

Role of histone deacetylases in pancreas: Implications for pathogenesis and therapy

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Abstract

In the last years, our knowledge of the pathogenesis in acute and chronic pancreatitis (AP/CP) as well as in pancreatic cancerogenesis has significantly diversified. Nevertheless, the medicinal therapeutic options are still limited and therapeutic success and patient outcome are poor. Epigenetic deregulation of gene expression is known to contribute to development and progression of AP and CP as well as of pancreatic cancer. Therefore, the selective inhibition of aberrantly active epigenetic regulators can be an effective option for future therapies. Histone deacetylases (HDACs) are enzymes that remove an acetyl group from histone tails, thereby causing chromatin compaction and repression of transcription. In this review we present an overview of the currently available literature addressing the role of HDACs in the pancreas and in pancreatic diseases. In pancreatic cancerogenesis, HDACs play a role in the important process of epithelial-mesenchymal-transition, ubiquitin-proteasome pathway and, hypoxia-inducible-factor-1-angiogenesis. Finally, we focus on HDACs as potential therapeutic targets by summarizing currently available histone deacetylase inhibitors.

Key words: Pancreatitis; Pancreatic cancer; Epigenetics; Histone deacetylase; Histone deacetylase inhibitor

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Core tip: Histone deacetylases (HDACs) are epigenetic regulators that play an essential role in organ develop-

ment and tissue homeostasis. Aberrant HDAC activity contributes to the development of several diseases, including acute and chronic pancreatitis as well as pancreatic cancer. In acute and chronic pancreatitis the inhibition of HDACs exerts significant positive effects of cytokine- and nuclear factor- κ B transmitted inflammation and tissue damage paralleled by reduced oxidative stress. HDACs are expressed in pancreatic cancer and were functionally linked to key processes of tumor progression (epithelial-mesenchymal-transition, the ubiquitin-proteasome pathway and angiogenesis), indicating a pleiotropic effect of HDACs in pancreatic cancerogenesis. Treatment of pancreatic cancer cells *in vitro* with HDAC inhibitors alone and/or in combination with conventional cancer agents resulted in diverse beneficial effects, including inhibition of proliferation and cell cycle as well as apoptosis. Therefore, inhibition of HDACs might be a promising strategy for treatment of pancreatic cancer.

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INTRODUCTION

The pancreas plays a key role in human physiology by its essential functions in gastrointestinal enzymatic digestion and endocrine glucose-dependent regulation of systemic energy metabolism *via* two main functions located in the histo-anatomical endocrine (islets of Langerhans) and exocrine (acinar - ductal) compartment of the pancreas (Figure 1)^[1]. The endocrine compartment releases hormones into the blood stream, thereby controlling blood glucose concentration, whereas the exocrine part produces and secretes digestive hydrolytic enzymes into the duodenum. These important physiological tasks of the pancreas become clinically evident, when an acute or chronic inflammatory process like pancreatitis as well as progressive carcinogenesis and subsequently necessary intensive surgery of pancreatic cancer lead to organ destruction and disturbance of the functional integrity of the pancreas (Figure 1)^[2-4]. As medicinal therapeutic options of pancreatitis and pancreas cancer are limited and mostly not associated with enhanced therapeutic success until now, the need for new approaches (such as epigenetic interactions) is still urgent in order to improve the quality of life and the outcome of patients with pancreatitis and pancreas cancer^[5,6]. In this review we give an overview of the role of epigenetic regulation by histone (de)acetylation in pancreatic inflammation as well as in development of pancreatic tumors. We will further discuss the potential of histone deacetylase inhibitors

(HDACis) as therapeutic approaches for treatment of these pancreatic diseases.

Epigenetic regulation of gene expression is a fundamental mechanism of eukaryotic organisms to ensure that only a subset of genes is actively expressed, thereby enabling the development of organs, specific tissues and their specialized physiologic functions. The term epigenetics describes all heritable changes in gene expression which act independently of the primary structure of the DNA, *i.e.*, the DNA sequence. The two major mechanisms of epigenetics are methylation of DNA and post-translational modification of histone tails^[7]. Histones are proteins that package the DNA in structural units called nucleosomes. There are five major classes of histones: H1, H2A, H2B, H3 and H4. H1 are linker histones, whereas two of each of the other four histone classes build the octameric core of the nucleosome^[8]. In general, DNA methylation is associated with gene silencing, whereas the effect of histone modifications is dependent on the modification itself, the position of the modification and other surrounding histone modifications^[7,9].

The two currently best known histone modifications are histone methylation and acetylation, of which methylation can lead to both, transcriptional activation and repression. Acetylation of histone tails, on the other hand mostly enhances gene expression^[8]. This can be explained by the fact that the addition of an acetyl group causes a neutralization of the positive charge of the histone, thereby loosening the contact between DNA and histones and facilitating accessibility of the DNA to transcription-promoting proteins^[8]. In contrast, the reverse process, called deacetylation, causes compaction of chromatin and repression of transcription^[10]. Deacetylation is performed by a group of enzymes, the histone deacetylases (HDACs), which can be further classified into four groups HDACs I-IV (for details of the different HDAC groups see^[10,11]). HDACs play a crucial role in proper development of organs by epigenetic repression of certain genes. However, aberrant activity of HDACs also contributes to development of various human malignancies^[10].

HDAC EXPRESSION IN PANCREATITIS

In the last years, intense efforts have been undertaken to gain more detailed insights into the role of HDACs in inflammation and their possible pathogenic involvement in chronic and destructive diseases. As reviewed in detail by others^[12-14], HDACs are centrally involved in inflammatory processes in numerous chronic and organ-destructive diseases such as inflammatory bowel disease, chronic respiratory conditions, rheumatoid arthritis and juvenile idiopathic arthritis, allergic diseases and atherosclerosis^[12-14]. Here, HDACs influence the antigen presentation, expression of inflammatory mediators and anti-viral responses either directly or indirectly, for instance *via* class II, major histocom-

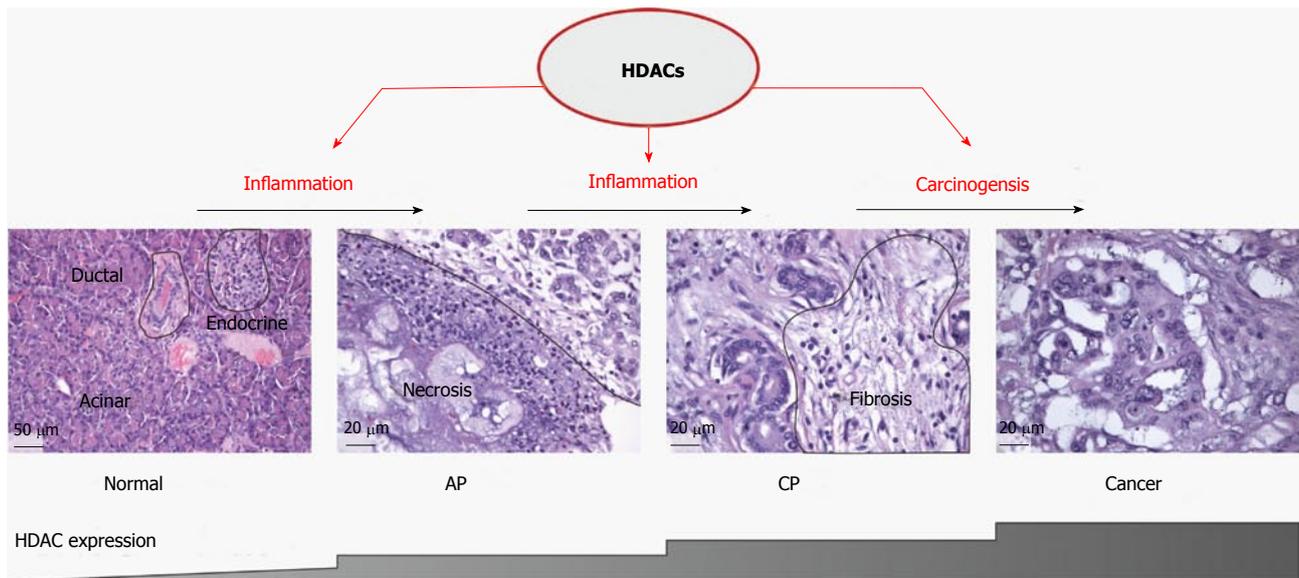


Figure 1 Histone deacetylases and histopathological correlates in the transition from normal to acute/chronic pancreatitis and pancreatic cancer. The trend to increased expression (based on pharmacological inhibition studies) of HDACs from normal to pancreatic cancer tissue is shown in the lower part of the figure. In pancreatic cancer, up-regulation of HDAC-1,-2,-3,-7,-8 could be demonstrated - see text for details - probably offering approaches for personalized therapies based on specific HDAC inhibition. HDAC: Histone deacetylases; AP: Acute pancreatitis; CP: Chronic pancreatitis.

patibility complex, transactivator (CIIT), Interleukin (IL)-10, nuclear factor (NF)- κ B, metastatic tumor antigen (MTA)1 or signal transducer and activator of transcription^[14,15]. To summarize the functional role of HDACs during pancreatic inflammation and pancreatitis, a recapitulation of relevant inflammatory pathways on cellular and molecular levels involved in acute or chronic pancreatitis (AP, CP) is given in short (reviewed in detail in^[16-18]) - in Figures 1 and 2: In the acute phase (AP), neutrophils, followed by monocytes and macrophages, represent the key inflammatory cells secreting the major cytokines and inflammatory mediators. These include, amongst others, tumor necrosis factor (TNF)- α , IL-1 β , IL-6, monocyte chemoattractant protein (MCP)-1 and platelet activating factor (PAF; being also produced in part by acinar cells)^[19]. For development of CP, activation of pancreatic stellate cells as well as infiltrating myeloid cells and particularly macrophages are important on cellular level, whereby NF- κ B plays a relevant role on molecular level initiating and promoting fibrosis and scarring of the pancreatic tissue, which results ultimately in loss of exocrine and endocrine functions of the pancreas^[20,21]. Additionally, detailed investigations of immune cells revealed that T-cell-subsets play a central role in the pathogenesis of CP by increased counts of CD4⁺ and CD8⁺ central memory T-cell subsets (especially CCR7⁺) which additionally show enhanced IL-10-based response activity towards pancreatitis-associated antigens (mediated *via* CD4⁺CD25^{high}FoxP3⁺CD127⁻)^[22,23].

The following selected experimental approaches showed an association between inflammatory members and HDACs during pathogenesis of AP and CP, using *in vitro* and *in vivo*-analysis with HDAC inhibitors.

In 2007, the group of Larsen *et al.*^[24] investigated

the possibly protective effect of HDAC inhibition on beta cells after cytokine-induced toxicity. They cultivated the INS-1 beta cell line and intact rat islets treated with the HDACis suberoylanilide hydroxamic acid (SAHA) or trichostatin A (TSA) in the absence or presence of IL-1 β and interferon (IFN)- γ . Based on insulin secretion, nitric oxide (NO) formation, inducible NO synthase (iNOS) levels and NF- κ B activity as well as viability and apoptosis, the authors could show that HDAC inhibition leads to cytokine-mediated decrease in insulin secretion, paralleled by reduced iNOS levels, NO formation and apoptosis. Furthermore, the IL-1 β -induced phosphorylation of the inhibitor protein kappa B α (I κ B α) was inhibited by HDACis. The authors concluded that application of HDACis had a preventive effect on cytokine-induced beta cell apoptosis and impaired beta cell function associated with a down-regulation of NF- κ B trans-activating activity.

In 2014, the group of Hartman *et al.*^[25] analyzed the role of HDAC in trypsin activation, inflammation, and tissue damage in severe acute pancreatitis. After induction of pancreatitis with taurocholic acid in C57Bl/6 mice, the effect of pretreatment with the HDAC inhibitor TSA on serum levels of amylase and IL-6 was determined as well as the pancreatic levels of macrophage inflammatory protein-2 (MIP-2), tissue morphology and myeloperoxidase activity, pro-inflammatory mediators, and trypsin activation in the pancreas and lungs. Using this experimental setting, the authors could demonstrate that pretreatment with TSA results in a significant decrease in amylase levels and a reduction of systemic IL-6 and pulmonary myeloperoxidase activity, as well as the taurocholate-induced gene expression of cyclooxygenase-2, MIP-2, MCP-1, IL-6, and IL-1 β in the pancreas. These findings

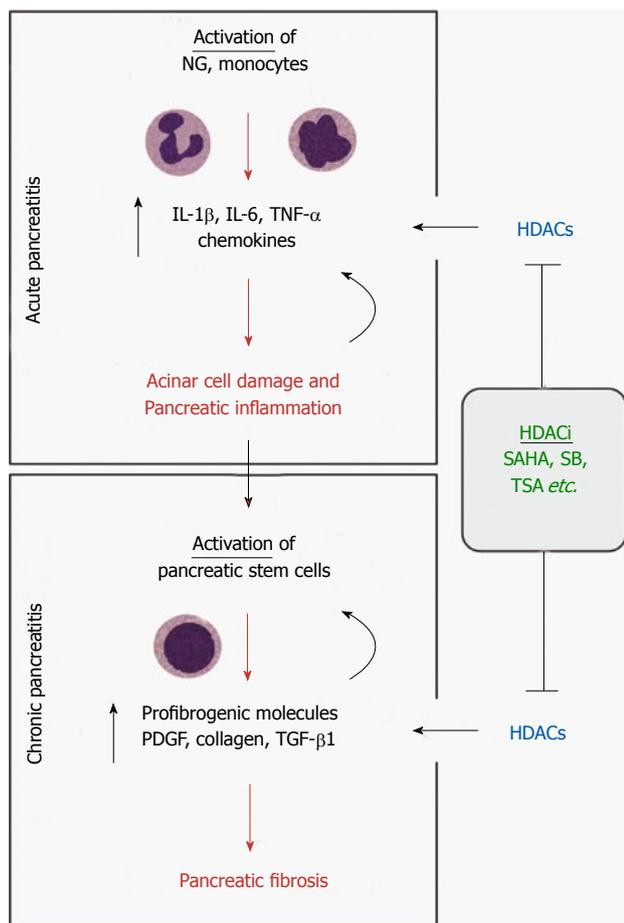


Figure 2 Histone deacetylases in acute and chronic pancreatitis. HDACs induce key pro-inflammatory mediators in AP and CP leading to destruction of pancreatic tissue with necrosis in case of AP and fibrosis/atrophy in case of CP. Inhibition of HDACs by HDACis was shown to significantly antagonize these effects *in vitro* and *in vivo*. HDAC: Histone deacetylases; HDACis: Histone deacetylases inhibitors; NG: Neutrophil granulocyte; IL: Interleukin; TNF: Tumor necrosis factor; PDGF: Platelet-derived growth factor; TGF: Transforming growth factor; SAHA: Suberoylanilide hydroxamic acid; SB: Sodium butyrate; TSA: Trichostatin A.

suggest that HDACs are involved in the pathogenetic process of AP such as inflammation and tissue damage.

Recently, the group of Kanika *et al.*^[26] studied the effect of HDAC inhibition on inflammation and fibrogenesis in L-Arginine(Arg)-induced pancreatitis and -associated fibrosis in Wistar rats. Looking at biochemical estimations, histological alterations, DNA damage, and the expression of various proteins, post-treatment with sodium butyrate (SB) decreased L-Arg-induced oxidative and nitrosative stress, DNA damage, histological alterations, and fibrosis. Interestingly, post-treatment with SB significantly decreased the expression of α -smooth muscle actin, IL-1 β iNOS, and 3-nitrotyrosine. Overall, the authors concluded that post-treatment with SB could alleviate L-Arg-induced pancreatic damage and fibrosis in rats^[26].

These findings are summarized in Figure 2: Taken together, the pre- or post-treatment of AP and CP with the three different HDAC inhibitory substances SAHA, TSA and SB resulted in a significant decrease

of inflammatory mediators in AP and CP with reduced disease progression compared to untreated controls. Interestingly, none of the mentioned experimental trials have carried out a sub-analysis of the HDAC classes and their members which could selectively be involved in this specific disease model. This approach could lead to the development of high selective HDAC-inhibitors to reduce systemic effects of pan-HDACis, because individual members of HDAC classes are specifically involved in the modulation of immune response in acute and chronic inflammatory diseases (reviewed in detail in^[14]).

HDAC EXPRESSION IN PANCREATIC TUMORS

The development from normal to cancerous cells is driven by complex modifications. Alternative pathways like epigenetic alterations become more and more interesting than progression models for mutations of different proto-oncogenes or tumor suppressor genes. One alternative way is the modification of histones by histone deacetylation. By removing acetyl groups from nucleosomes, histones, and non-histone proteins, HDACs do restrict the availability to access transcription factors or repressors^[27], implicating that over-expression of HDACs can lead to aberrant gene expression and carcinogenesis^[28].

Ductal adenocarcinoma of the pancreas, or simply called pancreatic cancer (PC), ranks among the most lethal of all malignancies in humans. In general, little is known about the role of HDACs in neoplasms derived from pancreatic endocrine and acinar cells; therefore the following paragraphs focus mainly on PC.

Recent studies revealed that under conditions of pancreatitis, adult exocrine acinar cells can differentiate and gain metaplastic ductal characteristics. This differentiation is also known as acinar-to-ductal metaplasia (ADM) and in mouse models, ADM is a precursor lesion of PC^[29,30]. Wauters *et al.*^[31] investigated the role of Sirtuine 1 (SIRT1) and its inhibition by Leptomycin B and nicotinamide in a mouse model and human pancreatic exocrine cell culture experiments. Localized in the nucleus of normal exocrine acinar cells, SIRT1 is inhibited by the protein deleted in breast cancer 1 (DBC1). In ADM, the co-localization of SIRT1 and DBC1 is disrupted and SIRT1 translocates into the cytoplasm, ending up in SIRT1-driven effects like cell differentiation and certain roles during multistage carcinogenesis^[32-34]. The Wnt/ β -catenin pathway plays an important role in embryonic acinar cell differentiation and Proliferation. Wauters *et al.*^[31] discovered that in pancreatic acini, SIRT1 is a regulator of the Wnt/ β -catenin signaling pathway and SIRT1 inhibition resulted in maintenance of Wnt/ β -Catenin signaling. In conclusion, Murtaugh *et al.*^[35] propose that in normal pancreas, DBC1 balances SIRT1 activity and acinar cells remain differentiated. In 2007, Nakagawa *et al.*^[36] investigated the expression profile of class I HDACs in human cancer tissues. Amongst others, they stained 20 PC samples with class

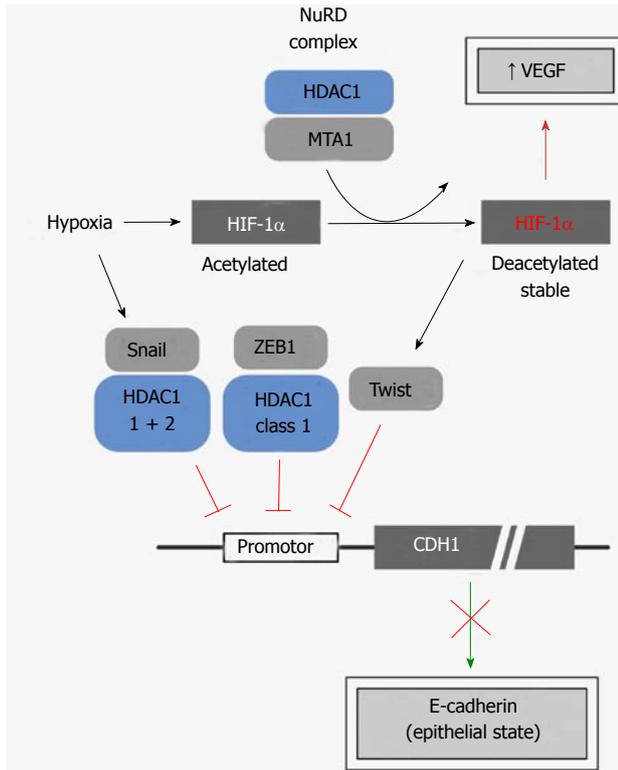


Figure 3 Histone deacetylase involvements in hypoxia inducible factor-1-mediated response to hypoxia. As suggested by Miyake *et al.*^[51], HIF-1 α is possibly regulated and stabilized by two subunits of the NuRD complex: HDAC1/MTA1. Stabilized HIF-1 α induces neo-angiogenesis by up-regulation of VEGF and, furthermore, contributes to EMT via Twist and subsequent inhibition of E-Cadherin expression (CDH1). Expression of E-Cadherin can be additionally repressed by complexes of either HDAC class I with ZEB1 or HDAC1 and 2 with Snail at the CDH1 promoter^[56]. HDAC: Histone deacetylases; HIF1: Hypoxia inducible factor-1; MTA1: Metastasis-associated protein 1; VEGF: Vascular endothelial growth factor; EMT: Epithelial-mesenchymal transition; ZEB1: Zinc finger E-box-binding homeobox 1.

I HDAC antibodies. Immunoreactivity was observable for HDAC1 in 17 PC (85%), for HDAC2 in 18 PC (90%), for HDAC3 in 20 PC (100%) and for HDAC 8 in 18 PC (90%) samples.

Lehmann *et al.*^[37] discovered a significant correlation between class I HDAC expression and an increased nuclear translocation of RelA/p65. RelA/p65 is a member of the NF κ B family transcription factors and a key regulator in pancreatic carcinogenesis. The NF κ B family is involved in the regulation of many genes which participate in functions like cell survival, proliferation, differentiation, and inflammation^[38]. In addition, Weichert *et al.*^[39] pointed out that high expression rates of RelA/p65 are correlated with the activation of the NF κ B pathway in PC. Furthermore, they linked their results on class I HDAC expression to higher tumor grades and poor prognosis.

It has been also reported that HDAC2 plays a role in therapeutic resistance in PC, since inhibition of HDAC2 leads to up-regulation of the BH3-only protein NOXA. This in turn makes PC cells vulnerable to etoposide-induced (topoisomerase II inhibitor) apoptosis as well as tumor necrosis factor-related apoptosis-inducing ligand

(TRAIL)-induced apoptosis^[40,41].

In addition, a relation between oncoproteins of the Myc family and HDAC2 up-regulation has been demonstrated: In PC, the c-Myc oncogene is highly expressed, whereas CCNG2 is under-expressed. CCNG2 is known to stop cell cycle progression by inducing G1/S phase cell cycle arrest^[42]. Marshall *et al.*^[43] showed that trichostatin A, a pan-HDACI, is able to improve CCNG2 expression and significantly elevates CCNG2 protein expression. On the contrary, they showed that transcriptional repression of CCNG2 contributes to N-Myc and HDAC2-induced cell proliferation. This suggests a potential benefit by using HDACIs in the treatment of PC as well.

In growing solid tumors, like PC, tumor cells experience specific microenvironmental conditions - in particular, a decreased oxygen level, called hypoxia^[44]. Hypoxia in the microenvironment of tumors can lead to radio/chemo-resistance and metastasis^[45-47]. Cellular response to hypoxia is controlled by many intracellular accumulating transcription factors, of which Hypoxia-inducible-factor-1 (HIF-1) plays an important role in the events induced by hypoxia^[44]. HIF-1 is composed by the HIF-1 α and HIF-1 β subunits^[48]. Denslow *et al.*^[49], Liu *et al.*^[50] and Miyake *et al.*^[51] showed that the expression of HDAC1 positively correlates with the expression of HIF-1 α and metastasis-associated protein 1 (MTA1) in PC and that the expression of HIF-1 α is possibly regulated through HDAC1/MTA1 subunits of the Nucleosome Remodeling Deacetylase (NuRD) complex, a HDAC containing repressor complex of proteins with the capability of ATP-dependent chromatin remodeling (Figure 3). As these changes are associated with poor prognosis, the inhibition of HDAC1 seems to be a promising therapeutic target^[51].

In recent studies, HDACs have been connected with epithelial-mesenchymal transition (EMT), a process that contributes to PC progression^[52]. EMT is described as a turning of tumor cells from an epithelial into a mesenchymal phenotype, thereby becoming more invasive - a process which can lead to the development of metastases^[53]. E-cadherin regulates metastasis of PC and is suppressed by a Snail/HDAC1/HDAC2 repressor complex. Similar to HIF-1, gene expression of *Snail* is upregulated by hypoxia^[54]. Von Burstin *et al.*^[55] showed that down-regulation of E-cadherin is associated with disorganization and loss of cell-cell adhesion in EMT and that inhibition of histone deacetylation seems to be one possibility to intervene E-cadherin down-regulation in PC. In cancer cells, E-cadherin is repressed by transcription repressors like Snail and Zinc finger E-box-binding homeobox 1 (ZEB1) which regulate the recruitment of HDAC1 and HDAC2 to the E-Cadherin promoter (Figure 3)^[56].

The ubiquitin-proteasome pathway regulates the degradation of intracellular proteins, including proteins which are involved in cell cycle regulation and differentiation. In order to survive, tumor cells are more dependent on the ubiquitin-proteasome pathway

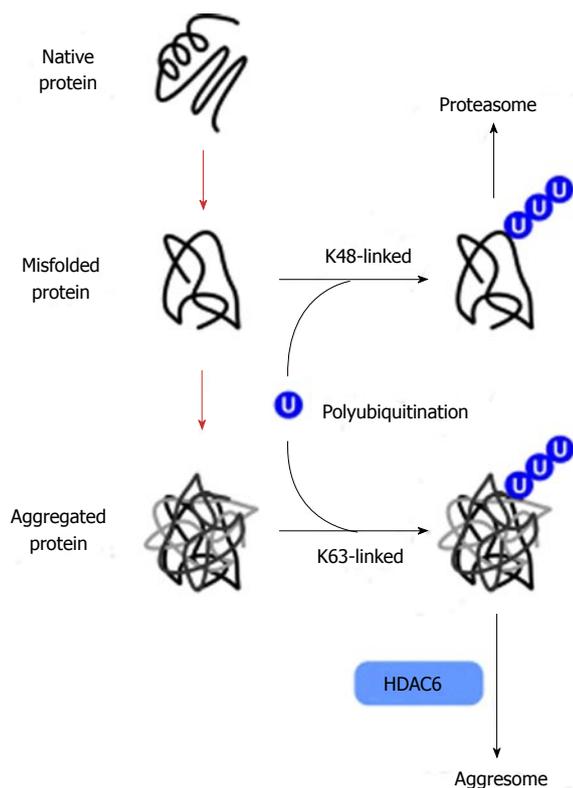


Figure 4 Role of histone deacetylase 6 in protein turnover. HDAC6 facilitates un-/misfolded protein degradation by recruiting ubiquitinated proteins to the aggresome or proteasome thus protecting tumor cells from apoptosis - see text for details^[58]. HDAC6: Histone deacetylase 6.

than healthy cells, because tumor cells show more accumulation of mis- or unfolded proteins than other cells^[57] - see also Figure 4. Aldana-Masangkay *et al.*^[58] detected that HDAC6 is able to bind ubiquitinated proteins and to activate the proteasome pathway. In consequence, HDAC6 protects tumor cells from apoptosis by helping to reduce the intercellular amount of mis- or unfolded proteins. As shown by Rodriguez-Gonzalez *et al.*^[57] HDAC6 inhibitors break up aggresomes, an aggregation of misfolded proteins, in PC. Furthermore, combination of HDAC6 and proteasome inhibitors increases proteasome-induced apoptosis in cancer cells (Figure 4)^[59]. Frankland-Searby *et al.*^[60] found that patients with a solid tumor like PC benefit from a combination of bortezomib (proteasome inhibitor) and a specific HDAC6 inhibitor.

The nerve growth factor IB, also known as Nur77, affects proliferation as well as apoptosis. *Nur77* gene encodes an orphan nuclear receptor that positively regulates antigen-induced apoptosis of thymocytes^[59]. HDAC7 was shown to be a key regulator in the negative selection of thymocytes and ensures down-regulation of the *Nur77* gene^[61]. Recently, Ouaissi *et al.*^[62] determined the expression pattern of *Nur77* gene simultaneously with the expression pattern of genes encoding for HDACs and SIRT1 in PC. They recognized an overexpression of *HDAC7* and *HDAC2* as well as *Nur77* in a significantly high percentage of PC compared

to benign tumors and chronic pancreatitis. Although the function of *Nur77* seems to be divergent and therefore further studies are needed to clarify the involvement of the *HDAC7/HDAC2/Nur77* axis in the pathogenesis of PC, those findings suggest new approaches in the design of anti-PC therapy^[62].

In summary, especially class I and II HDACs influence events involved in pancreatic cancerogenesis. Significant correlations of the NFκB-family member RelA/p65 and class I HDACs imply possible effects on functions like cell survival, proliferation, differentiation, and inflammation, which all play a role in cancerogenesis^[37,38]. Primarily class I HDACs show importance in the regulation of apoptosis and cell cycle in mainly three different ways: (1) inhibition of HDACs (HDAC2 and 7) induces up-regulation of BH-3 only protein *NOXA*, *CCNG2* gene expression and *Nur77*^[41,43,61]; (2) moreover, HDACs are involved in EMT of PC tumor cells *via* the *Snail/HDAC1/HDAC2* complex that suppresses E-Cadherin expression; and (3) in the oxygenation of PC microenvironment by regulating the expression *HIF-1α* through *HDAC1/MTA1*^[51,55]. All these findings suggest that HDAC inhibitors (HDACi) would interfere with cancerogenesis in PC on different points and are therefore a highly promising tool in anti-PC therapy.

HDAC-INHIBITORS: FROM THE BENCH TO THE BED

The development of HDACis as therapeutics for chronic diseases and cancer arose from the functional understanding of the underlying dys-regulation of HDACs. The acetylation status of histones is controlled by the opposing actions of two enzyme classes, the histone acetyltransferases (HATs), which transfer acetyl groups to lysine residues within the N-terminal tails of core histones, and the HDACs which remove the acetyl groups^[63]. Histone hyperacetylation is associated with transcriptional activity. The rate of regulation and affection through HDACis lies by 20% of all known genes, whereof almost one half is down-regulated and the other half is up-regulated^[64].

The family of HDACis includes naturally occurring and synthetically generated compounds which target the HDAC enzyme family. These compounds vary in their chemical structure, their biological activity, and their specificity. There are two HDACis - vorinostat (Zolinza[®]) and romidepsin (Istodax[®]) - which have received approval from the United States Food and Drug Administration (FDA) for treatment of cutaneous T-cell lymphoma (CTCL). Romidepsin also got approved for the treatment of peripheral T-cell lymphoma^[65,66].

The HDACis can be grouped by their structure into hydroxamic acid, cyclic peptide, bibenzimide, and short-chain fatty acid group (Table 1). The group of hydroxamates (vorinostat, givinostat, abexinostat, panobinostat, belinostat, and trachostatinA) exerts nonspecific HDAC inhibition by affecting all classes of

Table 1 Overview of histone deacetylase inhibitors based on their structure, class specificity; current clinical trials and suggested therapeutic effects^[63,77,84-88]

Structure class	HDAC class specificity	HDAC inhibitor	Clinical trials	Effects
Hydroxamic acid	I, II, IV	Trichostatin A	-	-
		Quisinostat	Phase I	-
		Vorinostat	FDA approved (2006), phase II, III	Vorinostat significantly sensitized pancreatic cancer cells for radiotherapy
		Panobinostat	Phase II, III	Panobinostat induced the death of pancreatic tumor cell by apoptosis
		Resminostat	Phase I, II	-
		Abexinostat	-	-
I, II	I, II	Belinostat	-	-
		Givinostat	-	The orally active HDAC inhibitor ITF2357 (givinostat) favors β -cell survival during inflammatory conditions
Cyclic peptide	I	Depsipeptide	FDA approved (2009), phase I, II	-
Benzamides	I HDAC1	Entinostat	Phase II	-
		Mocetinostat	Phase I, II	Mocetinostat + gemcitabine might be an effective treatment for gemcitabine-refractory pancreatic cancer
Fatty acid	I, II	Valporic acid	Phase I, II, III	Valporic acid may protect β -cells from palmitate-induced apoptosis and ER stress via GSK-3 β inhibition, independent of ATF4/CHOP pathway
		Butyrate	Phase II	Butyrate regulates both the survival and replication of human β -cells

HDAC: Histone deacetylase; FDA: Food and Drug Administration.

HDACs^[67,68].

The group of cyclic peptides includes compounds like depsipeptide (romidespin) and trapoxin. The benzamides include entinostat and mocetinostat. The hydroxamates, cyclic peptides and benzamides have potent inhibition properties in the nanomolar range. HDAC isotype-selective inhibitors like tubacin, mocetinostat and PC-34501 inhibit HDAC6; in addition HDAC1 and 8 are also becoming available^[69,70].

It is a current topic of discussion whether to choose a broad-spectrum HDACi or a class specific HDACi. Furthermore, there are emerging hypotheses about the combination of HDACis with other signaling compounds like miRNA inhibition, in order to obtain better inhibition outputs^[6].

The response to HDACis is complex and involves transcriptional effects as well as non-transcriptional effects in the cell: Lee *et al.*^[71] summarized the multi-modal effects through HDACis including apoptosis, cell-cycle arrest, necrosis, autophagy, differentiation, and migration. Normal cells are up to ten times more resistant to HDACi-induced cell death than transformed cells. As an example, they described that vorinostat induced DNA double strand breaks (DSB) in normal and transformed cells in the cell culture, but normal cells were able to repair the DSB without almost any loss in viability^[71].

In pancreatic cell lines, HDACis were shown to be potent anticancer drugs as single compounds but also as adjuvant drugs when combined with DNA-damaging agents, ionizing radiation or other approaches such as silencing through small interfering RNA^[72,73]. Vincent *et al.*^[74] showed that Drosophila Eyes Absent Homologue-2 (*EYA2*) is silenced in the majority of PC and investigated the role of epigenetic mechanisms of *EYA2* gene silencing in pancreatic cancers. Knockdown of *EYA2* increased cell proliferation in pancreatic cancer cell lines. Silencing of *EYA2* expression in pancreatic cancer cell lines correlated with histone deacetylation and was reversible with HDACis.

Peulen *et al.*^[75] described that HDAC inhibition in human pancreas cell lines with chemical inhibitors (SAHA, MS-275 and celecoxib) significantly impaired proliferation of a human pancreatic cell line (BxPC-3 cells) *in vitro*.

Yee *et al.*^[76] showed in human pancreatic adenocarcinoma cells that the combination of the HDACi suberoylanilide hydroxamic acid (SAHA) and ML-60218 (inhibitor of RNA polymerase III) led to suppression of colony formation and proliferation, cell cycle arrest, and apoptotic cell death. The enhanced cytotoxicity was accompanied by up-regulation of the pro-apoptotic regulator BAX and the cyclin-dependent kinase inhibitor p21 (CDKN1A).

Mhedi *et al.*^[77] examined human pancreatic cancer cell lines (Panc-1, BxPC-3, SOJ-6) and an immortalized epithelial cell line of a normal human pancreatic duct (HPDE/E6E7): A significant variation in HDACs and SIRT6 protein expression levels was seen among individual cell samples. The *in vivo* results showed that panobinostat

(LBH589) exhibited a tumor reduction efficacy similar to the chemotherapeutic drug gemcitabine. In line with its *in vitro* activity, panobinostat also achieved a significant reduction of tumor growth in a BxPC-3 pancreatic tumor cell line subcutaneous xenograft mouse model^[77].

In a xenograft model of pancreatic cancer, Lee *et al.*^[78] tested the effects of combined (vorinostat) SAHA and bortezomib treatment with or without gemcitabine on cell growth, apoptosis and expression of related proteins. The triple combination of vorinostat, bortezomib, and gemcitabine resulted in the strongest antitumor effects *in vitro*.

Currently, there are 7 clinical trials concerning HDACis in PC^[79]. In general, there are more than 80 clinical trials investigating more than eleven different HDACis in solid and hematological malignancies, either as mono-therapies or in combination with other antitumor agents^[63].

Vorinostat

The FDA approval for vorinostat was given after two phase II clinical trials in CTCL patients. Vorinostat showed similar effects as standard therapy in CTCL patients, but with a higher relief from pruritus. It was well tolerated with some adverse effects like diarrhea, fatigue and nausea. The response rates in solid cancer like breast, colorectal or lung cancer were poor. The use as a single agent has been unsuccessful, but the combination with conventional cancer agents seemed to be highly beneficial^[78,80,81].

Depsipeptide

The bicyclic peptide is connected with potent cytotoxic effects *in vitro* and *in vivo*. Depsipetide was tested in a range of clinical trials (phase I/II/III) in colorectal, renal, breast neoplasms as well as hematological malignancies; and showed limited activity as monotherapy in acute myeloid leukemia and myelodysplastic syndrome^[82,83].

CONCLUSION

The pancreas plays a key role in the exocrine and endocrine functional integrity of the organism which is severely affected by processes like acute or chronic inflammation as well as cancerogenesis. It is clear today that epigenetic regulators, such as HDACs are involved in development and progression of pancreatic diseases as shown during the last years in diverse *in vitro* and *in vivo* models. In this review, we investigated current literature to comprehensively summarize the role of HDACs in AP and CP as well as in PC. HDACs are overly expressed in PC and are associated with EMT, angiogenesis, and consequently with poor prognosis. HDACis were shown to have multifariously anti-tumor effects in PC, especially in combination with standard chemotherapeutics. Based on the data presented in this review, targeting HDACs can be a promising therapeutic option for treatment of PC and should be prospectively

assessed in future clinical trials.

REFERENCES

- 1 **Chandra R**, Liddle RA. Recent advances in the regulation of pancreatic secretion. *Curr Opin Gastroenterol* 2014; **30**: 490-494 [PMID: 25003603 DOI: 10.1097/MOG.000000000000099]
- 2 **Binker MG**, Cosen-Binker LI. Acute pancreatitis: the stress factor. *World J Gastroenterol* 2014; **20**: 5801-5807 [PMID: 24914340 DOI: 10.3748/wjg.v20.i19.5801]
- 3 **Maeda H**, Hanazaki K. Pancreatogenic diabetes after pancreatic resection. *Pancreatology* 2011; **11**: 268-276 [PMID: 21734430 DOI: 10.1159/000328785]
- 4 **Ohtsuka T**, Tanaka M, Miyazaki K. Gastrointestinal function and quality of life after pylorus-preserving pancreatoduodenectomy. *J Hepatobiliary Pancreat Surg* 2006; **13**: 218-224 [PMID: 16708298 DOI: 10.1007/s00534-005-1067-z]
- 5 **Neureiter D**, Jäger T, Ocker M, Kiesslich T. Epigenetics and pancreatic cancer: pathophysiology and novel treatment aspects. *World J Gastroenterol* 2014; **20**: 7830-7848 [PMID: 24976721 DOI: 10.3748/wjg.v20.i24.7830]
- 6 **Swierczynski S**, Klieser E, Illig R, Alinger-Scharinger B, Kiesslich T, Neureiter D. Histone deacetylation meets miRNA: epigenetics and post-transcriptional regulation in cancer and chronic diseases. *Expert Opin Biol Ther* 2015; **15**: 651-664 [PMID: 25766312 DOI: 10.1517/14712598.2015.1025047]
- 7 **Egger G**, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004; **429**: 457-463 [PMID: 15164071 DOI: 10.1038/nature02625]
- 8 **Strahl BD**, Allis CD. The language of covalent histone modifications. *Nature* 2000; **403**: 41-45 [PMID: 10638745 DOI: 10.1038/47412]
- 9 **Jenuwein T**, Allis CD. Translating the histone code. *Science* 2001; **293**: 1074-1080 [PMID: 11498575 DOI: 10.1126/science.1063127]
- 10 **Tang J**, Yan H, Zhuang S. Histone deacetylases as targets for treatment of multiple diseases. *Clin Sci (Lond)* 2013; **124**: 651-662 [PMID: 23414309 DOI: 10.1042/CS20120504]
- 11 **Haumaitre C**, Lenoir O, Scharfmann R. Directing cell differentiation with small-molecule histone deacetylase inhibitors: the example of promoting pancreatic endocrine cells. *Cell Cycle* 2009; **8**: 536-544 [PMID: 19197155]
- 12 **Woan KV**, Sahakian E, Sotomayor EM, Seto E, Villagra A. Modulation of antigen-presenting cells by HDAC inhibitors: implications in autoimmunity and cancer. *Immunol Cell Biol* 2012; **90**: 55-65 [PMID: 22105512 DOI: 10.1038/icb.2011.96]
- 13 **Dekker FJ**, van den Bosch T, Martin NI. Small molecule inhibitors of histone acetyltransferases and deacetylases are potential drugs for inflammatory diseases. *Drug Discov Today* 2014; **19**: 654-660 [PMID: 24269836 DOI: 10.1016/j.drudis.2013.11.012]
- 14 **Shakespeare MR**, Halili MA, Irvine KM, Fairlie DP, Sweet MJ. Histone deacetylases as regulators of inflammation and immunity. *Trends Immunol* 2011; **32**: 335-343 [PMID: 21570914 DOI: 10.1016/j.it.2011.04.001]
- 15 **Suárez-Álvarez B**, Baragaño Raneros A, Ortega F, López-Larrea C. Epigenetic modulation of the immune function: a potential target for tolerance. *Epigenetics* 2013; **8**: 694-702 [PMID: 23803720 DOI: 10.4161/epi.25201]
- 16 **Thrower E**, Husain S, Gorelick F. Molecular basis for pancreatitis. *Curr Opin Gastroenterol* 2008; **24**: 580-585 [PMID: 19122498 DOI: 10.1097/MOG.0b013e32830b10e6]
- 17 **Zheng L**, Xue J, Jaffee EM, Habtezion A. Role of immune cells and immune-based therapies in pancreatitis and pancreatic ductal adenocarcinoma. *Gastroenterology* 2013; **144**: 1230-1240 [PMID: 23622132 DOI: 10.1053/j.gastro.2012.12.042]
- 18 **Gukovskiy I**, Li N, Todoric J, Gukovskaya A, Karin M. Inflammation, autophagy, and obesity: common features in the pathogenesis of pancreatitis and pancreatic cancer. *Gastroenterology* 2013; **144**: 1199-209.e4 [PMID: 23622129 DOI: 10.1053/j.gastro.2013.02.007]
- 19 **Makhija R**, Kingsnorth AN. Cytokine storm in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2002; **9**: 401-410 [PMID: 12483260 DOI: 10.1007/s005340200049]

- 20 **Omary MB**, Lugea A, Lowe AW, Pandolfi SJ. The pancreatic stellate cell: a star on the rise in pancreatic diseases. *J Clin Invest* 2007; **117**: 50-59 [PMID: 17200706 DOI: 10.1172/JCI30082]
- 21 **Treiber M**, Neuhöfer P, Anetsberger E, Einwächter H, Lesina M, Rickmann M, Liang S, Kehl T, Nakhai H, Schmid RM, Algül H. Myeloid, but not pancreatic, RelA/p65 is required for fibrosis in a mouse model of chronic pancreatitis. *Gastroenterology* 2011; **141**: 1473-1485, 1485.e1-e7 [PMID: 21763242 DOI: 10.1053/j.gastro.2011.06.087]
- 22 **Schmitz-Winnenthal H**, Pietsch DH, Schimmack S, Bonertz A, Udonta F, Ge Y, Galindo L, Specht S, Volk C, Zgraggen K, Koch M, Büchler MW, Weitz J, Beckhove P. Chronic pancreatitis is associated with disease-specific regulatory T-cell responses. *Gastroenterology* 2010; **138**: 1178-1188 [PMID: 19931255 DOI: 10.1053/j.gastro.2009.11.011]
- 23 **Grundsten M**, Liu GZ, Permert J, Hjelmstrom P, Tsai JA. Increased central memory T cells in patients with chronic pancreatitis. *Pancreatology* 2005; **5**: 177-182 [PMID: 15849488 DOI: 10.1159/000085269]
- 24 **Larsen L**, Tonnesen M, Ronn SG, Størling J, Jørgensen S, Mascagni P, Dinarello CA, Billestrup N, Mandrup-Poulsen T. Inhibition of histone deacetylases prevents cytokine-induced toxicity in beta cells. *Diabetologia* 2007; **50**: 779-789 [PMID: 17265033 DOI: 10.1007/s00125-006-0562-3]
- 25 **Hartman H**, Wetterholm E, Thorlacius H, Regnér S. Histone deacetylase regulates trypsin activation, inflammation, and tissue damage in acute pancreatitis in mice. *Dig Dis Sci* 2015; **60**: 1284-1289 [PMID: 25492506 DOI: 10.1007/s10620-014-3474-y]
- 26 **Kanika G**, Khan S, Jena G. Sodium Butyrate Ameliorates L-Arginine-Induced Pancreatitis and Associated Fibrosis in Wistar Rat: Role of Inflammation and Nitrosative Stress. *J Biochem Mol Toxicol* 2015; **29**: 349-359 [PMID: 25774002 DOI: 10.1002/jbt.21698]
- 27 **Haberland M**, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. *Nat Rev Genet* 2009; **10**: 32-42 [PMID: 19065135 DOI: 10.1038/nrg2485]
- 28 **Kouzarides T**. Histone acetylases and deacetylases in cell proliferation. *Curr Opin Genet Dev* 1999; **9**: 40-48 [PMID: 10072350]
- 29 **Rooman I**, Real FX. Pancreatic ductal adenocarcinoma and acinar cells: a matter of differentiation and development? *Gut* 2012; **61**: 449-458 [PMID: 21730103 DOI: 10.1136/gut.2010.235804]
- 30 **Pérez-Mancera PA**, Guerra C, Barbacid M, Tuveson DA. What we have learned about pancreatic cancer from mouse models. *Gastroenterology* 2012; **142**: 1079-1092 [PMID: 22406637 DOI: 10.1053/j.gastro.2012.03.002]
- 31 **Wauters E**, Sanchez-Arévalo Lobo VJ, Pinho AV, Mawson A, Herranz D, Wu J, Cowley MJ, Colvin EK, Njicop EN, Sutherland RL, Liu T, Serrano M, Bouwens L, Real FX, Biankin AV, Rooman I. Sirtuin-1 regulates acinar-to-ductal metaplasia and supports cancer cell viability in pancreatic cancer. *Cancer Res* 2013; **73**: 2357-2367 [PMID: 23370328 DOI: 10.1158/0008-5472.CAN-12-3359]
- 32 **Strobel O**, Dor Y, Alsina J, Stirman A, Lauwers G, Trainor A, Castillo CF, Warsaw AL, Thayer SP. In vivo lineage tracing defines the role of acinar-to-ductal transdifferentiation in inflammatory ductal metaplasia. *Gastroenterology* 2007; **133**: 1999-2009 [PMID: 18054571 DOI: 10.1053/j.gastro.2007.09.009]
- 33 **Nin V**, Escande C, Chini CC, Giri S, Camacho-Pereira J, Matalonga J, Lou Z, Chini EN. Role of deleted in breast cancer 1 (DBC1) protein in SIRT1 deacetylase activation induced by protein kinase A and AMP-activated protein kinase. *J Biol Chem* 2012; **287**: 23489-23501 [PMID: 22553202 DOI: 10.1074/jbc.M112.365874]
- 34 **Song NY**, Surh YJ. Janus-faced role of SIRT1 in tumorigenesis. *Ann N Y Acad Sci* 2012; **1271**: 10-19 [PMID: 23050959 DOI: 10.1111/j.1749-6632.2012.06762.x]
- 35 **Murtaugh LC**, Law AC, Dor Y, Melton DA. Beta-catenin is essential for pancreatic acinar but not islet development. *Development* 2005; **132**: 4663-4674 [PMID: 16192304 DOI: 10.1242/dev.02063]
- 36 **Nakagawa M**, Oda Y, Eguchi T, Aishima S, Yao T, Hosoi F, Basaki Y, Ono M, Kuwano M, Tanaka M, Tsuneyoshi M. Expression profile of class I histone deacetylases in human cancer tissues. *Oncol Rep* 2007; **18**: 769-774 [PMID: 17786334]
- 37 **Lehmann A**, Denkert C, Budczies J, Buckendahl AC, Darb-Esfahani S, Noske A, Müller BM, Bahra M, Neuhaus P, Dietel M, Kristiansen G, Weichert W. High class I HDAC activity and expression are associated with RelA/p65 activation in pancreatic cancer in vitro and in vivo. *BMC Cancer* 2009; **9**: 395 [PMID: 19912635 DOI: 10.1186/1471-2407-9-395]
- 38 **van Essen D**, Engist B, Natoli G, Saccani S. Two modes of transcriptional activation at native promoters by NF-kappaB p65. *PLoS Biol* 2009; **7**: e73 [PMID: 19338389 DOI: 10.1371/journal.pbio.1000073]
- 39 **Weichert W**, Boehm M, Gekeler V, Bahra M, Langrehr J, Neuhaus P, Denkert C, Imre G, Weller C, Hofmann HP, Niesporek S, Jacob J, Dietel M, Scheiderei C, Kristiansen G. High expression of RelA/p65 is associated with activation of nuclear factor-kappaB-dependent signaling in pancreatic cancer and marks a patient population with poor prognosis. *Br J Cancer* 2007; **97**: 523-530 [PMID: 17622249 DOI: 10.1038/sj.bjc.6603878]
- 40 **Fritsche P**, Seidler B, Schüler S, Schnieke A, Göttlicher M, Schmid RM, Saur D, Schneider G. HDAC2 mediates therapeutic resistance of pancreatic cancer cells via the BH3-only protein NOXA. *Gut* 2009; **58**: 1399-1409 [PMID: 19528037 DOI: 10.1136/gut.2009.180711]
- 41 **Schüler S**, Fritsche P, Diersch S, Arlt A, Schmid RM, Saur D, Schneider G. HDAC2 attenuates TRAIL-induced apoptosis of pancreatic cancer cells. *Mol Cancer* 2010; **9**: 80 [PMID: 20398369 DOI: 10.1186/1476-4598-9-80]
- 42 **Bennin DA**, Don AS, Brake T, McKenzie JL, Rosenbaum H, Ortiz L, DePaoli-Roach AA, Horne MC. Cyclin G2 associates with protein phosphatase 2A catalytic and regulatory B' subunits in active complexes and induces nuclear aberrations and a G1/S phase cell cycle arrest. *J Biol Chem* 2002; **277**: 27449-27467 [PMID: 11956189 DOI: 10.1074/jbc.M111693200]
- 43 **Marshall GM**, Gherardi S, Xu N, Neiron Z, Trahair T, Scarlett CJ, Chang DK, Liu PY, Jankowski K, Iraci N, Haber M, Norris MD, Keating J, Sekyere E, Jonquieres G, Stossi F, Katzenellenbogen BS, Biankin AV, Perini G, Liu T. Transcriptional upregulation of histone deacetylase 2 promotes Myc-induced oncogenic effects. *Oncogene* 2010; **29**: 5957-5968 [PMID: 20697349 DOI: 10.1038/onc.2010.332]
- 44 **Nurwidya F**, Takahashi F, Minakata K, Murakami A, Takahashi K. From tumor hypoxia to cancer progression: the implications of hypoxia-inducible factor-1 expression in cancers. *Anat Cell Biol* 2012; **45**: 73-78 [PMID: 22822460 DOI: 10.5115/acb.2012.45.2.73]
- 45 **Xing F**, Okuda H, Watabe M, Kobayashi A, Pai SK, Liu W, Pandey PR, Fukuda K, Hirota S, Sugai T, Wakabayashi G, Koeda K, Kashiwaba M, Suzuki K, Chiba T, Endo M, Mo YY, Watabe K. Hypoxia-induced Jagged2 promotes breast cancer metastasis and self-renewal of cancer stem-like cells. *Oncogene* 2011; **30**: 4075-4086 [PMID: 21499308 DOI: 10.1038/onc.2011.122]
- 46 **Raval RR**, Lau KW, Tran MG, Sowter HM, Mandriota SJ, Li JL, Pugh CW, Maxwell PH, Harris AL, Ratcliffe PJ. Contrasting properties of hypoxia-inducible factor 1 (HIF-1) and HIF-2 in von Hippel-Lindau-associated renal cell carcinoma. *Mol Cell Biol* 2005; **25**: 5675-5686 [PMID: 15964822 DOI: 10.1128/MCB.25.13.5675-5686.2005]
- 47 **Huang LE**, Bindra RS, Glazer PM, Harris AL. Hypoxia-induced genetic instability--a calculated mechanism underlying tumor progression. *J Mol Med (Berl)* 2007; **85**: 139-148 [PMID: 17180667 DOI: 10.1007/s00109-006-0133-6]
- 48 **Semenza GL**. Hydroxylation of HIF-1: oxygen sensing at the molecular level. *Physiology (Bethesda)* 2004; **19**: 176-182 [PMID: 15304631 DOI: 10.1152/physiol.00001.2004]
- 49 **Denslow SA**, Wade PA. The human Mi-2/NuRD complex and gene regulation. *Oncogene* 2007; **26**: 5433-5438 [PMID: 17694084 DOI: 10.1038/sj.onc.1210611]
- 50 **Liu Y**, Long Y, Xing Z, Zhang D. C-Jun recruits the NSL complex to regulate its target gene expression by modulating H4K16

- acetylation and promoting the release of the repressive NuRD complex. *Oncotarget* 2015; **6**: 14497-14506 [PMID: 25971333]
- 51 **Miyake K**, Yoshizumi T, Imura S, Sugimoto K, Batmunkh E, Kanemura H, Morine Y, Shimada M. Expression of hypoxia-inducible factor-1 α , histone deacetylase 1, and metastasis-associated protein 1 in pancreatic carcinoma: correlation with poor prognosis with possible regulation. *Pancreas* 2008; **36**: e1-e9 [PMID: 18362831 DOI: 10.1097/MPA.0b013e31815f2c2a]
- 52 **Krantz SB**, Shields MA, Dangi-Garimella S, Munshi HG, Bentrem DJ. Contribution of epithelial-to-mesenchymal transition and cancer stem cells to pancreatic cancer progression. *J Surg Res* 2012; **173**: 105-112 [PMID: 22099597 DOI: 10.1016/j.jss.2011.09.020]
- 53 **Yang J**, Weinberg RA. Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell* 2008; **14**: 818-829 [PMID: 18539112 DOI: 10.1016/j.devcel.2008.05.009]
- 54 **Barrallo-Gimeno A**, Nieto MA. The Snail genes as inducers of cell movement and survival: implications in development and cancer. *Development* 2005; **132**: 3151-3161 [PMID: 15983400 DOI: 10.1242/dev.01907]
- 55 **von Burstin J**, Eser S, Paul MC, Seidler B, Brandl M, Messer M, von Werder A, Schmidt A, Mages J, Pagel P, Schnieke A, Schmid RM, Schneider G, Saur D. E-cadherin regulates metastasis of pancreatic cancer in vivo and is suppressed by a SNAIL/HDAC1/HDAC2 repressor complex. *Gastroenterology* 2009; **137**: 361-371, 371.e1-e5 [PMID: 19362090 DOI: 10.1053/j.gastro.2009.04.004]
- 56 **Aghdassi A**, Sandler M, Guenther A, Mayerle J, Behn CO, Heidecke CD, Friess H, Büchler M, Evert M, Lerch MM, Weiss FU. Recruitment of histone deacetylases HDAC1 and HDAC2 by the transcriptional repressor ZEB1 downregulates E-cadherin expression in pancreatic cancer. *Gut* 2012; **61**: 439-448 [PMID: 22147512 DOI: 10.1136/gutjnl-2011-300060]
- 57 **Rodriguez-Gonzalez A**, Lin T, Ikeda AK, Simms-Waldrup T, Fu C, Sakamoto KM. Role of the aggresome pathway in cancer: targeting histone deacetylase 6-dependent protein degradation. *Cancer Res* 2008; **68**: 2557-2560 [PMID: 18413721 DOI: 10.1158/0008-5472.CAN-07-5989]
- 58 **Aldana-Masangkay GI**, Sakamoto KM. The role of HDAC6 in cancer. *J Biomed Biotechnol* 2011; **2011**: 875824 [PMID: 21076528 DOI: 10.1155/2011/875824]
- 59 **Wu Q**, Li Y, Liu R, Agadir A, Lee MO, Liu Y, Zhang X. Modulation of retinoic acid sensitivity in lung cancer cells through dynamic balance of orphan receptors nur77 and COUP-TF and their heterodimerization. *EMBO J* 1997; **16**: 1656-1669 [PMID: 9130711 DOI: 10.1093/emboj/16.7.1656]
- 60 **Frankland-Searby S**, Bhaumik SR. The 26S proteasome complex: an attractive target for cancer therapy. *Biochim Biophys Acta* 2012; **1825**: 64-76 [PMID: 22037302 DOI: 10.1016/j.bbcan.2011.10.003]
- 61 **Dequiedt F**, Kasler H, Fischle W, Kiermer V, Weinstein M, Herdier BG, Verdin E. HDAC7, a thymus-specific class II histone deacetylase, regulates Nur77 transcription and TCR-mediated apoptosis. *Immunity* 2003; **18**: 687-698 [PMID: 12753745]
- 62 **Ouaïssi M**, Silvy F, Loncle C, Ferraz da Silva D, Martins Abreu C, Martinez E, Berthéze P, Cadra S, Le Treut YP, Hardwigen J, Sastre B, Sielezoeff I, Benkoel L, Delgrande J, Ouaïssi A, Iovanna J, Lombardo D, Mas E. Further characterization of HDAC and SIRT gene expression patterns in pancreatic cancer and their relation to disease outcome. *PLoS One* 2014; **9**: e108520 [PMID: 25275504 DOI: 10.1371/journal.pone.0108520]
- 63 **Ververis K**, Hiong A, Karagiannis TC, Licciardi PV. Histone deacetylase inhibitors (HDACIs): multitargeted anticancer agents. *Biologics* 2013; **7**: 47-60 [PMID: 23459471 DOI: 10.2147/BTT.S29965]
- 64 **Minucci S**, Pelicci PG. Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nat Rev Cancer* 2006; **6**: 38-51 [PMID: 16397526 DOI: 10.1038/nrc1779]
- 65 **Marks PA**, Breslow R. Dimethyl sulfoxide to vorinostat: development of this histone deacetylase inhibitor as an anticancer drug. *Nat Biotechnol* 2007; **25**: 84-90 [PMID: 17211407 DOI: 10.1038/nbt1272]
- 66 **Campas-Moya C**. Romidepsin for the treatment of cutaneous T-cell lymphoma. *Drugs Today (Barc)* 2009; **45**: 787-795 [PMID: 20126671 DOI: 10.1358/dot.2009.45.11.1437052]
- 67 **Marks PA**. The clinical development of histone deacetylase inhibitors as targeted anticancer drugs. *Expert Opin Investig Drugs* 2010; **19**: 1049-1066 [PMID: 20687783 DOI: 10.1517/13543784.2010.510514]
- 68 **Schneider-Stock R**, Ocker M. Epigenetic therapy in cancer: molecular background and clinical development of histone deacetylase and DNA methyltransferase inhibitors. *IDrugs* 2007; **10**: 557-561 [PMID: 17665331]
- 69 **Parmigiani RB**, Xu WS, Venta-Perez G, Erdjument-Bromage H, Yaneva M, Tempst P, Marks PA. HDAC6 is a specific deacetylase of peroxiredoxins and is involved in redox regulation. *Proc Natl Acad Sci USA* 2008; **105**: 9633-9638 [PMID: 18606987 DOI: 10.1073/pnas.0803749105]
- 70 **Tang W**, Luo T, Greenberg EF, Bradner JE, Schreiber SL. Discovery of histone deacetylase 8 selective inhibitors. *Bioorg Med Chem Lett* 2011; **21**: 2601-2605 [PMID: 21334896 DOI: 10.1016/j.bmcl.2011.01.134]
- 71 **Lee JH**, Marks PA. Histone deacetylase inhibitors in the therapy of cancer: much to learn. *Epigenomics* 2010; **2**: 723-725 [PMID: 22122077 DOI: 10.2217/epi.10.59]
- 72 **Fogg PC**, O'Neill JS, Dobrzycki T, Calvert S, Lord EC, McIntosh RL, Elliott CJ, Sweeney ST, Hastings MH, Chawla S. Class IIa histone deacetylases are conserved regulators of circadian function. *J Biol Chem* 2014; **289**: 34341-34348 [PMID: 25271152 DOI: 10.1074/jbc.M114.606392]
- 73 **Neureiter D**, Zopf S, Leu T, Dietze O, Hauser-Kronberger C, Hahn EG, 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 [PMID: 17190770 DOI: 10.1080/00365520600874198]
- 74 **Vincent A**, Hong SM, Hu C, Omura N, Young A, Kim H, Yu J, Knight S, Ayars M, Griffith M, Van Seuning I, Maitra A, Goggins M. Epigenetic silencing of EYA2 in pancreatic adenocarcinomas promotes tumor growth. *Oncotarget* 2014; **5**: 2575-2587 [PMID: 24810906]
- 75 **Peulen O**, Gonzalez A, Peixoto P, Turtoi A, Mottet D, Delvenne P, Castronovo V. The anti-tumor effect of HDAC inhibition in a human pancreas cancer model is significantly improved by the simultaneous inhibition of cyclooxygenase 2. *PLoS One* 2013; **8**: e75102 [PMID: 24040391 DOI: 10.1371/journal.pone.0075102]
- 76 **Yee NS**, Zhou W, Chun SG, Liang IC, Yee RK. Targeting developmental regulators of zebrafish exocrine pancreas as a therapeutic approach in human pancreatic cancer. *Biol Open* 2012; **1**: 295-307 [PMID: 23213420 DOI: 10.1242/bio.2012539]
- 77 **Mehdi O**, Françoise S, Sofia CL, Urs G, Kevin Z, Bernard S, Igor S, Anabela CD, Dominique L, Eric M, Ali O. HDAC gene expression in pancreatic tumor cell lines following treatment with the HDAC inhibitors panobinostat (LBH589) and trichostatin (TSA). *Pancreatol* 2012; **12**: 146-155 [PMID: 22487525 DOI: 10.1016/j.pan.2012.02.013]
- 78 **Lee JK**, Ryu JK, Yang KY, Woo SM, Park JK, Yoon WJ, Lee SH, Jeong KS, Kim YT, Yoon YB. Effects and mechanisms of the combination of suberoylanilide hydroxamic acid and bortezomib on the anticancer property of gemcitabine in pancreatic cancer. *Pancreas* 2011; **40**: 966-973 [PMID: 21487323 DOI: 10.1097/MPA.0b013e3182156d5a]
- 79 Proteasome Inhibitor NPI-0052 and Vorinostat in Patients With Non-small Cell Lung Cancer, Pancreatic Cancer, Melanoma or Lymphoma. [accessed 2014 Aug]. Available from: URL: <https://clinicaltrials.gov/ct2/show/NCT00667082?term=Pancreas and HDAC&rank=1>
- 80 **Olsen EA**, Kim YH, Kuzel TM, Pacheco TR, Foss FM, Parker S, Frankel SR, Chen C, Ricker JL, Arduino JM, Duvic M. Phase IIb multicenter trial of vorinostat in patients with persistent, progressive, or treatment refractory cutaneous T-cell lymphoma. *J Clin Oncol* 2007; **25**: 3109-3115 [PMID: 17577020 DOI: 10.1200/JCO.2006.10.2434]
- 81 **Duvic M**, Talpur R, Ni X, Zhang C, Hazarika P, Kelly C, Chiao JH, Reilly JF, Ricker JL, Richon VM, Frankel SR. Phase 2 trial of oral

- vorinostat (suberoylanilide hydroxamic acid, SAHA) for refractory cutaneous T-cell lymphoma (CTCL). *Blood* 2007; **109**: 31-39 [PMID: 16960145 DOI: 10.1182/blood-2006-06-025999]
- 82 **Sandor V**, Bakke S, Robey RW, Kang MH, Blagosklonny MV, Bender J, Brooks R, Piekarz RL, Tucker E, Figg WD, Chan KK, Goldspiel B, Fojo AT, Balcerzak SP, Bates SE. Phase I trial of the histone deacetylase inhibitor, depsipeptide (FR901228, NSC 630176), in patients with refractory neoplasms. *Clin Cancer Res* 2002; **8**: 718-728 [PMID: 11895901]
- 83 **Byrd JC**, Marcucci G, Parthun MR, Xiao JJ, Klisovic RB, Moran M, Lin TS, Liu S, Sklenar AR, Davis ME, Lucas DM, Fischer B, Shank R, Tejaswi SL, Binkley P, Wright J, Chan KK, Grever MR. A phase I and pharmacodynamic study of depsipeptide (FK228) in chronic lymphocytic leukemia and acute myeloid leukemia. *Blood* 2005; **105**: 959-967 [PMID: 15466934 DOI: 10.1182/blood-2004-05-1693]
- 84 **Deorukhkar A**, Shentu S, Park HC, Diagaradjane P, Puduvalli V, Aggarwal B, Guha S, Krishnan S. Inhibition of radiation-induced DNA repair and prosurvival pathways contributes to vorinostat-mediated radiosensitization of pancreatic cancer cells. *Pancreas* 2010; **39**: 1277-1283 [PMID: 20531243 DOI: 10.1097/MPA.0b013e3181dd63e1]
- 85 **Lewis EC**, Blaabjerg L, Størling J, Ronn SG, Mascagni P, Dinarello CA, Mandrup-Poulsen T. The oral histone deacetylase inhibitor ITF2357 reduces cytokines and protects islet β cells in vivo and in vitro. *Mol Med* 2011; **17**: 369-377 [PMID: 21193899 DOI: 10.2119/molmed.2010.00152]
- 86 **Sung V**, Richard N, Brady H, Maier A, Kelter G, Heise C. Histone deacetylase inhibitor MGCD0103 synergizes with gemcitabine in human pancreatic cells. *Cancer Sci* 2011; **102**: 1201-1207 [PMID: 21375679 DOI: 10.1111/j.1349-7006.2011.01921.x]
- 87 **Huang S**, Zhu M, Wu W, Rashid A, Liang Y, Hou L, Ning Q, Luo X. Valproate pretreatment protects pancreatic β -cells from palmitate-induced ER stress and apoptosis by inhibiting glycogen synthase kinase-3 β . *J Biomed Sci* 2014; **21**: 38 [PMID: 24884462 DOI: 10.1186/1423-0127-21-38]
- 88 **Tian J**, Dang H, Chen Z, Guan A, Jin Y, Atkinson MA, Kaufman DL. γ -Aminobutyric acid regulates both the survival and replication of human β -cells. *Diabetes* 2013; **62**: 3760-3765 [PMID: 23995958 DOI: 10.2337/db13-0931]

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