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Mini review

HDAC family: What are the cancer relevant targets?

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ABSTRACT

Histone deacetylases comprise a family of 18 genes, which are grouped into classes I–IV based on their homology to their respective yeast orthologues. Classes I, II, and IV consist of 11 family members, which are referred to as "classical" HDACs, whereas the 7 class III members are called sirtuins. Classical HDACs are a promising novel class of anti-cancer drug targets. First HDAC inhibitors have been evaluated in clinical trials and show activity against several cancer diseases. However, these compounds act unselectively against several or all 11 HDAC family members. As a consequence, clinical phase I trials document a wide range of side effects. Therefore, the current challenge in the field is to define the cancer relevant HDAC family member(s) in a given tumor type and to design selective inhibitors, which target cancer cells but leave out normal cells. Knockout of single HDAC family members in mice produces a variety of phenotypes ranging from early embryonic death to viable animals with only discrete alterations, indicating that potential side effects of HDAC inhibitors depend on the selectivity of the compounds. Recently, several studies have shown that certain HDAC family members are aberrantly expressed in several tumors and have non-redundant function in controlling hallmarks of cancer cells. The aim of this review is to discuss individual HDAC family members as drug targets in cancer taking into consideration their function under physiological conditions and their oncogenic potential in malignant disease.

1. Introduction

HDAC1 was identified using the HDAC inhibitor trapoxin as an affinity tag from nuclear extracts in 1996 [1]. It turned out that HDAC1 shares high sequence homology with yeast Rpd3, a global gene regulator and transcriptional co-repressor with histone deacetylase activity [2]. Subsequently, 18 HDAC family members have been identified in the human genome. Following recombinant expression or purification of the HDAC proteins, it was only recently possible to characterize the inhibitory profile of HDAC inhibitors, which have already been widely applied in cell culture and animal models. It turned out that most of the currently used HDAC inhibitors act rather unselective and inhibit either all or at least several members of the HDAC family.

Unselective HDAC inhibitors are now being evaluated in clinical trials and show promising results in adult patients with leukaemia's and solid tumors. Vorinostat (SAHA) was the first HDAC inhibitor to be approved by the US Food and Drug Administration for cutaneous T-cell lymphoma in 2006 [3]. However, first phase I and II studies demonstrate that pan-HDAC inhibitors may also cause numerous side effects such as bone marrow depression, diarrhea, weight loss, taste disturbances, electrolyte changes, disordered clotting, fatigue, and cardiac arrhythmias [4]. These observations are not surprising if one considers the central role of HDACs as key regulators of chromatin structure and posttranslational modifiers of numerous key proteins in any cell type and tissue. Thus, the question arises whether

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Abbreviations: HDAC, histone deacetylase; TNM, staging system according to local tumor invasion, lymph node involvement and metastasis.

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future drug development in the field should focus on selective targeting of individual HDAC family members, which possess a critical oncogenic function in cancer cells but have no such function in normal cells.

Here, we discuss the potential of individual HDACs as drug targets in cancer therapy. To this aim, we review the role of individual HDACs not only in cancer, but also in normal physiology and development in order to understand the potential side effects associated with their inhibition. Finally, we discuss the selectivity of currently used HDAC inhibitors, their molecular mode of action and their clinical effects.

2. Classification of HDAC family members

HDACs are grouped into class I, class II, class III and class IV based on their sequence homology to their yeast orthologues Rpd3, HdaI and Sir2, respectively [5,6]. Class I, II, and IV are referred to as "classical" HDACs and comprise 11 family members (Table 1), whereas class III members are named sirtuins [6]. Classical HDACs and sirtuins differ in their catalytic mechanisms. Classical HDACs are Zn^{2+}-dependent enzymes harboring a catalytic pocket with a Zn^{2+} ion at its base that can be inhibited by Zn^{2+} chelating compounds such as hydroxamic acids. In contrast, these compounds are not active against sirtuins as these class III enzymes have a different mechanism of action requiring NAD^{+} as an essential cofactor [5]. The term "HDAC inhibitors" is commonly used for compounds that target the "classical" class I, II, and IV HDACs and that are currently evaluated in clinical trials.

2.1. Basic biochemical and cellular functions of classical HDAC family members

The initially discovered and best studied enzymatic activity of HDACs is the deacetylation of histone proteins. Through this activity, HDACs control the interaction of positively charged histones with the negatively charged DNA, and thus regulate chromatin conformation and transcriptional activity. In general, high HDAC activity is associated with condensed, transcriptionally inactive chromatin. In addition to this epigenetic function of HDACs, it is now recognized that certain HDACs also exhibit important cytoplasmatic function by controlling the acetylation status and function of numerous cytoplasmatic proteins and transcription factors. Therefore, the more precise term for these enzymes would be "lysine deacetylases" to indicate that their substrates are not restricted to histone proteins [7].

2.2. Class I HDACs

HDAC family members 1, 2 and 3 are subunits of multiprotein nuclear complexes that are crucial for transcriptional repression and epigenetic landscaping. For example,

Table 1
HDACs: molecular function and role in normal development

	HDAC family member		Substrates	Binding Partners	Tissue Expression	mouse knock out phenotype
class I	HDAC1	N	p53, MyoD, E2F-1, Stat3, androgen	Sin3, Mi-2/NuRD, CoREST	ubiquitous	embryonic lethal day 9.5, p21 and p27 up-regulation, reduced overall HDAC activity
	HDAC2	N	Bcl-6, Stat3, glucocorticoid receptor, YY-1	Sin3, Mi-2/NuRD, CoREST	ubiquitous	viable until perinatal period, fatal mulitple cardiac defects, excessive hyperplasia of heart muscle, arrythmia
	HDAC3	N	GATA-1, RelA, Stat3, MEF2D, YY-1, SHP	N-CoR/SMRT	ubiquitous	embryonic lethal before day 9.5, defective cell cycle, DNA repair and apoptosis in embryonic fibroblasts. Conditional liver knock out results in hepatocyte hypertrophy and induction of metabolic genes
	HDAC8	N/C	nd	EST1B	ubiquitous	nd
class II A	HDAC4	N/C	GCMa, GATA-1, HP-1	ANKRA, RFXANK	heart, smooth muscle, brain	viable, premature and ectopic ossification, chondrocyte hypertrophy
	HDAC5	N/C	Smad7, HP-1, GCMa	REA, estrogen receptor	heart, smooth muscle, brain	myocardial hypertrophy, abnormal cardiac stress response
	HDAC7	N/C	FLAG1 and 2	HIF1a, Bcl-6,	heart, placenta, pancreas, smooth muscle	embryonic lethal, lack of endothelial cell-cell adhesion
	HDAC9	N/C	nd	FOX3P	smooth muscle, brain	viable at birth, spontaneous myocardial hypertrophy
class II B	HDAC6	C	a-Tubulin, HSP90, SHP, Smad7	nd	kidey, liver, heart, pancreas	viable, no significant defects, increase in global tubulin acetylation. MEFs fail to recover from oxidative stress
	HDAC10	C	HSP90?	nd	spleen, kidney, liver	nd
class IV	HDAC11	N/C	nd	HDAC6?	heart, smooth muscle, kidney, brain	nd

Abbreviations: MEFs, mouse embryonic fibroblasts; N, nuclear; C, cytoplsmic, N/C, nuclear and cytoplasmic; nd, no data

HDAC1 and 2 are components of the co-rest complex which inactivates the expression of neuronal genes in non-neuronal tissues [8]. Other complexes containing HDAC1 and 2 are the NURD and SIN3 repressor complexes [9]. HDAC3 is found within the N-COR and SMRT repressor complex [10]. Of note, class I member HDAC8 has not been found to be a component of any repressor complex so far, suggesting a particular function for this class I HDAC.

2.3. Class II HDACs

Class II HDAC family members are further subdivided into IIA and IIB (Table 1). Class IIA members HDAC4, 5, 7, 9 are defined by a large, functionally important N-terminal domain regulating nuclear-cytoplasmic shuttling and specific DNA-binding. The cellular trafficking of these HDACs is regulated by intrinsic nuclear import and export signals as well as binding sites for 14-3-3 proteins. HDAC4, 5, 7, 9 contain three conserved 14-3-3 binding sites. Binding of the 14-3-3 proteins stimulate the cytoplasmatic retention or nuclear export of the class IIA HDACs in a phosphorylation dependent manner, which in turn regulates the activity of transcription factors like the myocyte enhancing factor-2 (MEF2) [7,11,12]. Several signalling pathways, including Ca^{2+}/calmodulin-dependent kinases (CaMKs) [11], protein kinase-D [13], microtubule affinity-regulating kinases [14], salt-inducible kinases [15] and checkpoint kinase-1 (CHK1) [16] regulate phosphorylation of these 14-3-3 binding sites.

Class IIB HDAC6 contains two tandem deacetylase domains and a C-terminal zinc finger. HDAC6 has emerged as a major cytoplasmic deacetylase functioning as an α-tubulin deacetylase [17] and HSP90 deacetylase [18] thereby regulating cell motility, adhesion and chaperone function. In addition, HDAC6 exerts cellular functions independent from its deacetylase activity. Binding to ubiquitin via its zinc finger domain regulates aggresome function, autophagy, heat shock factor-1 (HSF-1) and platelet derived growth factor (PDGF) function [19,20]. HDAC10 is structurally related to HDAC6, but contains one additional catalytically inactive domain. Its function is largely unknown.

2.4. Class IV HDAC

Class IV comprises HDAC11 only. It is structurally related to both, class I and II HDACs. Very little information is available about its expression and function.

An increasing number of non-histone proteins are recognized as substrates of HDACs such as p53, E2Fs, GATA1, Bcl-6, Stat3, HMGs, HSP90, NF-κB, tubulin, importin, nuclear hormone receptors, and β-catenin (for review see [21]). For example, HDAC1 has been shown to regulate the activity of the transcription factor p53. Deacetylation reduces p53 stability, represses its interaction with DNA, and its transactivation activity. This in turn modulates p53-mediated cell growth arrest and apoptosis [22,23]. Thus, HDACs regulate the activity of cellular key players involved in regulation of transcription, signal transduction, cell cycle, apoptosis and others. This clearly indicates that HDACs regulate important cellular functions independent from their epigenetic role in controlling chromatin structure.

3. Classical HDAC family members in mouse development

Targeting of HDACs for therapeutic purposes requires knowledge about their function in normal tissues and during development in order to understand the potential side effects of this class of compounds. Several knockout mice targeting HDAC family members have been generated, providing valuable insights into their physiological function (Table 1).

3.1. Class I HDACs

3.1.1. HDAC1

Knockout of Hdac1 is embryonic lethal by day 9.5 and results in proliferation defects of embryonic stem cells. Expression of the cyclin-dependent kinase (CDK) inhibitors p21 and p27 is up-regulated and global histone deacetylase activity is downregulated. Loss of Hdac1 function in mice cannot be compensated by concomitant upregulation of Hdac2 and 3 [24,25].

3.1.2. HDAC2

Mice lacking Hdac2 survive until the perinatal period, when they die of multiple cardiac defects [25,26]. Cardiac-specific deletion of either Hdac1 or Hdac2 alone using a conditional knockout model does not produce a phenotype. However, cardiac-specific deletion of both genes simultaneously results in neonatal lethality, accompanied by cardiac arrhythmias and dilated cardiomyopathy [25].

3.1.3. HDAC3

Germline deletion of Hdac3 causes early embryonic lethality before day 9.5. Inactivation of Hdac3 led to a delay in cell cycle progression, cell cycle-dependent DNA damage and inefficient repair, and apoptosis in mouse embryonic fibroblasts [27]. Liver specific knockout of Hdac3 resulted in an enlarged organ, hepatocyte hypertrophy and disturbed fat metabolism [28].

3.2. Class IIA HDACs

3.2.1. HDAC4

Hdac4-null mice display premature ossification of developing bones due to ectopic and early onset chondrocyte hypertrophy. Overexpression of Hdac4 in proliferating chondrocytes *in vivo* inhibits chondrocyte hypertrophy and differentiation. Thus, Hdac4 is a central regulator of chondrocyte hypertrophy and endochondral bone formation [29], which acts in concert with MEF2C [30].

3.2.2. HDAC5

Mice lacking Hdac5 develop profoundly enlarged hearts in response to pressure overload resulting from aortic constriction or constitutive activation of cardiac stress signals [31].

3.2.3. HDAC7

Disruption of the Hdac7 gene in mice results in embryonic lethality due to a failure in endothelial cell–cell adhe-

sion and consequent dilatation and rupture of blood vessels. Hdac7 is specifically expressed in the vascular endothelium during early embryogenesis, where it maintains vascular integrity by repressing the expression of matrix metalloproteinase 10 by associating with myocyte enhancer factor-2 (MEF2) [32].

3.2.4. HDAC9

Hdac9 mutant mice develop normally and are viable at birth. By the age of 8 months mice develop spontaneous cardiac hypertrophy due to sensitisation to hypertrophic signals [33].

3.3. Class IIB HDACs

3.3.1. HDAC6

HDAC6 is now recognized as a tubulin-deacetylase. Mice lacking Hdac6 are viable, despite having highly elevated tubulin acetylation in multiple organs. Lack of Hdac6 results in minor changes in bone mineral density and immune response. Hdac6-deficient mouse embryo fibroblasts show increased Hsp90 acetylation resulting in its impaired function [34]. Hdac6 −/− MEFs fail to form stress granules and do not recover from oxidative stress. In the absence of intact Hdac6 function, cells that have been treated with arsenite undergo apoptosis [35].

HDAC8, 10, 11 knockout mice have not yet been published.

These knockout studies demonstrate, that gene deletion of Hdacs 1, 2, 3, and 7 produce a severe embryonic lethal phenotype most likely due to impaired cell cycle of early embryonic cells (Hdac 1, 2, 3), or impaired blood vessels development (Hdac7). In contrast, mice lacking Hdacs 4, 5, 6, and 9 are viable and show defects in the regulation of cellular hypertrophy, stress response and defects in muscle, cardiovascular, and bone development and differentiation, respectively (Table 1). From these data it can be concluded that (i) the function of individual HDACs can not be compensated by other members of the HDAC family, (ii) individual HDACs are fundamental for normal embryonic development and key cellular processes such as cell cycle control, DNA damage response, stress response, cell growth, metabolism and differentiation, (iii) conditional, tissue-specific knockout models need to be generated to determine the function of individual HDACs for normal tissue and cellular functions during later stages of development, (iv) the use of broad-spectrum HDAC inhibitors are likely to produce significant side effects if the therapeutic window is not wide enough.

4. Classical HDAC family members in cancer

Despite the broad application of HDAC inhibitors in cell culture, animal models, and early phase clinical trials, surprisingly little is known about the expression of their targets in cancer tissues. Additionally, systematic investigations of the function of all HDAC family members in a given tumor model are lacking. This information is of important clinical relevance, as a recent study demonstrates resistance to HDAC inhibitors in cells lacking HDAC2 expression [36]. In this section, we review our current knowledge on the genetics, expression and function of the classical HDAC family members 1–11 in cancer (Table 2).

Table 2
HDACs in cancer: expression and functional studies

	HDAC family member	Expression in tumor tissues	Function in cancer cells
class I	HDAC1	*gastric cancer*: elevated expression, associated with nodal spread and poor prognosis; *pancreatic cancer:* expression associated with de-differentiation, enhanced proliferation and poor prognosis; *colorectal cancer*: increased expression associated with poor prognosis; *prostate cancer*: increased in high grade, hormone refractory cancers; *hepatocellular carcinoma*: high expression associated with portal vein invasion, poor differentiation, advanced TNM stage	*cervical cancer cells*: HDAC1 knockdown results in inhibition of proliferation and induction of autophagy; *osteosarcoma and breast cancer cells*: knockdown causes cell cycle arrest, growth inhibition, apoptosis; *colon cancer cells*: knockdown suppresses growth; *prostate cancer:* overexpression increases proliferation and de-differentiation; *neuroblastoma cells:* knockdown sensitizes for chemotherapy; *CLL cells*: knockdown sensitizes for TRAIL-apoptosis
	HDAC2	*colorectal cancer*: upregulation in polyps, associated with poor prognosis; *cervical carcinoma*: high expression in dysplasia; *gastric and prostate cancer*: increased expression associated with advanced stage and poor prognosis	*cervical cancer cells:* HDAC2 knockdown results in differentiation, apoptosis and p53 independent p21 expression; *breast cancer cells:* increased p53 activity, inhibition of proliferation, induction of senescence, induction of apoptosis; *colon cancer cells:* knockdown causes growth arrest; *neuroblastoma cells*: knockdown induces apoptosis; genetic HDAC2 mutation reduces intestinal tumor development in APC mice in vivo; *CLL cells*: knockdown sensitizes for TRAIL-apoptosis
	HDAC3	*gastric, prostate, colorectal cancers*: high expression associated with poor prognosis (together with HDAC1 and 2)	APL cells: HDAC3 associated with PML-RARa fusion protein, knockdown induces differentiation genes; *AML:* AML-1-ETO binds HDAC3 (and HDACs 1, 2), disrupts cell cycle
	HDAC8	childhood *neuroblastoma:* high HDAC8 expression significantly correlates with advanced stage disease, clinical and genetic risk factors and poor long term survival	*neuroblastoma cells*: HDAC8 knockdown induces differentiation, cell cycle arrest and inhibits clonogenic growth; *lung, colon, cervical cancer cells*: knockdown of HDAC8 reduces proliferation
class II A	HDAC4	*breast cancer:* upregulation compared with renal, bladder, colorectal cancer	*APL cells*: HDAC4 interacts with PLZF-RARa fusion protein, represses differentiation genes; renal carcinoma cells: knockdown inhibits expression and functional activity of HIF-1a
	HDAC5	*colorectal cancer:* upregulation compared with renal, bladder, breats cancer	*erythroleukemia*: HDAC5 shuttles from nucleus to cytoplasm upon differentiation, interacts with GATA-1
	HDAC7	*colorectal cancer*: high expression compared with bladder, renal, breast cancer tissues	*endothelial cells:* HDAC7 silencing alters morphology, migration and tube-forming capacity
	HDAC9	nd	nd
class II B	HDAC6	*oral squamous cell cancer*: high expression, increased in advanced stage; *breast cancer*: high expression correlates with response to endocrine treatment, inverse correlation of expression with survival and tumor size	Targeted inhibition of HDAC6 leads to acetylation of HSP90 and disruption of its chaperone function, resulting in depletion of pro-growth and pro-survival client proteins including the Bcr-Abl oncoprotein in *K562 leukemic* cells; HDAC6 targeting blocks EGF induced nuclear translocation of ß-catenin and c-myc expression in *colon carcinoma* cells; knockdown of HDAC6 causes donwregulation of HIF-1a, VEGFR1/2; HDAC6 invovled in TGFb induced epithelial-mesenchymal transition of *lung carcinoma* cells
	HDAC10	nd	Knockdown of HDAC10 downregulates VEGFR
class IV	HDAC11	nd	nd

Abbreviations: CLL, chronic lymphatic leukemia; APL, acute promyelocytic leukemia; AML, acute myeloid leukemia; RARa, retinoic acid receptor alpha; CML, chronic myeloid leukemia; nd, no data

4.1. Germline HDAC polymorphisms and cancer risk

Germline variants of several HDACs have been studied in lung and breast cancer patients. Neither study found evidence for association of HDAC3, 4 and 5 variants with lung cancer risk [37], or HDAC2 and 5 with breast cancer risk [38]. An insertion of a CAG triplet in the 5′-UTR of HDAC2 was recently identified in 18% of 181 cancer samples investigated versus 10% of 192 normal DNA controls ($P < 0.01$) [39]. HDAC10 promoter polymorphism in 24 patients with hepatocellular carcinoma (HCC) resulted in increased promoter activity *in vitro* and was associated with development of HCC among chronic HBV patients [40].

4.2. Somatic HDAC mutations in cancer

Somatic mutations of the HDAC2 gene in human epithelial cancers with microsatellite instability have been identified in cell lines [36,39]. A HDAC2 truncating mutation was detected in 48/228 (21%) of investigated cancers with microsatellite instability associated with loss of HDAC2 protein expression. Interestingly, the mutation was shown in functional assays to confer resistance to the anti-proliferative and proapoptotic effects of HDAC inhibitors [36]. HDAC4 mutations have been identified in breast cancer samples at significant frequency in the recent large-scale sequencing study of breast and colorectal cancers [41].

4.3. Expression and function of classical HDACs in cancer

4.3.1. Class I HDACs

4.3.1.1. HDAC1 expression. In a first study of gastric cancer, HDAC1 expression was upregulated in 17/25 cases (60%) compared with normal tissue [42]. This observation was confirmed in a recent larger study, including 293 gastric cancer samples investigating the prognostic value of HDAC1, 2, 3 expressions [43]. In that study, elevated class I HDAC expression was significantly associated with nodal spread and was an independent prognostic marker for survival of patients with gastric cancer [43]. In pancreatic cancer, high HDAC1 expression together with HIF1a was associated with poor prognosis in a series of 39 pancreatic carcinomas [44]. Similarly, in a larger study involving 192 pancreatic carcinoma samples, high HDAC 1, 2, 3 expressions was associated with dedifferentiation and enhanced proliferation of pancreatic cancer cells [45]. In colorectal cancer, increased HDAC1 expression along with HDAC5, 7 were observed in contrast to breast, renal, and bladder cancer [39]. In a recent series of 140 colorectal cancer samples, high HDAC1, 2, 3 expression levels implicated significantly reduced patient survival, with HDAC2 expression being an independent prognostic factor [46]. In another small series of 14 prostate cancer samples, HDAC1 protein expression was higher in hormone refractory, high grade cancer compared with low grade cancer and benign prostatic hyperplasia. [47]. In hepatocellular carcinoma, high HDAC1 expression was associated with cancer cell invasion into the portal vein, a poorer histological differentiation, a more advanced TNM stage and poor survival of patients in 47 cases [48]. In lung cancer, a trend of higher HDAC1 expression in advanced stage disease compared with low stage, but no difference compared with normal lung tissue was reported in a series of 102 samples [49]. In 200 breast cancer samples, HDAC1 and 3 expression was found to correlate with oestrogen and progesterone receptor expression and HDAC-1 expression predicted better disease free survival. Multivariate analysis demonstrated that HDAC-1 was an independent prognostic marker [50]. In another series of 162 breast cancer samples, high HDAC1 expression correlated with better survival, negative lymph node status and small tumor size [51].

Taken together, these studies show that HDAC1 overexpression appears especially common in cancers of the gastrointestinal system and is associated with dedifferentiation, enhanced proliferation, invasion, advanced disease and poor prognosis. However, these studies mostly investigated only HDAC1 and not any other HDAC family member.

4.3.1.2. HDAC1. function. Essential function of HDAC1 in proliferation control and p21 and p27 CDK inhibitor repression has been described in mouse embryonic stem cells [24]. In cancer cells, several studies have similarly found an important function of HDAC1 in controlling cell proliferation. HDAC1 and 3 knockdown resulted in inhibition of cell proliferation of HeLa cells, whereas knockdown of HDAC4 and 7 did not lead to decreased cell numbers [52]. Knockdown of HDAC1 results in arrest either at the G(1) phase of the cell cycle or at the G(2)/M transition, causes loss of mitotic cells, cell growth inhibition, and an increase in the percentage of apoptotic cells in osteosarcoma and breast cancer cells [53]. On the contrary, HDAC2 knockdown showed no such effects in these cells [53]. Short interfering RNA-based inhibition of HDAC1 and HDAC2 suppressed growth of colon cancer cells *in vitro* [46]. HDAC1 overexpression led to an increase in proliferation and to an undifferentiated phenotype in cultured prostate cancer cells [47]. In addition to controlling cell cycle and apoptosis, HDAC1 might also be involved in multidrug resistance. HDAC1 was overexpressed in chemotherapy resistant neuroblastoma cells *in vitro* and siRNA knockdown sensitized cells for etoposide treatment [54].

HDAC1 knockdown by small interference RNA stimulated urokinase plasminogen activator expression and invasion of neuroblastoma cells *in vitro*, which was also observed using the unselective HDAC inhibitors TSA, butyrate and scriptaid [55]. However, recent findings shows that the HDAC inhibitor HC toxin efficiently inhibits migration and invasion of MYCN amplified neuroblastoma cells [56], which could be due to the unique features of this compound compared with other HDAC inhibitors investigated in the same culture model [56,57]. HDAC1 targeting was recently shown to induce autophagy in HeLa cells [58]. Knockdown of HDAC1 (and HDAC2) but not HDAC3, HDAC6, and HDAC8 sensitizes CLL cells for TRAIL-induced apoptosis [59].

4.3.1.3. HDAC2 expression. Upregulation of HDAC2 in colorectal cancer occurred early at the polyp stage, was more robust and occurred more frequently than HDAC1. Similarly, in cervical dysplasia and invasive carcinoma, HDAC2 expression showed a clear demarcation of high-intensity

staining at the transition region of dysplasia compared to HDAC1 [60]. HDAC2 expression together with HDAC1 and 3 was associated with advanced stage disease and poor prognosis in gastric, colorectal and prostate cancer [43,45,46].

4.3.1.4. HDAC2 function. HDAC2 knockdown in cervical cancer cells caused a differentiated phenotype, increase in apoptosis associated with increased $p21^{Cip1/WAF1}$ expression that was independent of p53 [60]. In breast cancer cells, HDAC2 knockdown increases the functional DNA binding activity of p53 associated with inhibition of proliferation and induction of cellular senescence [61]. Selective inhibition of HDAC2, but not HDAC1 or HDAC6, was sufficient to potentiate tamoxifen-induced apoptosis in estrogen/progesterone receptor-positive breast cancer cells through downregulation of both hormone receptors [62]. Selective depletion of HDAC2 resulted in simultaneous depletion of estrogen receptor and progesterone receptor, and potentiated the effects of anti-hormone therapy in estrogen receptor-positive cells. SiRNA mediated knockdown of HDAC2 and HDAC1 but not HDAC3 suppressed growth of colon cancer cells *in vitro* [46]. Knockdown of HDAC2 (and HDAC1) but not HDAC3, HDAC6, and HDAC8 sensitizes CLL cells for TRAIL-induced apoptosis [59]. HDAC2 knockdown induces apoptosis in neuroblastoma cells in contrast to HDAC8 [63]. Crossing of HDAC2-mutant with tumor-prone APC (min) mice revealed a rate-limiting role of HDAC2 for intestinal tumor development *in vivo* [64].

4.3.1.5. HDAC3 expression. Together with HDAC1 and 2, HDAC3 expression was significantly associated with poor prognosis in large series of gastric, prostate and colorectal cancer samples [43,45,46].

4.3.1.6. HDAC3 function. In acute promyelocytic leukemia cells, HDAC3 is a key component of the aberrant transcription regulation in PML-RARα-expressing cells. Knockdown of HDAC3 in these cells restores expression of retinoic acid dependent genes [65]. The AML t(8;21) fusion transcript AML-1-ETO binds class I HDACs HDAC1, 2, and 3 via ETO to repress transcription and disrupt the cell cycle of leukemia cells [66].

4.3.1.7. HDAC8 expression. In childhood neuroblastoma, high HDAC8 expression significantly correlated with advanced stage disease, poor prognostic markers and poor survival of children. In contrast, all other 10 HDAC family members investigated did not correlate with disease stage [63].

4.3.1.8. HDAC8 function. Proliferation of lung, colon and cervical cancer cell lines is reduced after HDAC8 knockdown [67]. HDAC8 has been implicated in the regulation of telomerase activity [68]. HDAC8 specific inhibitor selectively induces apoptosis in T-cell derived lymphoma and leukemic cells, but not in solid cancer cell lines [69]. In childhood neuroblastoma cells, knockdown of HDAC8 resulted in inhibition of proliferation, reduced clonogenic growth, cell cycle arrest and differentiation without affecting global histone acetylation or cellular HDAC activity [63]. Of note, in the same study, HDAC2 knockdown induced apoptosis but no signs of differentiation, suggesting that individual HDACs suppress different cancer relevant programs in a given tumor cell.

4.3.2. Class IIA HDACs

4.3.2.1. HDAC4 expression. HDAC4 expression was upregulated in breast cancer samples compared with renal, bladder and colorectal cancer [39].

4.3.2.2. HDAC4 function. In APL cells, HDAC4 was found to interact with the leukemic PLZF-RARa fusion protein and to mediate repression of differentiation associated genes [70]. HDAC4 (and HDAC6) were shown to bind to and regulate HIF-1α transcriptional activity in renal carcinoma cells and targeting of these HDACs could therefore be a means of tumor anti-angiogenesis [71].

4.3.2.3. HDAC5 expression. HDAC5 expression was upregulated in colorectal cancer in contrast to renal, bladder and breast cancer [39].

4.3.2.4. HDAC5 function. HDAC5 interacts with the transcription factor GATA-1 and shuttles from the nucleus to the cytoplasm upon erythroid differentiation of mouse erythroleukemia cells [72].

4.3.2.5. HDAC7 expression. HDAC7 is highly expressed in colorectal cancer in contrast to bladder, renal and breast cancer [39].

4.3.2.6. HDAC7 function. HDAC7 silencing in endothelial cells altered their morphology, their migration, and their capacity to form capillary tube-like structures *in vitro* but did not affect cell adhesion, proliferation, or apoptosis, suggesting that HDAC7 may represent a rational target for anti-angiogenesis in cancer [73].

4.3.2.7. HDAC9. No published data on HDAC9 expression and function in cancer are available.

4.3.3. Class IIB HDACs

4.3.3.1. HDAC6 expression. In oral squamous cell carcinoma, significantly higher HDAC6 expression was found in carcinomas versus normal oral squamous tissue, and HDAC6 expression was increased in advanced-stage cancers compared with early stage in 90 samples [74]. In a series of 135 breast cancer samples, HDAC6 expression correlated with better survival and was higher in small tumors, low histologic grade, and in estrogen and progesterone receptor-positive tumors. High levels of HDAC6 mRNA tended to be more responsive to endocrine treatment than those with low levels. HDAC6 may thus serve as a predictive indicator of responsiveness to endocrine treatment and also as a prognostic indicator for breast cancer progression [75]. In another study of 139 breast cancer tissues, HDAC6 protein expression revealed no significant prognostic differences based on HDAC6 expression. However, subset analysis of estrogen receptor-positive patients who received adjuvant treatment with tamoxifen ($n = 67$)

showed a statistically significant difference in relapse-free survival and overall survival in favor of the HDAC6-positive group and HDAC6 expression was an independent prognostic indicator [76].

4.3.3.2. HDAC6 function. HDAC6 overexpression leads to increased migration of embryonic fibroblasts. Specific inhibition of HDAC6 reduced migration of fibroblasts, but did not alter cell cycle progression [77]. Targeted inhibition of HDAC6 leads to acetylation of HSP90 and disruption of its chaperone function, resulting in depletion of progrowth and prosurvival client proteins, including the Bcr-Abl oncoprotein in K562 leukemic cells [78]. HDAC6 was found to play a role in epidermal growth factor (EGF)-induced nuclear localization of β-catenin and subsequent c-myc activation in colon carcinoma cells [79]. HDAC6 (and HDAC4) were shown to bind to and regulate HIF-1α transcriptional activity and targeting of these HDACs could therefore be a means of tumor anti-angiogenesis [71]. Knockdown of HDAC6 (and HDAC10) via siRNA transfection induced depletion of VEGFR1 or VEGFR2 proteins and may play a role in HSP-mediated proteasomal degradation of VEGFRs in anti-angiogenesis [80]. Very recently, HDAC6 was shown to be involved in epithelial-mesenchymal transition of lung carcinoma cell metastasis *in vitro* by influencing the TGF-β SMAD3 cascade [81].

Thus, HDAC6 is a good example for an HDAC family member regulating multiple cancer-relevant cell biological function, which is non-epigenetic in nature.

4.3.3.3. HDAC10. Expression of HDAC10 in cancer samples has not been reported to data. Knockdown of HDAC10 (and HDAC6) reduces VEGF receptor 1 and 2 expression in cancer cells and it was suggested that these HDACs play pivotal roles in heat shock protein 90-mediated proteasomal degradation of vascular endothelial growth factor receptors [80].

4.3.4. Class IV HDAC

4.3.4.1. HDAC11. No published data on HDAC11 expression in tumor tissues and its cellular function is available.

Taken together, expression of specific HDAC family members was correlated with cancer progression and patient survival in several entities. Although most initial studies included only small number of patient samples, recent papers involved 100 or more specimen providing statistically significant data. The functional experiments based on selective knockdown of individual HDACs through RNA interference in cell culture models demonstrate that HDACs are involved in regulating hallmarks of cancer cell biology such as cell cycle, differentiation, apoptosis, but also migration, invasion and angiogenesis.

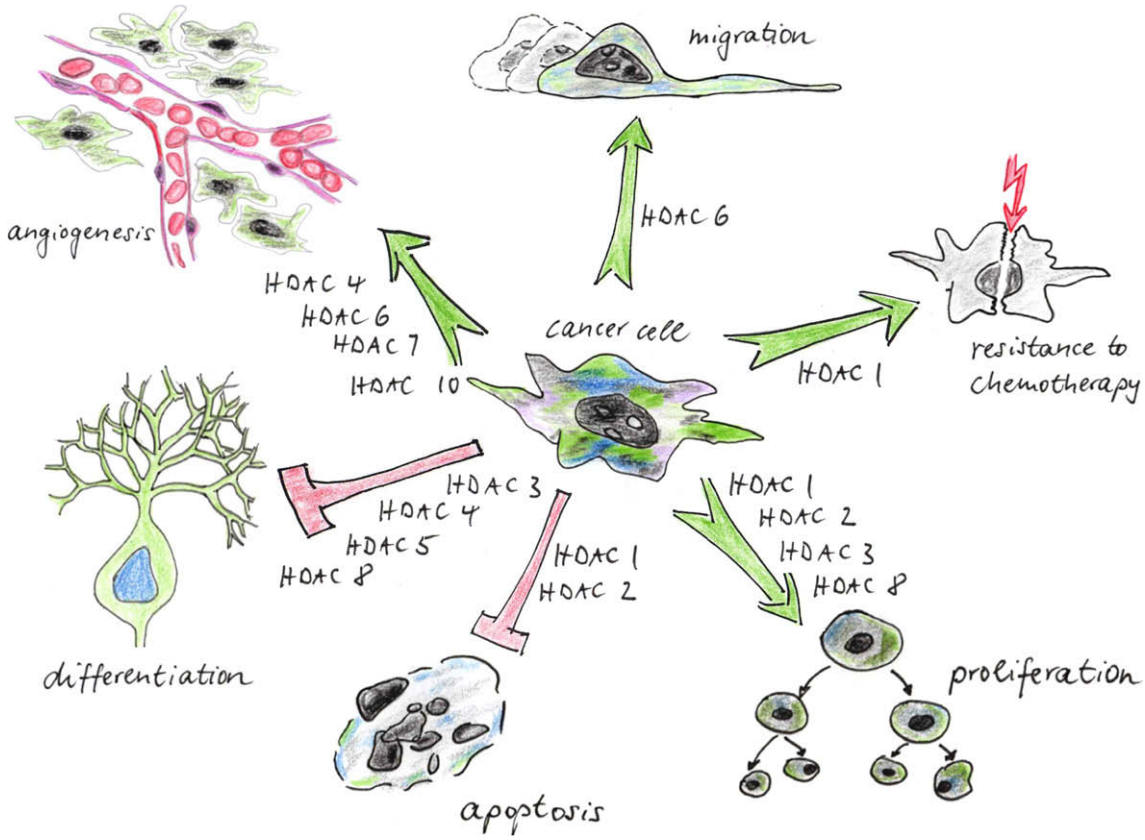

Fig. 1. HDAC family members control hallmarks of cancer cell biology. Selective targeting of individual HDACs cause differentiation, apoptosis, cell cycle inhibition, inhibition of migration, susceptibility to chemotherapy and anti-angiogenesis. See text for further information and references.

Similar to the knockout studies in mice discussed above, the function of each HDAC appears not to be redundant and the cancer cell obviously can not compensate loss of expression of a given HDAC. Targeting of class I HDACs 1, 2, and 3 inhibits cell cycle progression and promotes apoptosis. This phenotype in cancer cells is in line with the early embryonic lethal phenotype in knockout mice most likely caused by impaired cell cycle defects in early embryonal progenitor cells as has been shown for embryonic fibroblasts. HDAC8 and class II HDACs rather control specific functions such as differentiation, cell signalling, migration, cell adhesion, protein stability and function, and angiogenesis. Fig. 1 summarizes the functional role of individual HDACs in cancer cell biology based on data of siRNA knockdown or enforced expression. It should be noted, however, that targeting of the same HDAC can have different biological effects depending on the cellular context.

5. Selective HDAC inhibitors

The activity of HDAC inhibitors on cancer cells have been extensively reviewed elsewhere and it is now generally accepted that these compounds have a broad spectrum of anti-tumoral activity against a variety of cancer cells in cell culture and animal models [82,83]. The pan-HDAC inhibitor Vorinostat is the first compound of its class, which was approved by the FDA for clinical use in cutaneous T-cell lymphoma [84]. The broad-spectrum HDAC inhibitors such as Vorinostat and trichostatin A often have a hydroxamic acid based structure and affect the expression of 2–10% of all genes investigated, corresponding to several hundreds to thousand genes in the human genome. Thus, there is an ongoing discussion whether selective HDAC inhibitors would be advantageous for clinical use.

In this chapter, we will review the more selective HDAC inhibitors targeting classical HDAC family members from classes I, II, and IV. HDAC-inhibitors act through binding into the active site pocket and chelation of the catalytic zinc-ion located at its base [85–87]. Due to the highly conserved nature of the enzymatic pocket, most HDAC-inhibitors do not selectively inhibit individual HDAC isoenzymes and either inhibit all HDACs or at least several members simultaneously. Characterization of the inhibitory-profile of currently used inhibitors within the entire HDAC family is hampered by the fact that recombinant production of purified, active enzymes is difficult [82]. The situation becomes even more complicated as many HDACs require multi-protein complexes or interaction with other HDACs for full enzymatic activity and therefore, *in vitro* HDAC activity is likely not to reflect the true intracellular situation.

Table 3 summarizes the inhibitory profile of several HDAC inhibitors considered to be pan (unselective), class

Table 3
Inhibitory profile of pan- and class-selective HDAC inhibitors

	Inhibitor	class I				class II A				class II B		class IV
		HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC5	HDAC7	HDAC9	HDAC6	HDAC10	HDAC11
pan-inhibitors	TSA						nd				nd	nd
	Vorinostat (SAHA)						nd				nd	nd
	NVP-LAQ824						nd				nd	nd
	Panbinostat						nd				nd	nd
	Belinostat						nd				nd	nd
	PCI-24781					nd	nd	nd	nd			nd
class I inhibitors	MS-275						nd				nd	nd
	MGCD0103								nd		nd	nd
	Depsipeptide			nd	nd		nd	nd	nd		nd	nd
	Apicidin						nd				nd	nd
	Valproic acid					nd	nd				nd	nd
	Trapoxin		nd	nd	nd		nd	nd	nd		nd	nd
	SB-429201		nd			nd	nd	nd	nd	nd	nd	nd
	Bispyridinum diene				nd		nd	nd	nd	nd	nd	nd
	SHI-1:2								nd		nd	nd
	R306465		nd	nd		nd	nd	nd	nd		nd	nd
	SB-379278A		nd			nd	nd	nd	nd	nd	nd	nd
	PCI-34051					nd	nd	nd	nd			nd
	Cpd2		nd	nd		nd	nd	nd	nd		nd	nd
class II inhibitors	APHA derivatives		nd	nd	nd		nd	nd	nd	nd	nd	nd
	Tubacin		nd	nd	nd	nd	nd	nd	nd		nd	nd
	Mercaptoacetamide			nd		nd	nd	nd	nd			nd
	NCT-10a/14a		nd	nd	nd		nd	nd	nd		nd	nd

Depicted are relative inhibitory potency of several pan-, class I selective, and class II selective compounds against HDACs1-11

strong inhibition (EC50 < 5fold x EC50 relative to most sensitive HDAC isoform)

weak inhibition (EC50 > 5fold x EC50 relative to most sensitive HDAC isoform)

no inhibition (EC50 > 100fold x EC50 relative to most sensitive HDAC isoform)

nd no data published

I selective, and class II selective inhibitors, respectively in a qualitative manner at a glance. The colour code reflects relative inhibitory potency against HDACs 1–11 based on inhibition of recombinantly expressed HDAC isoforms or immunoprecipitated HDACs in cell free assays. For example, TSA shows strong inhibitory potency against HDAC1, 2, 3, 4, 7, 9, and 6 (black). TSA shows only relative weak inhibitory activity against HDAC8 (dark grey), compared with the most TSA-sensitive isoform, being HDAC1 [88]. No data are published for the activity of TSA against HDAC5, 10 and 11.

5.1. Class I inhibitors

Class I HDACs are considered by many authors as the most relevant targets for cancer therapy because inhibitors which possess activity against HDACs 1, 2, and 3 usually show strong anti-proliferative and apoptosis-inducing activity.

5.1.1. HDAC1 and 2 selective inhibitors

The benzamide MS-275 is relative selective for HDAC1 compared with the other class I HDACs 2 and 3. However, it was also found to inhibit HDAC9 with an EC50 only 2- to 3-fold higher than HDAC1 [88]. The compound induces histone but not tubulin-acetylation and is currently evaluated in phase I/II clinical trials. MGCD0103, structurally related to MS-275, is very potent against HDAC1 and 2 acting in the nanomolar range, but less effective against other class I and II family members [88]. The compound exerts growth inhibitory activity against various cancer cells and is being tested in phase II clinical trials. The cyclic tetrapeptide depsipeptide (FK228, romidepsine) is particularly active against HDAC1 and to a lesser extent against HDAC2 [89]. At higher concentrations, the compound also inhibits HDAC4. Depsipeptide is active in several cancer cell models and is currently in phase II trials. Trapoxin and other cyclic tetrapeptides reveal high activity against HDAC1 being several 100- to 1000-fold more potent against HDAC1 compared with HDAC6 [90]. However, activity against other class I, II, and IV family members has not been determined so far. SB-429201 was found to be a HDAC1 selective inhibitor compared with HDAC3 and 8 [91]. Again, the activity against other class I, II, and IV HDACs was not determined. Bispyridinum diene selectively inhibits HDAC1 compared with HDAC3 and 4 and does not alter tubulin-acetylation suggesting that HDAC6 is also not a target of this compound [92]. Very recently, HDAC1 and 2 selective biaryl benzamides have been described. These agents exhibit selectivity over other class I HDACs and class II HDAC4–7 and displayed tumor growth inhibition activity in a HCT-116 xenograft model [93]. R306465 is a novel hydroxamate-based HDAC inhibitor showing selectivity against HDAC1 and 8 over HDAC6. The compound displayed broad spectrum anti-tumor activity against solid and hematological malignancies in preclinical models [94].

5.1.1.1. Molecular mechanism. Class I selective HDAC inhibitors induce cell cycle block and inhibition of proliferation. A key player in this scenario is the induction of the cyclin dependent kinase inhibitor 1A (p21Waf1/Cip1) [95–98]. All unselective pan-HDAC inhibitors have been shown to rapidly induce p21Waf1/Cip1 mRNA and protein expression in a p53 independent manner. Class I selective inhibitors targeting HDAC1 or HDAC2 such as MS275 [99,100], apicidin [96], and depsipeptide [101] are also potent inducers of p21Waf1/Cip1 *in vitro* and *in vivo* suggesting that targeting of HDACs 1 and 2 is sufficient to activate expression of this critical tumor suppressor gene. This observation is in line with genetic studies demonstrating that deletion of HDAC1 results in proliferation defect and p21Waf1/Cip1 upregulation in embryonic fibroblasts [24]. Induction of p21Waf1/Cip1 through HDAC inhibition is associated with acetylation of lysines 5, 8, 12 of histone H4, acetylation of lysines 9 and 14 of histone H3, and methylation of lysine 4 of histone 3 in the p21Waf1/Cip1 promoter region [102]. These epigenetic changes are associated with induction of an open chromatin structure indicated by increased DNase I hypersensitivity. The protein complex associated with the proximal region of the p21Waf1/Cip1 promoter includes HDAC1, HDAC2, myc, BAF155, Brg-1, GCN5, p300, and SP1 [102]. Induction of p21Waf1/Cip1 by HDAC inhibition is critical for mediating the anti-proliferative effect of these compounds [98].

The quality of the cellular response to class I HDAC inhibitor MS 275 is concentration dependent. At low concentration, MS-275 induced p21Waf1/Cip1 expression, cell cycle block and differentiation [99]. However, at higher concentrations, the compound causes marked induction of reactive oxygen species (ROS), mitochondrial damage, caspase activation and apoptosis [99].

In addition to a direct activation of p21Waf1/Cip1, the class I HDAC inhibitor depsipeptide can also stimulate the activity of p53 mediated p21 transcription. Depsipeptide causes p53 acetylation at lysines 373/382 thereby protecting it from proteasomal degradation [103]. Acetylated p53 in turns recruits histone acetyl transferases to the p21Waf1/Cip1 promoter with subsequent transcriptional activation [103].

Recently, it was shown that depsipeptide not only increases histone acetylation, but also efficiently inhibits DNA-demethylation, adding an additional layer of epigenetic mechanisms of action [104]. Depsipeptide demethylates the promoters of several genes, including p16, SALL3, and GATA4 by decreasing the binding of DNMT1 to the promoter of these genes. This was associated with a decrease of di- and tri-methylated H3K9 around the promoters of these genes due to suppression of histone methyltransferases G9A and SUV39H1 expression. Depsipeptide reduced loading of heterochromatin-associated protein 1 to methylated H3K9 and binding of DNMT1 to these genes [104]. Interestingly, pan-HDAC inhibitor trichostatin A did not induce DNA-demethylation, pointing to a specific function of class I HDAC inhibitors.

5.1.1.2. Clinical application. First phase I clinical trials have been published for class I selective HDAC inhibitors MS275 [105–107], depsipeptide (romidespin) [108–110] including children [111], and MGCD0103 [112,113] in patients with advanced solid tumors, lymphomas and leukaemias. Pharmacodynamic investigations document induction of his-

tone acetylation in PBMCs of patients as a surrogate parameter for *in vivo* HDAC inhibition. However, this observation was inconsistent in some patients. Clinical responses in these heavily pretreated patient populations were moderate. However, certain entities such as cutaneous T-cell lymphoma showed dramatic responses in some cases. To better understand treatment responses to HDAC inhibitors, a current challenge is to define response prediction parameters. Toxicity profile of the class I selective HDAC inhibitors is similar to the one observed with unselective pan-HDAC inhibitors und includes fatigue, nausea, vomiting, diarrhea, thrombocytopenia, and neutropenia. Depsipeptide revealed cardiotoxicity in early clinical studies, which later on could not be confirmed [114].

5.1.2. HDAC8 selective inhibitors

Three HDAC8 selective inhibitors have been identified so far. SB-379278A inhibits HDAC8, but not HDAC1 and 3 [91]. PCI-34051 was found to selectively target HDAC8, but not HDACs1, 2, 3, 6, and 10 [69].

PCI-34051 induces apoptosis in T-cell lymphoma or leukemia cell lines but not in cells derived from solid tumors suggesting a tumor-type selective activity [69]. In neuroblastoma cells, the HDAC8 selective compound Cpd2 [115] induces neuronal differentiation, inhibition of proliferation, and decreased clonogenic growth [63]. All three HDAC8 selective compounds do not alter histone acetylation in contrast to class I HDAC selective inhibitors. This is in line with the observation that knockdown of HDAC8 does not increase histone acetylation in contrast to knockdown of HDAC2. Despite not affecting histone acetylation, HDAC8 selective inhibitors nevertheless can induce particular phenotypes in tumor cells such as apoptosis, cell cycle block, and differentiation [69]. This suggests that HDAC8 selective inhibitors do not act via induction of histone acetylation at certain promoter sites, but may rather act by changing the acetylation level of non-histone target proteins. The molecular mechanism of PCI-34051 in inducing apoptosis of T-cell lymphoma cells involves phospholipase Cγ1 signalling, intracellular mobilization of calcium from endoplasmic reticulum and cytochrome *c* release from mitochondria [69]. In neuroblastoma cells, selective inhibition of HDAC8 does not induce apoptosis, but stimulates neurite outgrowth and expression of MAP2, neurofilament, neurotrophin receptor A kinase and downregulates the stem cell marker nestin indicating induction of neuronal differentiation. Thus, selective HDAC8 inhibition can activate apoptosis or differentiation programs in cancer cells independent from bulk histone acetylation.

5.1.3. Other class I selective inhibitors

The cyclic tetrapeptide apicidin inhibits HDAC2 and 3, and to a lesser extent HDAC8, but does not target HDAC1 or other class II members [88]. The short chain fatty acid valproic acid inhibits class I HDACs 1, 2, 3, and 8 in the millimolar range, but was ineffective against class II HDACs [88]. Valproic acid has long been used for the treatment of seizure disorders and is currently tested in several cancer trials. However, one major drawback is the very high concentration required for its anti-tumoral activity.

5.2. Class II inhibitors

5.2.1. HDAC6 selective inhibitors

Tubacin was the first discovered selective HDAC inhibitor. The compound inhibits HDAC6 leading to α-tubulin acetylation in mammalian cells without affecting histone acetylation [77]. Tubacin treatment of cells resulted in inhibition of migration due to altered cell adhesion in NIH-3T3 fibroblasts [116]. In breast cancer cells, tubacin prevents estradiol-stimulated cell migration [76]. Tubacin also inhibits epithelial-mesenchymal transition of tumor cells, a process promoting cell motility and invasion [81]. The molecular basis for this effect involves interference with the TGFβ-SMAD3 signalling pathway [81]. Tubacin also blocks aggresome function, which is dependent on HDAC6 activity to degrade unfolded and misfolded ubiquitinated proteins similar to the proteasomal pathway [117]. In multiple myeloma cells, tubacin induces marked accumulation of ubiquitinated proteins, and synergistically augments bortezomib-induced cytotoxicity by c-Jun NH_2-terminal kinase/caspase activation [117]. Recently, thiolate analogues NCT-10a and NCT-14a were discovered as HDAC6 selective inhibitors [118,119]. The compound themselves did not display growth inhibitory activity but significantly increased the effect of paclitaxel on cancer cell growth [119] and inhibited the growth of estrogen-dependent breast cancer cells. Mercaptoacetamides have been recently described to selectively inhibit HDAC6 compared with HDAC1, 2, 8, 10 and to protect cultured neurons from oxidative stress [120].

Similar to HDAC8 selective inhibitors, HDAC6 selective inhibitors act independently from changing histone acetylation. In contrast to pan-HDAC inhibitors, HDAC6 selective inhibition did not significantly change gene expression signatures in microarray analysis, did not alter cell cycle progression, and did not lead to aberrant mitotic spindle formation [77]. This clearly shows a rather selective effect of HDAC6 inhibitors on cell biology. Interestingly, HDAC6 targeted deletion in mice does not impair normal development or major organ functions [34], suggesting that HDAC6 inhibition in clinical settings would not cause major side effects in contrast to inhibition of other HDACs, in particular class I HDACs.

Thus, tubacin and other HDAC6 selective inhibitors may be useful in cancer treatment by inhibition of tumor cell migration and metastasis, and in combination with drugs inducing stress response pathways in cancer cells. These compounds exert their effects on cells without changing histone acetylation-dependent epigenetic processes through acetylation of cytoplasmic proteins such as α-tubulin and heat shock proteins.

Other class II selective inhibitors include APHA derivatives displaying 120-fold selectivity for HDAC4 over HDAC1 [121] and recently identified Trifluoroacetylthiophenes showing HDAC4 and HDAC6 selectivity [122]. In cell based assays using U937 leukemic cells, HDAC4 selective APHA derivative did not induce apoptosis, cell cycle arrest or differentiation in contrast to SAHA (Vorinostat) [121].

Table 3 summarizes the selectivity of the discussed HDAC inhibitors. The compounds are grouped into pan-HDAC inhibitors, class I, and class II HDAC selective inhib-

itors, respectively. From these data it is evident that most of the selective inhibitors have not been tested against the whole panel of the HDAC family. In particular, information on the inhibitory activity against HDAC5, 10, and 11 is largely lacking. Table 3 also illustrates, that application of a given HDAC inhibitor at an inappropriate high concentration is likely to produce "off-target" effects on multiple HDACs. Thus, at present there are only very few HDAC inhibitors which have been proven to be truly selective.

6. Summary and outlook

The classical human HDAC family consist of 11 class I, II and IV members with non-redundant functions in normal development and cancer biology. In tumor tissues, expression of distinct HDAC family members is up-regulated and correlates with clinical outcome of patients. Class I HDACs have been considered the main enzymes relevant as anticancer drug targets. Indeed, targeted disruption in mice and siRNA-mediated knockdown in cultured cancer cells revealed strong anti-proliferative and proapoptotic effects. However, there is now increasing evidence that class II HDACs are also promising drug targets involved in regulation of differentiation, proliferation, stress response, migration, and angiogenesis. This suggests that the plethora of anti-tumor effects observed in cancer cells following exposure to pan-HDAC inhibitors can be explained as the sum effect of targeting non-redundant HDAC functions in cancer cells. This could be of benefit for anti-cancer treatment as the tumor cell is hit at multiple cellular key functions simultaneously. Currently, pan-HDAC inhibitors are in phase I–III clinical trials and Vorinostat was the first HDAC inhibitor to be approved for treatment of a malignant disease. However, these trials also show that treatment of patients with unselective pan-HDAC inhibitors is associated with toxicities of the gastrointestinal, hematopoietic, cardiac, and nervous system. Therefore, it remains to be shown whether selective inhibitors will be equally effective but harbor less side effects. So far, phase I trials of class I selective HDAC inhibitors show similar clinical efficacy and toxicity compared with pan-HDAC inhibitors. It will be now important to also evaluate class II selective HDAC inhibitors in animal models and clinical trials. An interesting strategy for the development of future treatment concepts involving HDAC inhibitors may be to identify the most relevant HDAC driving tumorigenesis in a given tumor in an individual patient and subsequently select the appropriate combination of compounds from a library of selective inhibitors for optimal treatment.

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