62].

5.3. HDAC inhibitor and suppression of angiogenesis

The anti-angiogenic property of HDAC inhibitors is intimately associated with the regulation of hypoxia-inducible factor-1 alpha (HIF-1α) and vascular endothelial growth factor (VEGF). The transcription factor HIF-1α alters the expression of various genes implicated in angiogenesis [63]. Recent study revealed that treatment with belinostat inhibited HIF transcription activity through down regulating the expression of HIF-1α and VEGF in pancreatic cancer cell lines [42]. HDACs have a prominent role in the stabilization and activation of HIF-1α [29]. Inhibition of HDAC6 leads to acetylation of HSP90 (heat shock protein 90) and loss of its chaperon function, resulting in degradation of HIF-1α [63,64]. HDAC7 is another key modulator of HIF-1α function, which translocates from the cytoplasm to the nucleus and binds to HIF-1α under hypoxic conditions, promoting the expression of HIF-1αand its transcriptional product VEGF [65,66]. Wang and colleagues revealed that VEGF induced phosphorylation of HDAC7, which is uniquely required for activation of VEGF-responsive genes and endothelial cell functions [67]. Additionally, HDACIs suppress the angiogenesis by targeting endothelial cells. HDACIs can directly inhibit the

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proliferation and migration of endothelial cells [67,68]. Moreover, it can inhibit the differentiation and mobilization of endothelial cells through decreasing the expression of CRCX4, a chemokine receptor participating in circulating endothelial cells to sites of neovascularization [69]. The exploration of HDAC inhibitors and its ability to suppress angiogenesis has implications for the therapeutic options of pancreatic cancer.

5.4. HDAC inhibitor and inhibition of metastasis

The role of HDAC inhibitors in pancreatic cancer metastasis focused on the reverse of epithelial to mesenchymal transition (EMT). EMT is a key regulatory factor of tumor invasion and metastasis [70–72]. The hall marker of EMT is loss of E-cadherin, a transmembrane glycoprotein maintaining the cellular polarity and cell–cell adhesion [73,74]. Some studies have shown that HDAC inhibitor including TSA [71] and SAHA [72] enhanced E-cadherin expression in pancreatic cancer cell lines. Recent study observed that reduced E-cadherin expression in pancreatic cancer correlate with poor survival. They also found that transcriptional factor ZEB1 attached to the E-cadherin promoter and responsible for recruitment of HDAC, leading to deacetylation of histones and suppression of E-cadherin. Furthermore, either treatment of HDAC inhibitor or knockdown of ZEB1 was able to restore expression of E-cadherin in pancreatic cancer cell lines [71]. HDAC1 and HDAC2 can also form a repressor complex with another transcriptional factor Snail and inhibited E-cadherin expression in pancreatic cancer [75].

Additionally, previous studies have shown HDACIs such as TSA, NaB and SAHA inhibited cancer cell metastasis by restoring the expression of reversion-inducing cysteinerich protein with Kazal motif (RECK) and inhibited the cancer cell metastasis and invasion. Since RECK is a membrane-bound negative regulator of matrix metalloproteinase (MMP). Down-regulation of MMP2 and MMP9 was also observed. Knockdown of HDAC using small interfering RNA further validated the critical role of HDAC inhibitor in regulation of RECK and MMP [76,77]. Altogether, these data suggest that HDAC inhibitors are promising agents to suppress high metastasis potential of pancreatic cancer.

6. Combination therapy with HDAC inhibitors in pancreatic cancer

HDAC inhibitors as single agents show significant therapeutic response in patient with hematological malignancies, unfortunately, it is less effective when treating solid tumors. Since HDACIs elicit diverse anti-neoplastic effect through multiple mechanisms and pathways, it seemed that the therapeutic potential of HDACIs might be fully exploited in combination with other cancer therapeutics [78]. A growing body of studies have shown that HDACIs exert additive or synergistic effects on many drugs commonly used for pancreatic cancer, including traditional cytotoxic agents such as gemcitabine, 5-FU [79], etoposide as well as targeted therapy including bortezomib, gefitinib. Substantial effort has been put into exploring novel rational based therapeutic approaches for pancreatic cancer. Due to encouraging results of preclinical studies, activity of HDAC inhibitors in clinical trials are evaluated (Table 1).

6.1. HDAC inhibitors in combination with gemcitabine

Gemcitabine is widely accepted as golden standard therapy for patients suffering from advanced pancreatic cancer. Unfortunately, most of these patients cannot benefit from gemcitabine treatment alone. Many clinical trials have been conducted to ascertain the optimum therapy utilizing gemcitabine in combination with traditional anticancer agents such as 5-FU [87], irinotecan [88], cisplatin [89], but almost none of them was proved to be more effective compared with gemcitabine alone for treating pancreatic cancer. Thus, there is an urgent need to identify appropriate agents to realize the full potential of gemcitabine.

Table 1
Activity of HDAC inhibitors in clinical trials for treatment of patients with pancreatic cancer.

HDACI	Combination therapy	Phase	Patients	Dosage schedule (HDAC inhibitor)	Outcome	Reference/trial identifier
CI-994	Gemcitabine	II	174	6 mg/m^2 daily Day 1–21	8PR 60PD Grade3–4 AEs: hematologic and gastrointestinal	[80]
Vorinostat	NPI0052	I	4	300 mg/day	3SD Common AEs: fatigue, vomiting, diarrhea, anorexia	[81]
Romidepsin	Gemcitabine	I	9	10 mg/m^2	5SD DLT led to dose reduction	[82]
Panobinostat[a]	Bortezomib	II	7	20 mg three times weekly 2 weeks	No response Early treatment related toxicty	[83]
Vorinostat[b]	Radiation Capecitabine	I	18	100/200/300/400 mg daily M-F 4 weeks	2SD for 6 cycles Most common AEs: lymphopenia, GI toxicity, fatigue	[84]
Vorinostat	Radiation	I/II	3	200 mg/day M-F 6 weeks	Terminated (slow accrual)	NCT00831493
Vorinostat	Radiation 5-FU	I/II	–	Dose depend on time of enrollment and tolerance	Ongoing	NCT00948688
LAQ824	–	I	3	6–100 mg/m^2/day d1–3, every 21 day	3PD Grade3–4 AEs: hematologic and gastrointestinal	[85]
CI-994	Capecitabine	I	4	4–10 mg/m^2/day 3 weeks 6 mg/m^2/day 6 weeks 4–8 mg/m^2/day 3 weeks	4PD Thrombocyto penia increased with increasing CI-994 dose	[86]

PD: progressive disease; SD: stable disease; PR: partial response; DLT: dose limit toxicity; M-F: Monday–Friday; AE: adverse event.
[a] The patient with pancreatic cancer progressed on gemcitabine-based therapy.
[b] The combination therapy was evaluated in non-metastatic pancreatic cancer.

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HDAC inhibitor, given its pleiotropic effect, appears to be a promising candidate. One study have shown combination of gemcitabine and belinostat, a pan-HDAC inhibitor, potently enhanced the apoptotic activity of either agent alone in T3M4 and Panc-1 pancreatic cancer cell lines [90]. Another study revealed that when combined with gemcitabine, TSA synergistically suppressed cellular proliferation through activation of cell death but not cell cycle arrest. Enhanced apoptosis was coincided with induction of pro-apoptotic protein Bim and production of ROS. In a pancreatic cancer (T3M4 cells) xenograft model, the combination therapy led to a 50% reduction of mean tumor weight relative to that of either agent alone. Importantly, adverse effect such as mice death, weight loss and other apparent signs of toxicity were not observed [56]. Recent study revealed a novel HDAC inhibitor, chidamide, markedly enhanced gemcitabine-mediated growth inhibition and apoptosis in pancreatic cancer cell lines through inhibition of anti-apoptotic Mcl-1 and subsequent disruption of mitochondrial membrane. Furthermore, the combinated-treatment of both agents also suppressed the expression of checkpoint kinase1 (CHK1) and DNA double strand break (DSB) repair, leading to increase of gemcitabine-mediated cell cycle arrest and DNA damage [44].

However, these encouraging preclinical results failed to translate into clinical success. A phase II trial of gemcitabine alone or in combination with CI199 was carried out in patients with advanced pancreatic cancer. The combination therapy did not offer improvement in terms of overall survival, response rate or time to progression. Moreover, patients receiving CI199/gemcitabine combination had a higher incidence of grade 3–4 adverse effect including thrombocytopenia (25% versus 11%), anemia (13% versus 5%) and leucopenia (30% versus 16%) compared with gemcitabine alone [80].

The disappointing clinical outcome can be in part explained by the weak inhibitory activity of CI199. Patients might benefit from the combination treatment employing novel and potent HDAC inhibitor. These data also indicate the urgency of identifying biomarkers to select responsive patients. Additionally, whether the dosing schedule of HDACI applied in this trial is appropriate remains illusive. Taken together, despite the negative result of current trial, the encouraging preclinical data suggest the strategy of combining HDACIs with gemcitabine remains a promising approach warrant further exploration.

6.2. HDAC inhibitors in combination with topoisomerase inhibitors

Topoisomerase inhibitors such as irinotecan, epribicin, etoposide are available for treating many cancers and exert anti-tumor effect by inducing DNA strand breaks and cell death [91]. Owning to the role of lowering the apoptotic threshold in transformed cells [35], HDAC inhibitors can potentiate the DNA damage and apoptosis induced by topoisomerase inhibitor. The effect of co-treatment with HDACI VPA and topoisomerase II inhibitor etoposide was evaluated in pancreatic cancer cell MiaPaCa2 and Panc1. VPA increased etoposide-mediated apoptosis as determined by fluorescence microscopy after Hoechst staining and caspase3/7assay. Their data also revealed that VPA markedly up-regulated the pro-apoptotic BH3-only protein NOXA expression in a dose dependent manner. Using ChIP assays, they demonstrated depletion of HDAC2 induced acetylation of histone H3 at the NOXA promoter and consequent relaxation of chromatin configuration, leading to increase of NOXA expression [27]. Another study have shown that topoisomerase I inhibitor irinotecan worked synergistically with TSA through increasing irinotecan-induced growth arrest in a panel of pancreatic adenocarcinoma cell lines [92].

6.3. HDAC inhibitors in combination with proteasome inhibitors

Many studies have demonstrated that HDAC inhibitors worked synergistically with proteasome inhibitors in pancreatic cancer. When combined with proteasome inhibitor bortezomib, the HDACI induced dramatic enhancement of apoptosis compared with either agent alone as measured by the activity of caspase3/7 [93]. PS341, a selective inhibitor of 26S proteasome, interact with TSA to induce synergistic effect in eight pancreatic adenocarcinoma cell lines. The combination treatment induced markedly increase of cell death and cooperatively activated caspases as well as stimulated PARP cleavage. The data suggested the observed synergism was likely through the disruption of NF-κB signaling and reduction of anti-apoptotic Bcl-xl expression. Furthermore, the combination treatment impaired the MAPK pathway as evidenced by decreased level of several key kinases [94].

Mechanisms have been reported to elucidate the observed additive effect on apoptosis. Some studies suggested aggresome disruption was closely associated with the synergistic effect [95]. It has been clearly demonstrated that proteasome inhibitors block the degradation of damaged proteins and cause accumulation of misfolded proteins, which can be removed via formation of aggresome, a cytoprotective mechanism. Deacetylation of α-tublin by HDAC6 is uniquely required in this pathway [96]. Thus, treatment with the HDAC inhibitors disrupt the aggresome formation and led to endoplasmic reticulum (ER) stress with subsequent apoptosis [97]. To determine the potential of SAHA/bortezomib combination therapy *in vivo*, pancreatic tumors were induced by implanting L3.6pl cell into pancreas of nude mice. Combination therapy led to a significantly decreased tumor weight compared with the single agent. Moreover, combine SAHA with bortezomib markedly induced apoptosis *in vivo* as confirmed by TUNEL staining and LSC analysis. Notably, both *in vitro* and *in vivo* experiments have observed the synergistic effect was tumor selective with little adverse effect detected [95].

A phase I trial assessing the combined treatment of vorinostat and proteasome inhibitor marizomib demonstrated co-administration of two agents was feasible and tolerable in patients with lung cancer, pancreatic and melanoma. Clinical benefit was encouraging, 61% of patients (including 3 of 4 patients with pancreatic cancer) achieve stable disease, with 39% of patients having reduction of tumor measurements [81]. Since only 4 pancreatic cancer patients are included in this trial, studies recruiting more patients suffering from pancreatic cancer are required to comprehensively determine the feasibility and efficacy of these therapies.

6.4. HDAC inhibitors in combination with other anticancer agents

Pancreatic cancer is characterized by extensive tumor-associated stroma and studies have revealed stroma cells were associated with pancreatic tumor progression [98]. The Hedgehog (Hh) pathway exert effect not only on tumor cells but also on peri-tumoral stroma, making it a prominent regulator in the progression of pancreatic cancer [99]. Study showed that inhibition of both Hedgehog pathway and HDAC by SANT-1 and SAHA respectively yielded a supra-additive suppression of proliferation in gemcitabine-resistant pancreatic cancer cell lines. SAHA enhanced SANT-1 mediated cell cycle arrest consistent with enhancement of p27 and p21 and reduction of cyclinD1. These evidences proposed that concomitant inhibition of HDAC and Hedgehog pathway might be a novel HDACI-based combination for treating pancreatic cancer [100].

Recent studies have demonstrated the class III HDAC Sirt1 had an oncogenic role in pancreatic cancer and worked cooperatively with EGFR inhibitor gefitinib to induce cell growth [32,101]. As compared with either agent alone, co-treatment of Sirt1 inhibitor

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nicotinamide and gefitinib inflicted a pronounced growth inhibition in pancreatic cancer cell lines [32]. Although the mechanism underpin the synergistic effect is not yet known, their data reveals that the class III HDAC Sirt1 represent as a promising therapeutic target for pancreatic cancer.

7. Predictors of therapeutic response to HDAC inhibitors

To improve the clinical efficiency of HDACI-based treatment, it is increasingly important to identify biomarkers which assist in predicting the therapeutic response to HDACIs and selecting appropriate patients for treatment [102]. To date, an established biomarker of HDACI-based treatment is HR23B, which significantly over expressed in cutaneous T-cell lymphoma (CTCL), a malignancy sensitive to HDACI-based treatment. Patients identified with high HR23B level are more likely to benefit from HDACI treatment [103].

Over expression of HDAC is often associated with poorly differentiated cancer, indicating HDAC expression has prognostic significance. However, whether HDAC expression is capable of monitoring the therapeutic response to HDACI-based therapy remains unknown [104]. Studies demonstrated that HDACIs enhanced acetylation of histones especially H3 and H4 in a time- and dose-dependent manner [56]. Clinical trial revealed that a similar increase of histone acetylation was present in both responsive and non-responsive patients. These observations suggested that histone acetylation can be used to monitor HDAC activity but did not correlate with therapeutic response [105,106].

8. Concluding remarks

Aberrant expression of HDACs represents an attractive therapeutic target for pancreatic cancer. Owning to the ability to reactivate epigenetically silenced genes, HDAC inhibitors elicit a variety of biological responses in pancreatic cancer as shown above. Different from hematological malignancies, the full potential of HDACIs in treatment of pancreatic cancer is probably realized in combination with other molecules, such as traditional anticancer agents and targeted regimens.

Despite initial optimism of HDACI-based therapy *in vitro* and *in vivo* for pancreatic cancer, many combination treatments using HDACIs failed to produce therapeutic benefit in patients with pancreatic cancer. Since the clinical trials evaluating the efficacy of HDACIs in pancreatic cancer patients are limited, a lack of optimal dosing schedule is in part responsible for the unsatisfactory clinical response. Although many molecular events have been reported, the mechanism underpin the epigenetic alteration in pancreatic cancer is still obscure. A detailed understanding of biological mechanism is critical for the improvement of clinical outcome along with low toxicity.

Since patients with pancreatic cancer respond poorly to most anticancer agents. The identification of responsive patients is important, further studies are required to surrogate potential biomarkers to stratify candidates who will benefit the most from HDACI-based treatment. Because of high recurrence occurs in patients with curative resection, adjuvant chemotherapy is required to improve long-term survival. Whether postoperative patients might be more sensitive to HDACIs-based treatment remains further investigation.

It is proposed that selective inhibition of HDAC could yield more potent efficacy and fewer side effects than pan-HDAC inhibitor. So the development of isoform specific HDAC inhibitor is shedding light on the investigation of optimal therapeutic approaches for pancreatic cancer and high quality clinical trials are required to determine the efficacy of novel combination incorporating the isoform selective HDAC inhibitors.

Conflict of Interest

The authors declare no conflict of interest.

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References

[1] A. Vincent, J. Herman, R. Schulick, R.H. Hruban, M. Goggins, Pancreatic cancer, Lancet 378 (2011) 607–620.
[2] R. Siegel, D. Naishadham, A. Jemal, Cancer statistics, 2012, CA Cancer J. Clin. 62 (2012) 10–29.
[3] H.R. Burris, M.J. Moore, J. Andersen, M.R. Green, M.L. Rothenberg, M.R. Modiano, M.C. Cripps, R.K. Portenoy, A.M. Storniolo, P. Tarassoff, Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial, J. Clin. Oncol. 15 (1997) 2403–2413.
[4] F. Rivera, S. López-Tarruella, M.E. Vega-Villegas, M. Salcedo, Treatment of advanced pancreatic cancer: from gemcitabine single agent to combinations and targeted therapy, Cancer Treat. Rev. 35 (2009) 335–339.
[5] M.J. Moore, D. Goldstein, J. Hamm, A. Figer, J.R. Hecht, S. Gallinger, H.J. Au, P. Murawa, D. Walde, R.A. Wolff, Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group, J. Clin. Oncol. 25 (2007) 1960–1966.
[6] T. Troiani, E. Martinelli, A. Capasso, F. Morgillo, M. Orditura, F. De Vita, F. Ciardiello, Targeting EGFR in pancreatic cancer treatment, Curr. Drug Targets 13 (2012) 802–810.
[7] G. Schneider, R. Hamacher, S. Eser, H. Friess, R.M. Schmid, D. Saur, Molecular biology of pancreatic cancer-new aspects and targets, Anticancer Res. 28 (2008) 1541–1550.
[8] T. Ito, T. Kojima, H. Yamaguchi, D. Kyuno, Y. Kimura, M. Imamura, A. Takasawa, M. Murata, S. Tanaka, K. Hirata, Transcriptional regulation of claudin-18 via specific protein kinase C signaling pathways and modification of DNA methylation in human pancreatic cancer cells, J. Cell. Biochem. 112 (2011) 1761–1772.
[9] E. Giovannetti, N. Funel, G.J. Peters, M. Del Chiaro, L.A. Erozenci, E. Vasile, L.G. Leon, L.E. Pollina, A. Groen, A. Falcone, MicroRNA-21 in pancreatic cancer: correlation with clinical outcome and pharmacologic aspects underlying its role in the modulation of gemcitabine activity, Cancer Res. 70 (2010) 4528–4538.
[10] A. Vincent, N. Omura, S.-M. Hong, A. Jaffe, J. Eshleman, M. Goggins, Genome-wide analysis of promoter methylation associated with gene expression profile in pancreatic adenocarcinoma, Clin. Cancer Res. 17 (2011) 4341–4354.
[11] S. Fukushige, A. Horii, Road to early detection of pancreatic cancer: attempts to utilize epigenetic biomarkers, Cancer Lett. (2012).
[12] A. Li, N. Omura, S.-M. Hong, A. Vincent, K. Walter, M. Griffith, M. Borges, M. Goggins, Pancreatic cancers epigenetically silence SIP1 and hypomethylate and overexpress miR-200a/200b in association with elevated circulating miR-200a and miR-200b levels, Cancer Res. 70 (2010) 5226–5237.
[13] K.-H. Lee, C. Lotterman, C. Karikari, N. Omura, G. Feldmann, N. Habbe, M.G. Goggins, J.T. Mendell, A. Maitra, Epigenetic silencing of MicroRNA miR-107 regulates cyclin-dependent kinase 6 expression in pancreatic cancer, Pancreatology 9 (2009) 293–301.
[14] S. Zhang, J. Hao, F. Xie, X. Hu, C. Liu, J. Tong, J. Zhou, J. Wu, C. Shao, Downregulation of miR-132 by promoter methylation contributes to pancreatic cancer development, Carcinogenesis 32 (2011) 1183–1189.
[15] A.L. McCleary-Wheeler, G.A. Lomberk, F.U. Weiss, G. Schneider, M. Fabbri, T.L. Poshusta, N.J. Dusetti, S. Baumgart, J.L. Iovanna, V. Ellenrieder, Insights into the epigenetic mechanisms controlling pancreatic carcinogenesis, Cancer Lett. (2012).
[16] B.E. Bernstein, A. Meissner, E.S. Lander, The mammalian epigenome, Cell 128 (2007) 669–681.
[17] B. Venugopal, T. Evans, Developing histone deacetylase inhibitors as anti-cancer therapeutics, Curr. Med. Chem. 18 (2011) 1658–1671.
[18] M.T. Epping, R. Bernards, Molecular basis of the anti-cancer effects of histone deacetylase inhibitors, Int. J. Biochem. Cell Biol. 41 (2009) 16–20.
[19] A.A. Lane, B.A. Chabner, Histone deacetylase inhibitors in cancer therapy, J. Clin. Oncol. 27 (2009) 5459–5468.
[20] S. Minucci, P.G. Pelicci, Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer, Nat. Rev. Cancer 6 (2006) 38–51.
[21] C. Choudhary, C. Kumar, F. Gnad, M.L. Nielsen, M. Rehman, T.C. Walther, J.V. Olsen, M. Mann, Lysine acetylation targets protein complexes and co-regulates major cellular functions, Science 325 (2009) 834–840.
[22] L. Ellis, P.W. Atadja, R.W. Johnstone, Epigenetics in cancer: targeting chromatin modifications, Mol. Cancer Ther. 8 (2009) 1409–1420.

[23] S. Spange, T. Wagner, T. Heinzel, O.H. Krämer, Acetylation of non-histone proteins modulates cellular signalling at multiple levels, Int. J. Biochem. Cell Biol. 41 (2009) 185–198.
[24] P.A. Marks, Histone deacetylase inhibitors: a chemical genetics approach to understanding cellular functions, Biochim. Biophys. Acta (BBA) – Gene Regul. Mech. 1799 (2010) 717–725.
[25] D.S. Schrump, Cytotoxicity mediated by histone deacetylase inhibitors in cancer cells: mechanisms and potential clinical implications, Clin. Cancer Res. 15 (2009) 3947–3957.
[26] A. Lehmann, C. Denkert, J. Budczies, A.-C. Buckendahl, S. Darb-Esfahani, A. Noske, B. Müller, M. Bahra, P. Neuhaus, M. Dietel, High class I HDAC activity and expression are associated with RelA/p65 activation in pancreatic cancer in vitro and in vivo, BMC Cancer 9 (2009) 395.
[27] P. Fritsche, B. Seidler, S. Schüler, A. Schnieke, M. Göttlicher, R.M. Schmid, D. Saur, G. Schneider, HDAC2 mediates therapeutic resistance of pancreatic cancer cells via the BH3-only protein NOXA, Gut 58 (2009) 1399–1409.
[28] M. Nakagawa, Y. Oda, T. Eguchi, S.-I. Aishima, T. Yao, F. Hosoi, Y. Basaki, M. Ono, M. Kuwano, M. Tanaka, Expression profile of class I histone deacetylases in human cancer tissues, Oncol. Rep. 18 (2007) 769–774.
[29] K. Miyake, T. Yoshizumi, S. Imura, K. Sugimoto, E. Batmunkh, H. Kanemura, Y. Morine, M. Shimada, Expression of hypoxia-inducible factor-1 [alpha], histone deacetylase 1, and metastasis-associated protein 1 in pancreatic carcinoma: correlation with poor prognosis with possible regulation, Pancreas 36 (2008) e1–e9.
[30] W. Wang, J. Gao, X.-H. Man, Z.-S. Li, Y.-F. Gong, Significance of DNA methyltransferase-1 and histone deacetylase-1 in pancreatic cancer, Oncol. Rep. 21 (2009) 1439–1447.
[31] M. Ouaissi, I. Sielezneff, B. Sastre, L. Dahan, N. Pirrò, High histone deacetylase 7 (HDAC7) expression is significantly associated with adenocarcinomas of the pancreas, Ann. Surg. Oncol. 15 (2008) 2318–2328.
[32] A. Stenzinger, V. Endris, F. Klauschen, B. Sinn, K. Lorenz, A. Warth, B. Goeppert, V. Ehemann, A. Muckenhuber, C. Kamphues, High SIRT1 expression is a negative prognosticator in pancreatic ductal adenocarcinoma, BMC Cancer 13 (2013) 450.
[33] J. Tang, H. Yan, S. Zhuang, Histone deacetylases as targets for treatment of multiple diseases, Clin. Sci. 124 (2013) 651–662.
[34] A.L. Abujamra, M.P. dos Santos, R. Roesler, G. Schwartsmann, A.L. Brunetto, Histone deacetylase inhibitors: a new perspective for the treatment of leukemia, Leukemia Res. 34 (2010) 687–695.
[35] J.E. Bolden, M.J. Peart, R.W. Johnstone, Anticancer activities of histone deacetylase inhibitors, Nat. Rev. Drug Discovery 5 (2006) 769–784.
[36] O. Khan, N.B. La Thangue, HDAC inhibitors in cancer biology: emerging mechanisms and clinical applications, Immunol. Cell Biol. 90 (2011) 85–94.
[37] M. Duvic, R. Talpur, X. Ni, C. Zhang, P. Hazarika, C. Kelly, J.H. Chiao, J.F. Reilly, J.L. Ricker, V.M. Richon, Phase 2 trial of oral vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL), Blood 109 (2007) 31–39.
[38] I. Koutsounas, C. Giaginis, E. Patsouris, S. Theocharis, Current evidence for histone deacetylase inhibitors in pancreatic cancer, World J. Gastroenterol.: WJG 19 (2013) 813.
[39] G. Schneider, O.H. Krämer, P. Fritsche, et al., Targeting histone deacetylases in pancreatic ductal adenocarcinoma[J], Journal of cellular and molecular medicine 14 (6a) (2010) 1255–1263.
[40] I. Koutsounas, C. Giaginis, S. Theocharis, Histone deacetylase inhibitors and pancreatic cancer: are there any promising clinical trials?, World J Gastroenterol.: WJG 19 (2013) 1173.
[41] M. Ocker, R. Schneider-Stock, Histone deacetylase inhibitors: signalling towards p^{21} cip^{1}/waf^{1}, Int. J. Biochem. Cell Biol. 39 (2007) 1367–1374.
[42] W. Chien, D.H. Lee, Y. Zheng, P. Wuensche, R. Alvarez, D.L. Wen, A.M. Aribi, S.M. Thean, N.B. Doan, J.W. Said, Growth inhibition of pancreatic cancer cells by histone deacetylase inhibitor belinostat through suppression of multiple pathways including HIF, NFkB, and mTOR signaling in vitro and in vivo, Mol. Carcinogen. (2013).
[43] V. Sung, N. Richard, H. Brady, A. Maier, G. Kelter, C. Heise, Histone deacetylase inhibitor MGCD0103 synergizes with gemcitabine in human pancreatic cells, Cancer Sci. 102 (2011) 1201–1207.
[44] Z. Qiao, S. Ren, W. Li, X. Wang, M. He, Y. Guo, L. Sun, Y. He, Y. Ge, Q. Yu, Chidamide, a novel histone deacetylase inhibitor, synergistically enhances gemcitabine cytotoxicity in pancreatic cancer cells, Biochem. Biophys. Res. Commun. (2013).
[45] P.S. Moore, S. Barbi, M. Donadelli, C. Costanzo, C. Bassi, M. Palmieri, A. Scarpa, Gene expression profiling after treatment with the histone deacetylase inhibitor trichostatin A reveals altered expression of both pro-and anti-apoptotic genes in pancreatic adenocarcinoma cells, Biochim. Biophys. Acta (BBA) – Mol. Cell Res. 1693 (2004) 167–176.
[46] J.K. Ryu, W.J. Lee, K.H. Lee, J.-H. Hwang, Y.-T. Kim, Y.B. Yoon, C.Y. Kim, SK-7041, a new histone deacetylase inhibitor, induces G_2-M cell cycle arrest and apoptosis in pancreatic cancer cell lines, Cancer Lett. 237 (2006) 143–154.
[47] G. Wang, J. He, J. Zhao, W. Yun, C. Xie, J.W. Taub, A. Azmi, R.M. Mohammad, Y. Dong, W. Kong, Class I and class II histone deacetylases are potential therapeutic targets for treating pancreatic cancer, PLoS ONE 7 (2012) e52095.
[48] A. Arlt, S.S. Müerköster, H. Schäfer, Targeting apoptosis pathways in pancreatic cancer, Cancer Lett. 332 (2013) 346–358.
[49] J. Kauh, S. Fan, M. Xia, P. Yue, L. Yang, F.R. Khuri, S.-Y. Sun, C-FLIP degradation mediates sensitization of pancreatic cancer cells to TRAIL-induced apoptosis by the histone deacetylase inhibitor LBH589, PLoS ONE 5 (2010) e10376.
[50] S. Schüler, P. Fritsche, S. Diersch, A. Arlt, R.M. Schmid, D. Saur, G. Schneider, Research HDAC2 attenuates TRAIL-induced apoptosis of pancreatic cancer cells, Mol. Cancer 9 (2010) 80.
[51] F. Natoni, L. Diolordi, C. Santoni, M.S. Gilardini Montani, Sodium butyrate sensitises human pancreatic cancer cells to both the intrinsic and the extrinsic apoptotic pathways, Biochim. Biophys. Acta (BBA) – Mol. Cell Res. 1745 (2005) 318–329.
[52] P. Marks, W.S. Xu, Histone deacetylase inhibitors: potential in cancer therapy, J. Cell Biochem. 107 (2009) 600–608.
[53] C. Dell'Aversana, I. Lepore, L. Altucci, HDAC modulation and cell death in the clinic, Exp. Cell Res. 318 (2012) 1229–1244.
[54] A. Newbold, R.K. Lindemann, L.A. Cluse, K.F. Whitecross, A.E. Dear, R.W. Johnstone, Characterisation of the novel apoptotic and therapeutic activities of the histone deacetylase inhibitor romidepsin, Mol. Cancer Ther. 7 (2008) 1066–1079.
[55] A.P. Wiegmans, A.E. Alsop, M. Bots, L.A. Cluse, S.P. Williams, K.-M. Banks, R. Ralli, C.L. Scott, A. Frenzel, A. Villunger, Deciphering the molecular events necessary for synergistic tumor cell apoptosis mediated by the histone deacetylase inhibitor vorinostat and the BH3 mimetic ABT-737, Cancer Res. 71 (2011) 3603–3615.
[56] M. Donadelli, C. Costanzo, S. Beghelli, M.T. Scupoli, M. Dandrea, A. Bonora, P. Piacentini, A. Budillon, M. Caraglia, A. Scarpa, Synergistic inhibition of pancreatic adenocarcinoma cell growth by trichostatin A and gemcitabine, Biochim. Biophys. Acta (BBA) – Mol. Res. 1773 (2007) 1095–1106.
[57] N. Mitsiades, C.S. Mitsiades, P.G. Richardson, C. McMullan, V. Poulaki, G. Fanourakis, R. Schlossman, D. Chauhan, N.C. Munshi, T. Hideshima, Molecular sequelae of histone deacetylase inhibition in human malignant B cells, Blood 101 (2003) 4055–4062.
[58] P. García-Morales, A. Gómez-Martínez, A. Carrato, I. Martínez-Lacaci, V.M. Barberá, J.L. Soto, E. Carrasco-García, M.P. Menéndez-Gutierrez, M.D. Castro-Galache, J.A. Ferragut, Histone deacetylase inhibitors induced caspase-independent apoptosis in human pancreatic adenocarcinoma cell lines, Mol. Cancer Ther. 4 (2005) 1222–1230.
[59] P.A. Marks, Thioredoxin in cancer—role of histone deacetylase inhibitors, in: Seminars in Cancer Biology, Elsevier, 2006, pp. 436–443.
[60] J. Ungerstedt, Y. Du, H. Zhang, D. Nair, A. Holmgren, In vivo redox state of Human thioredoxin and redox shift by the histone deacetylase inhibitor suberoylanilide hydroxamic acid (SAHA), Free Radical Biol. Med. (2012).
[61] J. Ungerstedt, Y. Sowa, W.-S. Xu, Y. Shao, M. Dokmanovic, G. Perez, L. Ngo, A. Holmgren, X. Jiang, P. Marks, Role of thioredoxin in the response of normal and transformed cells to histone deacetylase inhibitors, Proc. Nat. Acad. Sci. USA 102 (2005) 673–678.
[62] B. Wang, X.-b. Wang, L.-y. Chen, L. Huang, R.-Z. Dong, Belinostat-induced apoptosis and growth inhibition in pancreatic cancer cells involve activation of TAK1-AMPK signaling axis, Biochem. Biophys. Res. Commun. (2013).
[63] L. Ellis, H. Hammers, R. Pili, Targeting tumor angiogenesis with histone deacetylase inhibitors, Cancer Lett. 280 (2009) 145–153.
[64] T. Hideshima, Histone Deacetylase Inhibitors in Multiple Myeloma. Myeloma Therapy, Springer, 2009. pp. 379–392.
[65] L. Chen, A. Endler, F. Shibasaki, Hypoxia and angiogenesis: regulation of hypoxia-inducible factors via novel binding factors, Exp. Mol. Med. 41 (2009) 849–857.
[66] L. Ellis, R. Pili, Histone deacetylase inhibitors: advancing therapeutic strategies in hematological and solid malignancies, Pharmaceuticals 3 (2010) 2441–2469.
[67] S. Wang, X. Li, M. Parra, E. Verdin, R. Bassel-Duby, E.N. Olson, Control of endothelial cell proliferation and migration by VEGF signaling to histone deacetylase 7, Proc. Nat. Acad. Sci. 105 (2008) 7738–7743.
[68] T. Liu, S. Kuljaca, A. Tee, G.M. Marshall, Histone deacetylase inhibitors: multifunctional anticancer agents, Cancer Treat. Rev. 32 (2006) 157–165.
[69] D.Z. Qian, Y. Kato, S. Shabbeer, Y. Wei, H.M. Verheul, B. Salumbides, T. Sanni, P. Atadja, R. Pili, Targeting tumor angiogenesis with histone deacetylase inhibitors: the hydroxamic acid derivative LBH589, Clin. Cancer Res. 12 (2006) 634–642.
[70] H.J. Maier, T. Wirth, H. Beug, Epithelial–mesenchymal transition in pancreatic carcinoma, Cancers (Basel) 2 (2010) 2058–2083.
[71] A. Aghdassi, M. Sendler, A. Guenther, J. Mayerle, C.-O. Behn, C.-D. Heidecke, H. Friess, M. Büchler, M. Evert, M.M. Lerch, Recruitment of histone deacetylases HDAC1 and HDAC2 by the transcriptional repressor ZEB1 downregulates E-cadherin expression in pancreatic cancer, Gut 61 (2012) 439–448.
[72] T. Kumagai, N. Wakimoto, D. Yin, S. Gery, N. Kawamata, N. Takai, N. Komatsu, A. Chumakov, Y. Imai, H.P. Koeffler, Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (Vorinostat, SAHA) profoundly inhibits the growth of human pancreatic cancer cells, Int. J. Cancer 121 (2007) 656–665.
[73] M. Iwatsuki, K. Mimori, T. Yokobori, H. Ishi, T. Beppu, S. Nakamori, H. Baba, M. Mori, Epithelial–mesenchymal transition in cancer development and its clinical significance, Cancer Sci. 101 (2010) 293–299.
[74] T. Arumugam, V. Ramachandran, K.F. Fournier, H. Wang, L. Marquis, J.L. Abbruzzese, G.E. Gallick, C.D. Logsdon, D.J. McConkey, W. Choi, Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer, Cancer Res. 69 (2009) 5820–5828.
[75] J. von Burstin, S. Eser, M.C. Paul, B. Seidler, M. Brandl, M. Messer, A. von Werder, A. Schmidt, J. Mages, P. Pagel, E-cadherin regulates metastasis of pancreatic cancer in vivo and is suppressed by a SNAIL/HDAC1/HDAC2 repressor complex, Gastroenterology 137 (2009) 361–371. e365.

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[76] H.W. Jeon, Y.M. Lee, Inhibition of histone deacetylase attenuates hypoxia-induced migration and invasion of cancer cells via the restoration of RECK expression, Mol. Cancer Ther. 9 (2010) 1361–1370.
[77] L.-T. Liu, H.-C. Chang, L.-C. Chiang, W.-C. Hung, Histone deacetylase inhibitor up-regulates RECK to inhibit MMP-2 activation and cancer cell invasion, Cancer Res. 63 (2003) 3069–3072.
[78] K.T. Thurn, S. Thomas, A. Moore, P.N. Munster, Rational therapeutic combinations with histone deacetylase inhibitors for the treatment of cancer, Future Oncol. 7 (2011) 263–283.
[79] S. Iwahashi, H. Ishibashi, T. Utsunomiya, Y. Morine, T.L. Ochir, J. Hanaoka, H. Mori, T. Ikemoto, S. Imura, M. Shimada, Effect of histone deacetylase inhibitor in combination with 5-fluorouracil on pancreas cancer and cholangiocarcinoma cell lines, J. Med. Invest.: JMI 58 (2011) 106–109.
[80] D. Richards, K. Boehm, D. Waterhouse, D. Wagener, S. Krishnamurthi, A. Rosemurgy, W. Grove, K. Macdonald, S. Gulyas, M. Clark, Gemcitabine plus CI-994 offers no advantage over gemcitabine alone in the treatment of patients with advanced pancreatic cancer: results of a phase II randomized, double-blind, placebo-controlled, multicenter study, Ann. Oncol. 17 (2006) 1096–1102.
[81] M. Millward, T. Price, A. Townsend, C. Sweeney, A. Spencer, S. Sukumaran, A. Longenecker, L. Lee, A. Lay, G. Sharma, Phase 1 clinical trial of the novel proteasome inhibitor marizomib with the histone deacetylase inhibitor vorinostat in patients with melanoma, pancreatic and lung cancer based on in vitro assessments of the combination, Invest. New Drug 30 (2012) 2303–2317.
[82] H. Doss, S. Jones, J. Infante, D. Spigel, N. Willcutt, R. Lamar, J. Barton, M. Keegan, H. Burris III, A phase I trial of romidepsin in combination with gemcitabine in patients with pancreatic and other advanced solid tumors, J. Clin. Oncol. 26 (2008) 25–67.
[83] H. Wang, Q. Cao, A.Z. Dudek, Phase II study of panobinostat and bortezomib in patients with pancreatic cancer progressing on gemcitabine-based therapy, Anticancer Res. 32 (2012) 1027–1031.
[84] E. Chan, L.R. Arlinghaus, D.B. Cardin, L.W. Goff, T.E. Yankeelov, J. Berlin, P. McClanahan, M. Holloway, A.A. Parikh, R.G. Abramson, Phase I trial of chemoradiation with capecitabine and vorinostat in pancreatic cancer, J. Clin. Oncol. (Meeting Abstracts) (2013) 225.
[85] J.S. de Bono, R. Kristeleit, A. Tolcher, P. Fong, S. Pacey, V. Karavasilis, M. Mita, H. Shaw, P. Workman, S. Kaye, Phase I pharmacokinetic and pharmacodynamic study of LAQ824, a hydroxamate histone deacetylase inhibitor with a heat shock protein-90 inhibitory profile, in patients with advanced solid tumors, Clin. Cancer Res. 14 (2008) 6663–6673.
[86] S. Undevia, H. Kindler, L. Janisch, S. Olson, R. Schilsky, N. Vogelzang, K. Kimmel, T. Macek, M. Ratain, A phase I study of the oral combination of CI-994, a putative histone deacetylase inhibitor, and capecitabine, Ann. Oncol. 15 (2004) 1705–1711.
[87] F. Di Costanzo, P. Carlini, L. Doni, B. Massidda, R. Mattioli, A. Iop, E. Barletta, L. Moscetti, F. Recchia, P. Tralongo, Gemcitabine with or without continuous infusion 5-FU in advanced pancreatic cancer: a randomised phase II trial of the Italian oncology group for clinical research (GOIRC), Brit. J. Cancer 93 (2005) 185–189.
[88] M.H. Kulke, M.A. Tempero, D. Niedzwiecki, D.R. Hollis, H.L. Kindler, M. Cusnir, P.C. Enzinger, S.M. Gorsch, R.M. Goldberg, R.J. Mayer, Randomized phase II study of gemcitabine administered at a fixed dose rate or in combination with cisplatin, docetaxel, or irinotecan in patients with metastatic pancreatic cancer: CALGB 89904, J. Clin. Oncol. 27 (2009) 5506–5512.
[89] G. Colucci, R. Labianca, F. Di Costanzo, V. Gebbia, G. Cartenì, B. Massidda, E. Dapretto, L. Manzione, E. Piazza, M. Sannicolò, Randomized phase III trial of gemcitabine plus cisplatin compared with single-agent gemcitabine as first-line treatment of patients with advanced pancreatic cancer: the GIP-1 study, J. Clin. Oncol. 28 (2010) 1645–1651.
[90] D.I. Dovzhanskiy, S.M. Arnold, T. Hackert, I. Oehme, O. Witt, K. Felix, N. Giese, J. Werner, Experimental in vivo and in vitro treatment with a new histone deacetylase inhibitor belinostat inhibits the growth of pancreatic cancer, BMC Cancer 12 (2012) 226.
[91] J.L. Nitiss, Targeting DNA topoisomerase II in cancer chemotherapy, Nat. Rev. Cancer 9 (2009) 338–350.
[92] P. Piacentini, M. Donadelli, C. Costanzo, P.S. Moore, M. Palmieri, A. Scarpa, Trichostatin A enhances the response of chemotherapeutic agents in inhibiting pancreatic cancer cell proliferation, Virchows Arch. 448 (2006) 797–804.
[93] J. Spratlin, T. Pitts, G. Kulikowski, M. Morelli, J. Tentler, N. Serkova, S. Eckhardt, Synergistic activity of histone deacetylase and proteasome inhibition against pancreatic and hepatocellular cancer cell lines, Anticancer Res. 31 (2011) 1093–1103.
[94] J. Bai, A. Demirjian, J. Sui, W. Marasco, M.P. Callery, Histone deacetylase inhibitor trichostatin A and proteasome inhibitor PS-341 synergistically induce apoptosis in pancreatic cancer cells, Biochem. Biophys. Res. Commun. 348 (2006) 1245–1253.
[95] S.T. Nawrocki, J.S. Carew, M.S. Pino, R.A. Highshaw, R.H. Andtbacka, K. Dunner, A. Pal, W.G. Bornmann, P.J. Chiao, P. Huang, Aggresome disruption: a novel strategy to enhance bortezomib-induced apoptosis in pancreatic cancer cells, Cancer Res. 66 (2006) 3773–3781.
[96] M. Bots, R.W. Johnstone, Rational combinations using HDAC inhibitors, Clin. Cancer Res. 15 (2009) 3970–3977.
[97] J.J. Wright, Combination therapy of bortezomib with novel targeted agents: an emerging treatment strategy, Clin. Cancer Res. 16 (2010) 4094–4104.
[98] R.F. Hwang, T. Moore, T. Arumugam, V. Ramachandran, K.D. Amos, A. Rivera, B. Ji, D.B. Evans, C.D. Logsdon, Cancer-associated stromal fibroblasts promote pancreatic tumor progression, Cancer Res. 68 (2008) 918–926.
[99] K.P. Olive, M.A. Jacobetz, C.J. Davidson, A. Gopinathan, D. McIntyre, D. Honess, B. Madhu, M.A. Goldgraben, M.E. Caldwell, D. Allard, Inhibition of Hedgehog signaling enhances delivery of chemotherapy in a mouse model of pancreatic cancer, Science 324 (2009) 1457–1461.
[100] S.G. Chun, W. Zhou, N.S. Yee, Combined targeting of histone deacetylases and hedgehog signaling enhances cytoxicity in pancreatic cancer, Cancer Biol. Ther. 8 (2009) 1328–1339.
[101] G. Zhao, J. Cui, J. Zhang, Q. Qin, Q. Chen, T. Yin, S. Deng, Y. Liu, L. Liu, B. Wang, SIRT1 RNAi knockdown induces apoptosis and senescence, inhibits invasion and enhances chemosensitivity in pancreatic cancer cells, Gene Ther. 18 (2011) 920–928.
[102] L. Stimson, N.B. La Thangue, Biomarkers for predicting clinical responses to HDAC inhibitors, Cancer Lett. 280 (2009) 177–183.
[103] O. Khan, S. Fotheringham, V. Wood, L. Stimson, C. Zhang, F. Pezzella, M. Duvic, D.J. Kerr, N.B. La Thangue, HR23B is a biomarker for tumor sensitivity to HDAC inhibitor-based therapy, Proc. Nat. Acad. Sci. 107 (2010) 6532–6537.
[104] W. Weichert, HDAC expression and clinical prognosis in human malignancies, Cancer Lett. 280 (2009) 168–176.
[105] G. Garcia-Manero, H. Yang, C. Bueso-Ramos, A. Ferrajoli, J. Cortes, W.G. Wierda, S. Faderl, C. Koller, G. Morris, G. Rosner, Phase 1 study of the histone deacetylase inhibitor vorinostat (suberoylanilide hydroxamic acid [SAHA]) in patients with advanced leukemias and myelodysplastic syndromes, Blood 111 (2008) 1060–1066.
[106] C.P. Miller, M.M. Singh, R.-D. Valle, C.A. Manton, J. Chandra, Therapeutic strategies to enhance the anticancer efficacy of histone deacetylase inhibitors, J. Biomed. Biotechnol. 2011 (2011).

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