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HDACs and the epigenetic plasticity of cancer cells: Target the complexity

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ABSTRACT

Cancer cells must adapt to the hostile conditions of the microenvironment in terms of nutrition, space, and immune system attack. Mutations of DNA are the drivers of the tumorigenic process, but mutations must be able to hijack cellular functions to sustain the spread of mutant genomes. Transcriptional control is a key function in this context and is controlled by the rearrangement of the epigenome. Unlike genomic mutations, the epigenome of cancer cells can in principle be reversed. The discovery of the first epigenetic drugs triggered a contaminating enthusiasm. Unfortunately, the complexity of the epigenetic machinery has frustrated this enthusiasm. To develop efficient patient-oriented epigenetic therapies, we need to better understand the nature of this complexity. In this review, we will discuss recent advances in understanding the contribution of HDACs to the maintenance of the transformed state and the rational for their selective targeting.

Contents

1. Introduction

The drawing and maintenance of complexity in multicellular organisms, as well as the adaptive responses that characterize the life of cells in tissues, are achieved through the control of gene expression. The first step in the long journey by which genes determine/control phenotype is transcriptional control. Access to the DNA sequence is a fundamental decision that enables the initiation of transcription and is operated through the control of chromatin compaction.

Changes in the transcriptional landscape are associated with almost all pathological conditions. Cancer is an altered cellular fate that is

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CdLS, Cornelia de Lange Syndrome; CSCs, cancer stem cells; DDR, DNA damage response; DLBCL, diffuse large B-cell lymphoma; EMT, epithelial-mesenchimal transition; ER, estrogen receptor; GEP-NET, gastroenteropancreatic neuroendocrine tumors; GBM, glioblastoma multiforme; HCC, hepatocellular carcinoma; HDACs, histone deacetylases; HDACIs, histone deacetylase inhibitors; KATs, lysine acetyl-transferases; KD, knock-down; KO, knock-out; MDSC, myeloid-derived suppressor cells; MEF2, myocyte enhancer factor 2; MM, multiple myeloma; NHEJ, non-homologous end joining; SE, super-enhancers; TCA, tricarboxylic acid cycle; TE, typical enhancers; TFs, transcription factors.

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constantly evolving. DNA mutations are the major causes of cancer occurrence and progression. With few exceptions, most mutations of cancer driver genes show their deleterious effects in only one or a few tissue types. It has been hypothesized that, among other factors, the pre-existing epigenetic landscapes that characterize different tissues may or may not allow mutant oncogenes or tumor suppressor genes to exert their malignant outcomes (Haigis, Cichowski, & Elledge, 2019). As recently observed, the transforming ability of the oncogenic mutation in $BRAF^{V600E}$ is robustly manifested in neural crest and melanoblast populations, whereas melanocytes are much less responsive. This oncogenic competence depends on chromatin-modifying enzymes such as ATAD2, which binds the transcription factors (TFs) SOX10 and MYC (Baggiolini et al., 2021).

Histones are the major structural proteins associated with DNA and the major targets of epigenetic control. Throughout evolution, several post-translational modifications (PTMs) of histones have been selected to regulate chromatin accessibility rapidly and reversibly. Among the various PTMs, acetylation acts as a distinct switch to favor TFs access to DNA. As is always the case with PTMs, acetylation is under the supervision of antagonizing enzymes: the histone acetyl-transferases or lysine acetyl-transferases (KATs) and histone deacetylases (HDACs) or lysine deacetylases (Drazic, Myklebust, Ree, & Arnesen, 2016).

Although originally defined as enzymes involved in the dynamic regulation of acetylation, it is currently emerging that additional PTMs can be monitored by HDACs and KATs. Short-chain lysine acylations can characterize histones and non-histone proteins. Formylation, propionylation, butyrylation, crotonylation, 2-hydroxyisobutyrylation, β-hydroxybutyrylation, succinylation, malonylation, glutarylation, and benzoylation dynamics have been shown to be under the supervision of KATs and HDACs (Aramsangtienchai et al., 2016; Kelly et al., 2018; Sabari, Zhang, Allis, & Zhao, 2017; Zhao, Zhang, & Li, 2018)

There are two main reasons that make HDACs promising targets for cancer therapy: i) their activities need to be hijacked to maintain the transformation state; ii) they are directly responsible for transformation events.

2. HDACs

Since their discovery more than 25 years ago, histone deacetylases have attracted much attention as potential targets for cancer therapies. Indeed, even before the cloning of the first HDAC, small natural compounds were known to increase histone acetylation, alter transcription, and induce growth arrest and cell death. Moreover, these compounds were fundamental to the identification of HDACs (Taunton, Hassig, & Schreiber, 1996). The use of HDAC inhibitors (HDACIs) in clinic, as single agent, is limited to few hematological tumors: the cutaneous T-cell lymphoma and the peripheral T-cell lymphoma (Bose, Dai and Grant, 2014; Moskowitz and Horwitz, 2017). After the initial enthusiasm for the treatment of these malignancies, the subsequent failures of several trials have led to a rethinking of the potential use of HDACIs in the clinic (Fig. 1A/B). Currently, several clinical trials are redesigning the use of HDACIs in combination therapies (Karagiannis & Rampias, 2021). All approved HDACIs are pan-selective inhibitors of zinc-dependent HDACs and interfere with the active site (Fig. 1C/D). The non-selective action of these inhibitors could be an explanation for the side effects, also severe, experienced by some patients.

In mammals, 18 different HDACs are expressed, 11 of which are zinc-dependent. In general, they are part of multiprotein complexes and can regulate lysine deacetylation of a relatively wide range of substrates, although histones remain an important target.

HDACs are classified into five subfamilies based on their sequence homology, catalytic activities, and phylogenetic criteria (Fig. 2). There are several excellent reviews discussing the structural differences, the specific regulatory mechanisms, and the various multiprotein complexes in which HDACs are found (Emmett & Lazar, 2019; Porter & Christianson, 2019; Bahl & Seto, 2021; Li, Tian, & Zhu, 2020, Millard, Watson, Fairall, & Schwabe, 2017; Di Giorgio & Brancolini, 2016). Here, we will focus on the most recent studies addressing the contribution of zinc dependent epigenetic HDACs to cancer development and the potential for targeting them in cancer therapy.

3. Class I HDACs

3.1. Structural features and rational of targeting

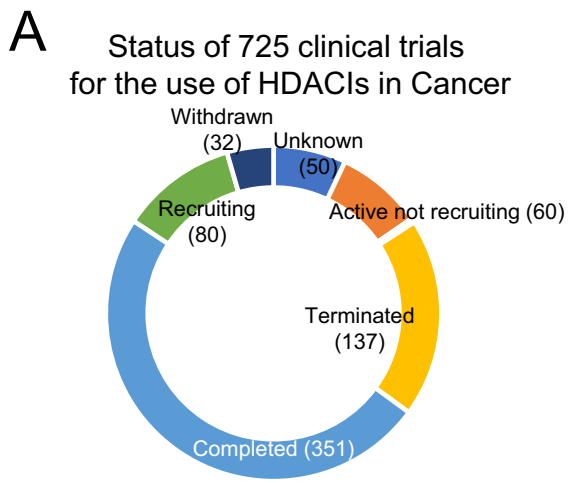
To better understand the contribution of these family members, which include HDAC1/2/3/8, to carcinogenesis and to rationally develop therapeutic strategies, it is important to recognize that they mainly do not act as isolated enzymes but as part of multiprotein complexes. The composition of these complexes is variable and can change according to cellular requirements. HDAC8 is the exception. It has strong catalytic activity even when not in complex with other partners. HDAC8 is subject to allosteric regulation, with populations moving back and forth between active and inactive states. A helix-loop-helix forms the "allosteric domain" that coincides with the distal region of the enzyme. This region is responsible for interaction with partners and is subject to regulatory post-translational modification (Lee, Rezai-Zadeh and Seto, 2004; Millard et al., 2017; Werbeck et al., 2020). Structural studies have revealed further peculiarities of HDAC8 in the catalytic pocket. In class I HDACs, a narrow hydrophobic channel allows acetyl-lysine to enter the catalytic chamber where the zinc ion is located. A second channel is perpendicular to the first channel and has been termed the 'foot pocket'. It is believed that the foot pocket forms the exit pathway for the acetate product (Millard et al., 2017; Wang, Wiest, Helquist, Lan-Hargest, & Wiech, 2004; Whitehead et al., 2011). In HDAC8, the presence of a tryptophan limits the available space in the foot pocket. The structural features of the foot pocket have been exploited to develop isoform-specific inhibitors. Benzamides and in particular MS-275/entinostat (Fig. 3) is considered a specific class I inhibitor and shows some selectivity for HDAC1/2/3 over HDAC8 (Hu et al., 2003).

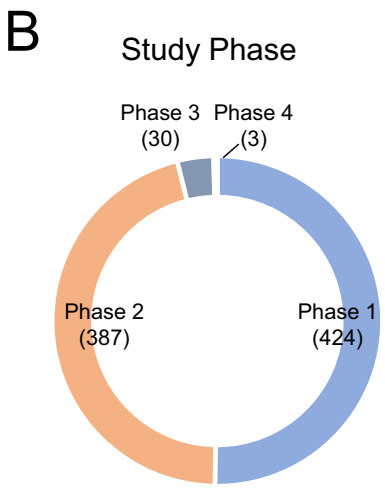
4. HDAC1 and HDAC2

HDAC1/2 are recruited to a multiprotein complex that promotes the acquisition of fully competent enzymatic activity. HDAC1/2 can remove the acetyl or other acyl groups from target lysines that are not restricted to histone proteins. These two HDACs are highly homologous (> 80% aa identity), often fully interchangeable, and are recruited to various multiprotein complexes involved in epigenetic control of gene expression (Millard et al., 2017). There are also reports showing independent actions of HDAC1 and HDAC2. For example, HDAC2, but not HDAC1 and HDAC6, represses the formation of primary cilia in pancreatic ductal carcinoma cells (Kobayashi et al., 2017). The primary cilium is a microtubule-based structure that is present on the surface of many cells and is often lost from cancer cells. As a sensor, it coordinates signaling responses to environmental stimuli and its repression sustains cell proliferation (Peixoto, Richard, Pant, Biswas, & Gradilone, 2020).

The most investigated HDAC1/2-containing complexes are the SIN3 (switch-independent 3) [Hassig, Fleischer, Billin, Schreiber, & Ayer, 1997; Clark et al., 2015; Banks et al., 2020; Laherty et al., 1997) the MiDAC (mitotic deacetylase) (Bantscheff et al., 2011; Itoh et al., 2015), the CoREST (co-repressor of REST) (You, Tong, Grozinger, & Schreiber, 2001; Song et al., 2020), the MIER (mesoderm induction early response) (Ding, Gillespie, & Paterno, 2003), and the RERE (arginine-glutamic acid dipeptide repeats) (Plaster, Sonntag, Schilling, & Hammerschmidt, 2007) (Fig. 4). These complexes contain scaffold proteins that, not only enhance the enzymatic activity of HDAC1/2, but also mediate the interaction with selected TFs.

The contribution of HDAC1/2 to cancer is not easy to assess. Direct dysfunction of these HDACs is frequently observed in the form of expression levels in various tumors, whereas mutations are rare (http://www.cbioportal.org). However, because these HDACs assemble in





C
Studies Phase 4

Study Title	Status	Interventions	Conditions
PD-1 Antibody, Chidamide, Lenalidomide and Etoposide for Relapsed or Refractory NK/T Cell Lymphoma	Unknown	Drug: PD-1 Antibody, chidamide, lenalidomide and etoposide	NK/T Cell Lymphoma
PD-1 Antibody, Chidamide, Lenalidomide and Gemcitabine for Peripheral T-cell Lymphoma	Recruiting	Drug: PD-1 blocking antibody, chidamide, lenalidomide and gemcitabine	Peripheral T-cell Lymphoma
CINC424A2X01B Rollover Protocol	Recruiting	Drug: ruxolitinib tablets or oral pediatric formulation, panobinostat capsules. Drug: ruxolitinib tablets or oral pediatric formulation	Primary Myelofibrosis, Chronic Idiopathic Myelofibrosis, Post Polycythemia Vera Myelofibrosis

Fig. 1. HDACIs in clinic.
A) Summary of the status for all clinical trials using HDACIs for cancer treatment.
B) Summary of all clinical trials using HDACIs for cancer treatment subdivided for the specific phase.
C) Description of the phase 4 clinical trials. Data were obtained from https://www.clinicaltrials.gov

distinct multiprotein complexes, dysfunction in any member of the complex could be responsible for affecting deacetylase activities during the tumorigenic process. Importantly, in cancer cells, additional copies of HDAC1/2 could interact with noncanonical partners or alternatively induce proteotoxic stress (Brancolini & Iuliano, 2020). There are several reports highlighting a role for HDAC1, HDAC2, or both in certain aspects of the transformation process, such as the pro-angiogenic switch or regulation of TP53 functions (Hulsurkar et al., 2017; Stojanovic et al., 2017). HDAC1/2 complexes can also influence the acetylation status of non-histone proteins. A well-known example is GLI1, a downstream TF of the Hedgehog pathway involved in tumorigenesis (Hui & Angers, 2011). HDAC1/2 deacetylate K518ac of GLI1 to allow its association with chromatin and activation of transcription (Canettieri et al., 2010). Control of acetylation status is important for the sequestration of GLI1 at the nuclear lamina (Mirza et al., 2019).

4.1. HDAC1/2, typical enhancers and super-enhancers

H3K4me1 and H3K27ac mark typical enhancers (TE) and super-enhancers (SE). SE are clusters of stretched TE, in which binding of multiple TFs can enhance transcriptional output. These distal regulatory elements control cell identity by regulating differentiation-specific genes (Aranda-Orgilles et al., 2016, Di Stefano et al., 2016). The epigenetic architecture that coordinates the activities of TE and SE is frequently altered in cancer to maintain the oncogenic transcriptional program (Mathison et al., 2021).

The CoREST complex coordinates various epigenetic modifications to repress gene expression. Via LSD1/KDM1A (lysine-specific demethylase 1), it catalyzes the demethylation of mono- and di-methylated lysine 4 of histone H3, while via HDAC1/2 it can deacetylate H3K27ac (Shi et al., 2004). The CoREST complex is therefore the ideal molecular machinery to silence TE and SE. In leukemia cells of the erythro-megakaryocyte lineage, CoREST can control the expression of TFs involved in myeloid differentiation, including GFI1, a master regulator of neutrophil development (Karsunky et al., 2002). This effect is achieved by silencing a super-enhancer known as GFI1-SE and can be controlled pharmacologically (Tatsumi et al., 2020).

The repressive action of HDAC1 at enhancers, as important event in the tumorigenic process, has also been observed in acute myeloid leukemia (AML). Here a repressive multiprotein complex composed of the Forkhead factor FOXC1, RUNX1, the Groucho repressor TLE3 and HDAC1 inhibits the activity of enhancers controlling the monocyte/

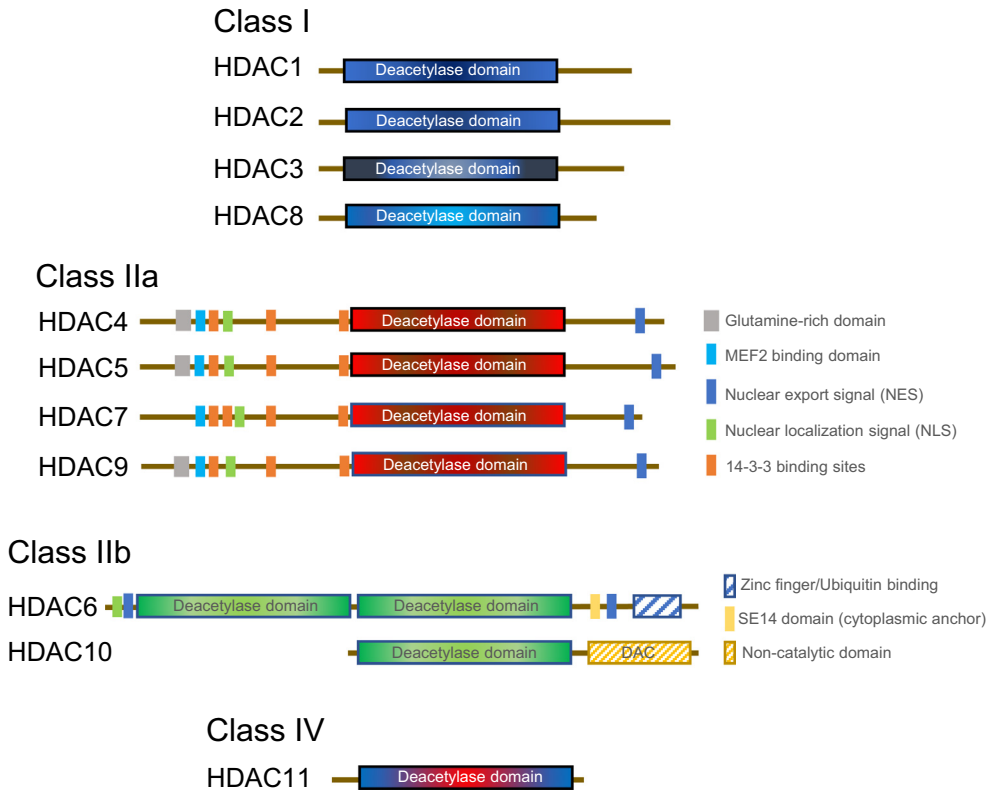


Fig. 2. Schematic representation of the major domains or regulatory regions in the 11 metal (zinc)-dependent human HDACs.
In mammals, HDACs are classified based on homology to the yeast's deacetylases Rpd3, Hda1, and Sir2. Class I is structurally related to Rpd3 (Reduced potassium dependency 3) and includes HDAC1, 2, 3, and 8. Class II is related to Hda1 and is further divided into two subclasses: Class IIa (HDAC4, 5, 7, and 9) and class IIb (HDAC6 and 10). In vertebrates, class IIa members contain a His/Tyr substitution in the catalytic site that nearly abolishes enzymatic activity. They are characterized by nuclear/cytoplasmic shuttling. Class IIb are largely cytosolic enzymes that act mainly on non-histone proteins such as microtubules or on polyamines (Hai, Shinsky, Porter, & Christianson, 2017). Class IV includes only HDAC11, which has sequence similarities to class I and II proteins. The class III includes enzymes that share homologies with Sir2 (Silent Information Regulator 2) and the seven sirtuins that are NAD -dependent protein deacetylases and/or ADP ribosylases are not shown.

macrophage differentiation and promotes the tumorigenic process (Simeoni et al., 2021).

HDAC1/2 may also indirectly sustain SE reprogramming. In glioblastoma multiforme (GBM) HDAC1/2 activities are required to support expression of the MYC oncogene. MYC in turn organizes SE that feed glycolysis and the Warburg effect. Non-selective HDACIs affect the expression of MYC, the organization of SE and the induction of TFs that are master regulators of the oxidative metabolism. In patient-derived xenografts, this metabolic rewiring can be addressed with inhibitors of fatty acid oxidation in combination with HDACIs. The combination of epigenetic resetting and metabolic inhibition may more effectively reduce tumor growth (Nguyen et al., 2020).

4.2. Glioblastoma multiforme (GBM) and the paradigm of the complexity

The pro-oncogenic activity of an epigenetic regulator can be attributed to different targets as part of different protein complexes. In GBM, silencing of HDAC1, but not HDAC2, profoundly affects proliferation of glioma stem cells by reactivating TP53 activities. Under these conditions, the cells reduce their proliferation and eventually die. However, when the same GBM cells are transplanted in a different microenvironment *in vivo*, the absence of HDAC1 leads to a more aggressive/invasive phenotype through activation of STAT3 (Lo Cascio et al., 2021). This study confirms the involvement of HDAC1 in GBM but opens a new scenario and gives rise to new cautions for the use of selective HDAC inhibitors (Puchalski et al., 2018, Qazi et al., 2017). Given the extreme heterogeneity of GBM, which is evident also between the margin and the core of the tumor, these results need to be validated with different patient-derived cells and ideally with knowledge of the specific mutational burden.

Tumor-initiating cells may be present at the periphery/edge of the tumor and cause recurrence from there (Minata et al., 2019). GBM spheres generated with these peripheral/marginal cells show higher expression of HDAC1 compared with nucleated spheres. Accordingly, high HDAC1 expression correlates with poorer prognosis. In contrast, HDAC2 expression correlates with a better prognosis. The aggressiveness and transcriptional profile that characterize these tumor-initiating cells require the presence of HDAC1.

Proteomic studies aimed at mapping the multiprotein complexes present in GBM cell lines have shown that HDAC1/2 belongs to the "classical CoREST complex" with NCOR1/LSD1. In addition, HDAC1 can also be isolated as part of a complex with PARP1, Ku70/Ku80, and CHD4 involved in the non-homologous end-joining (NHEJ) DNA repair pathway (Connelly, Hedrick, Paschoal Sobreira, Dykhuizen, & Aryal, 2018). Consequently, impairment of HDAC1/2 activities both genetically and pharmacologically impairs cell proliferation and survival (Was et al., 2019).

4.3. HDAC1/2 and the metastatic process: hopes for therapies

The treatment of metastatic cancer is still an unsolved therapeutic problem. Dysregulations of HDAC1 activity have recently been discovered in metastatic breast cancer. Chromatin remodeling complexes monitor access to DNA by hydrolyzing ATP and modulating the topology of nucleosomes. The SWI/SNF ATPase complex was first identified in yeast and is the best characterized chromatin remodeler. (Centore,

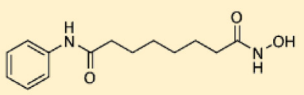
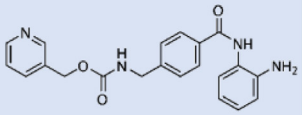
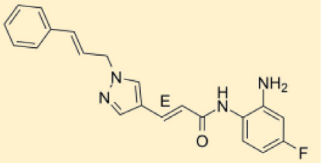
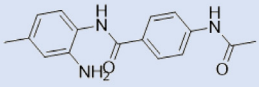
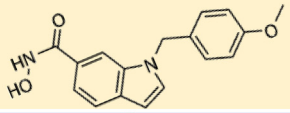
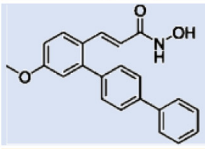
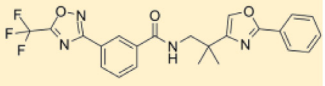
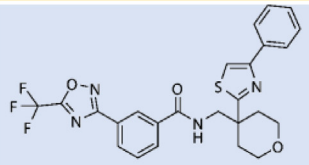
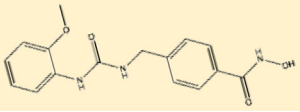
Inhibitor	Structure	Target	Main rerference	Clinical trials
Vorinostat (SAHA)		pan-HDAC Inhibitor	Richon V.M. et al. Second generation hybrid polar compounds are potent inducers of transformed cell differentiation. Proc Natl Acad Sci U S A. 1996;93(12):5705-5708. doi:10.1073/pnas.93.12.5705	❖ Breast Cancer Phase II NCT00365599 ❖ Prostate Cancer Phase II NCT00330161 ❖ Lung Cancer Phase I NCT00821951
Entinostat (MS-275)		HDAC1 HDAC2 HDAC3	Suzuki T. et al. Synthesis and histone deacetylase inhibitory activity of new benzamide derivatives. J Med Chem. 1999;42(15):3001-3003. doi:10.1021/jm980565u	❖ Breast Cancer Phase I NCT02820961 ❖ Colorectal Cancer Phase I/II NCT03215264 ❖ Lung Cancer Phase I NCT01594398
RGFD966		HDAC3	Malvaez M, et al. HDAC3-selective inhibitor enhances extinction of cocaine-seeking behavior in a persistent manner. Proc Natl Acad Sci U S A. 2013;110(7):2647-2652. doi:10.1073/pnas.1213364110	none available
BRD4097		HDAC1 HDAC2 HDAC3 HDAC8	Mondello P. et al. Selective Inhibition of HDAC3 Targets Synthetic Vulnerabilities and Activates Immune Surveillance in Lymphoma. Cancer Discov. 2020;10(3):440-459. doi:10.1158/2159-8290.CD-19-0116	none available
PCI-34051		HDAC8	Suzuki T. et al. Rapid discovery of highly potent and selective inhibitors of histone deacetylase 8 using click chemistry to generate candidate libraries. J Med Chem. 2012;55(22):9562-9575. doi:10.1021/jm300837y	none available
22D		HDAC8	Huang W.J.et al. Synthesis and biological evaluation of ortho-aryl N-hydroxycinnamides as potent histone deacetylase (HDAC) 8 isoform-selective inhibitors. ChemMedChem. 2012;7(10):1815-1824. doi:10.1002/cmdc.201200300	none available
TMP-195		Class IIa	Lobera M. et al. Selective class IIa histone deacetylase inhibition via a nonchelating zinc-binding group. Nat Chem Biol. 2013;9(5):319-325. doi:10.1038/nchembio.1223	none available
TMP-269		Class IIa	Lobera M. et al. Selective class IIa histone deacetylase inhibition via a nonchelating zinc-binding group. Nat Chem Biol. 2013;9(5):319-325. doi:10.1038/nchembio.1223	none available
Elevenostat		HDAC11	Huang J. et al. Histone/protein deacetylase 11 targeting promotes Foxp3+ Treg function. Sci Rep. 2017;7(1):8626. Published 2017 Aug 17. doi:10.1038/s41598-017-09211-3	none available

Fig. 3. Chemical structures, HDACs targets, references, and current clinical trials for the HDACIs mentioned in the text.

Sandoval, Soares, Kadoch, & Chan, 2020, Reyes, Marcum, & He, 2021). In human there are three SWI/SNF complexes known as BAF (BRG1/BRM-associated factor), p-BAF and ncBAF (non-canonical BAF) (Mashtalir et al., 2018; Michel et al., 2018). BRG1/SMARCA4 and BRM/SMARCA2 provide the ATPase activity which generates the energy necessary for nucleosome sliding and eviction, mutually exclusive to all complexes. Furthermore, a constellation of additional partners characterizes the different chromatin remodelers. ARID1A is a specific subunit of the BAF complex. Recently, inactivating mutations in this gene have been reported in treatment-resistant tumours and metastases (Yates et al., 2017) as well as in ER+ breast cancer (Pereira et al., 2016). In breast cancer, the pioneer factor FOX1A recruits ARID1A to chromatin to modulate expression of ER-target genes from enhancer elements, independent of nuclear receptor activation. HDAC1 interacts with ARID1A and tamoxifen may promote recruitment of HDAC1 to ER complexes to mediate a repressive effect (Papachristou et al., 2018). Patient-derived xenograft models mutant for ARID1A are characterized by a reduction in interactions between ER, HDAC1, and BAF components. Finally, loss of ARID1A is associated with a lack of recruitment of HDAC1 and an increase in histone H4 acetylation in regulated genomic regions (Nagarajan et al., 2020). Although it is unclear which HDAC1 subcomplex plays a role in this context, this discovery opens a new scenario for the treatment of patients carrying ARID1A mutations. In these patients, instead of HDAC inhibitors, BET inhibitors should be more effective (Bechter & Schöffski, 2020).

ARID1A also affects HDAC2 activities independently of HDAC1. ARID1A mutations are characteristic of more than 50% of ovarian cancers. These mutations enable the interaction between HDAC2 and the

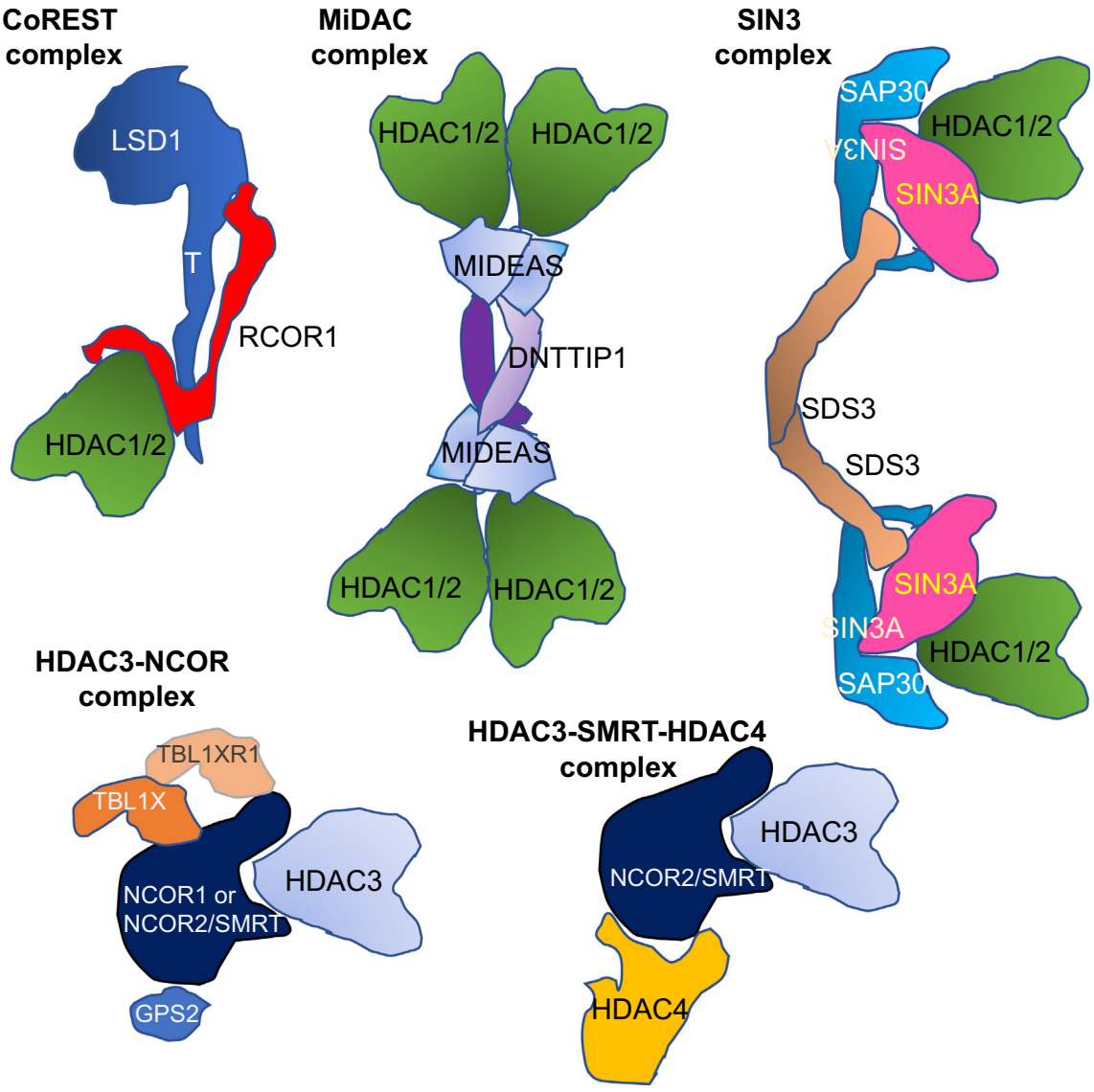


Fig. 4. Different multiprotein complexes containing HDAC1/2 or HDAC3 activities
Some of different multiprotein complexes which assemble HDACs are described. HDAC4 was selected as an example of class IIa HDACs for the interaction with NCOR2/HDACs (Park et al., 2018)

catalytic subunit EZH2 of PRC2 (Polycomb repressive complex 2). HDAC2 can affect the expression of a subset of genes regulated by EZH2/ARID1A, and SAHA/vorinostat can increase survival in mice with ARID1A-mutated cancer (Fukumoto et al., 2018).

Another complex of HDAC1/2 has been shown to be involved in melanoma metastasis. Here, the SIN3A-HDAC1/2 complex silences BMP6 expression and promotes metastatic spread and tumor growth by suppressing BMP6-activated SMAD5 signaling (Min et al., 2020). Similarly, in colon cancer, miR-500a-5p targeting the 3' end of *HDAC2* mRNA is downregulated during cancer progression and HDAC2 is necessary to maintain proliferation and invasion of neoplastic cells (Tang et al., 2019). Again, the molecular complexes and specific mechanisms by which HDAC2 is involved in colon cancer cell aggressiveness remain to be elucidated.

4.4. HDAC1/2 as tumor suppressors

Depending on the context HDACs can also exert tumor suppressive actions. High-risk B-cell acute lymphoblastic leukemia is frequently characterized by loss of IKAROS/IKFZ1 functions. IKAROS binds to the promoter of the anti-apoptotic gene BCL2L1/BCLXL where it recruits HDAC1, to repress transcription (Song et al., 2020). In this context, targeting HDAC1 activity could have a deleterious effect on tumor growth due to apoptotic resistance. A role that has also been observed in previous studies (Paz-Priel, Houng, Dooher, & Friedman, 2011). Focusing on a single target gene could lead to misinterpretation, as HDAC1/2 multiprotein complexes can also repress the expression of pro-apoptotic genes (Contreras et al., 2013; Piazza et al., 2013; Ramsey, He, Forster, Ory, & Ellisen, 2011). Cancer cells could switch the repressive influence of HDAC1 on pro-apoptotic genes by controlling the phosphorylation status of HDAC1. The catalytic activity of HDAC1 is modulated by serine phosphorylation (Pflum, Tong, Lane, & Schreiber, 2001) and cancer cells can sustain it (Citro, Miccolo, Meloni, & Chiocca, 2015). HDAC1 is also phosphorylated at multiple tyrosines. In particular, Tyr72 plays an important role in regulating HDAC1 stability, and can be phosphorylated by EGFR. This phosphorylation impinges the anti-apoptotic role of HDAC1 and the repression of the pro-apoptotic BH3-only family member BIM1 (Bahl et al., 2021).

Further studies reported the tumor suppressive effect of HDAC1/2 in a specific context. Aggressive forms of mantle cell lymphoma express

high levels of the neuronal transcription factor SOX11. CyclinD1/CCND1 has been shown to bind HDAC1/2 and sequester it into the cytoplasm. Although there are several aspects of this mechanism that deserve further validation, knockdown of HDAC1/2 is sufficient to increase acetylation of H3K9/14 at the SOX11 promoter and increase its expression. (Mohanty et al., 2019).

The TF Sall4, also known as stem cell factor (Zhan et al., 2006), is strongly upregulated in hyperplastic, melanoma-prone murine melanocytes, where it sustains cell proliferation (Diener et al., 2021). Although Sall4 is required for primary tumor growth, loss of Sall4 results in increased metastatic burden. The anti-invasive effect of Sall4 is explained by a repressive effect on a number of melanoma-specific invasiveness genes. The repressive influence of Sall4 is enabled by the establishment of a complex with Hdac2 at the regulatory elements of invasive genes. Here, the Sall4/Hdac2 complex represses their expression through an epigenetic switch involving the reduction of H3K27ac levels (Diener et al., 2021). This result contrasts with the pro-metastatic role of HDAC1/2 in melanoma through BMP6 regulation discussed above (Smart, Oleksak, & Hartsough, 2021). Clearly, further studies are needed to rationalize the use of specific inhibitors targeting the metastatic behavior of melanoma. Fig. 5 summarizes some of the pro-oncogenic and tumor suppressive actions of HDAC1/2.

4.5. HDAC1/2, the DNA damage response (DDR) and the genome stability

In response to genotoxic insults epigenetic modifications cooperate with the DNA damage response to orchestrate an efficient repair (Mir et al., 2021; Van & Santos, 2018). DNA double-strand breaks (DSB) are the most deleterious type of DNA damage. They are repaired mainly by two major pathways: nonhomologous end-joining (NHEJ) and homologous recombination (HR) (Gavande et al., 2016). Lysine 85 of linker histone H1 is dynamically acetylated in response to DNA damage by the antagonistic activities of the acetyl-transferase p300/CBP-associated factor (PCAF/KAT2B) and HDAC1. Acetylation at this residue promotes chromatin compaction through greater interaction with nuclear histones and recruitment of HP1 (heterochromatin protein 1). A condition that makes cells more susceptible to death in response to genotoxic agents, possibly due to an increased amount of DNA damage (Li et al., 2018).

HDAC1/2 can also control the acetylation status of core histones during DDR. By deacetylating of H3K56, they promote NHEJ repair (Miller et al., 2010). An activity monitored by USP38. This deubiquitylase binds to HDAC1 and removes the K63-linked ubiquitin chain. Within the CoREST complex, deubiquitylation of HDAC1 increases its interaction with SIN3 (Yang et al., 2020). It is possible, but currently unknown, that HDAC1/2 might also modulate acetylation of proteins involved in NHEJ repair. In this context, the use of isoform-specific HDACs inhibitors in combination with inhibitors of the various DDR signaling pathways should be investigated.

Other studies have discovered additional contributions of HDAC1/2 complexes during NHEJ. Chromodomain helicase DNA binding protein 7 (CHD7) is recruited to damaged sites in a PARP-dependent manner to aid repair. Chromatin at the damaged site must undergo a cycle of decondensation and condensation to promote the recruitment of protein complexes involved in DDR and NEHJ repair. PARP triggers the relaxation of chromatin. This relaxation must be spatially controlled to initiate NHEJ repair. Here, it was proposed that the deacetylase activities of HDAC1/2 act as limiting factors to concentrate the classical NHEJ complexes at the cleaved site by preventing their spread along the chromosome (Rother et al., 2020).

Regulation of the stability of class I HDACs has also been associated with DNA damage. HDAC2, together with HDAC3, can form a noncanonical complex with PACS-1 (phosphofurin acidic cluster sorting protein-1). PACS-1 is a well-known multifunctional regulator of membrane trafficking that shuttles between the Golgi and the early endosome (Thomas et al., 2017). During the cell cycle, PACS-1 can accumulate in the nucleus, where it maintains genome stability by buffering replication stress. The action of PACS-1 is important in protecting HDAC2 and HDAC3 from degradation in replication forks and thus can support the establishment of the desired chromatin microenvironment (Mani et al., 2020).

HDAC1/2 are also responsible for preserving the genome stability during mitosis. The MiDAC complex contains as core components MIDEAS (mitotic deacetylase associated SANT domain protein) and DNTTIP1 (deoxynucleotidyltransferase terminal interacting protein

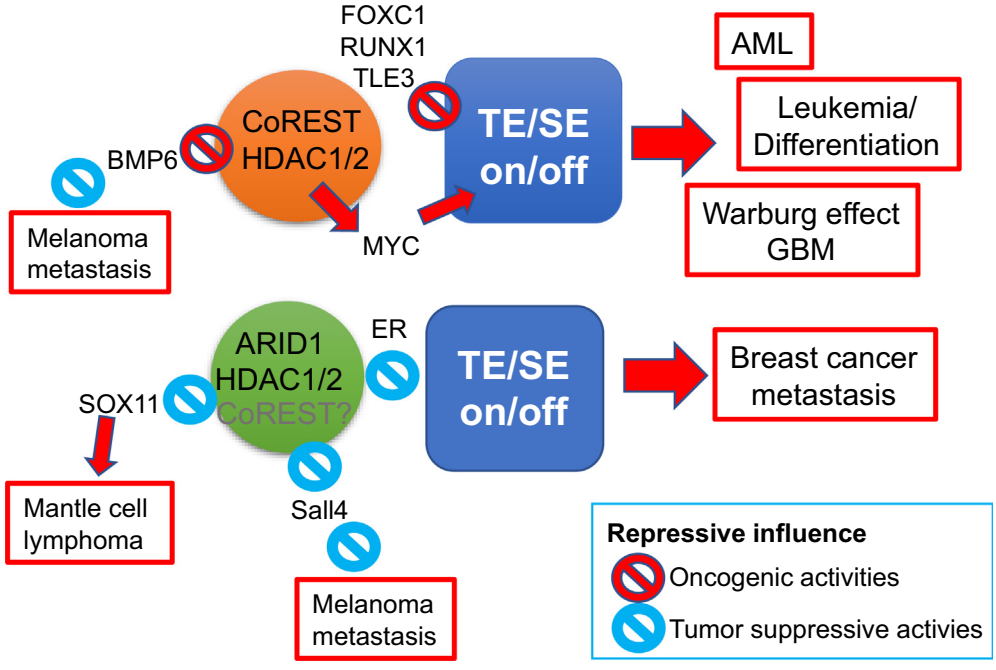


Fig. 5. Some of the pro-oncogenic and tumor suppressive activities of HDAC1/2.

1) which binds and oriented HDAC1/2 (Huttlin et al., 2017; Joshi et al., 2013) The MiDAC complex plays an important role in cell cycle control. Indeed, knockdown of MIDEAS or DNTTIP1 in cancer cells leads to misalignment of chromosomes during mitosis. (Turnbull et al., 2020). Accordingly, the KO of HDAC1 and/or HDAC2 in mouse ES cells results in lagging chromosomes at the anaphase, micronuclei formation, and monopolar spindles (Jamaladdin et al., 2014). A cryo-EM study suggests that DNNTIP coordinates the assembly of the complex by binding MiDEAS and determining the orientation of the HDACs (Fig. 4). The MIDAC complex is quite unique in that it is responsible for the assembly of four HDACs subunits. The authors suggest that the peculiarities of this complex may guarantee the alignment of specific chromatin conformations (Turnbull et al., 2020).

It has been underestimated that the activities of HDAC1/2 related to DDR can be targeted with specific inhibitors. It is very possible that targeting these epigenetic regulators may improve the efficacy of genotoxic therapies (Groselj et al., 2018, Tharkar-Promod et al., 2018). Whether or not manageable in terms of toxicity needs to be carefully evaluated.

4.6. Class I HDACs, the example of entinostat and the immunotherapy

Benzamides and entinostat in particular have recently experienced a new renaissance as HDAC1/2/3 subclass-specific inhibitors (Fig. 3). The new frontier on which many hopes are pinned is the improvement of immunotherapy. Initial studies have shown that a small group of patients with advanced lung cancer benefit from a combination of epigenetic drugs (azacytidine plus entinostat) and nivolumab, a checkpoint inhibitor, targeting PD -L1 (Juergens et al., 2011; Topalian et al., 2012). Preclinical studies have confirmed these initial observations. In syngeneic mouse models of lung and renal cell carcinoma, entinostat alone inhibited the immunosuppressive function of both polymorphonuclear and monocytic-myeloid-derived suppressor cell populations (Orillion et al., 2017).

Tregs are key components for immune system homeostasis (Josefowicz, Lu, & Rudensky, 2012). In cancer, these cells can dampen host anti-tumor immunity, reducing the effectiveness of tumor immune surveillance (Bauer et al., 2014). In patients with metastatic clear cell renal cell carcinoma, entinostat in combination with high dose of IL2 led to downregulation of Fox3p expression and a reduction in the number of Tregs. An effect that enhanced anti-tumor immunity and showed promising clinical activity (Pili et al., 2017). The ability of HDACIs to reinforce immunotherapy has been demonstrated in other studies. Entinostat can suppress the immunosuppressive effect of tumor-infiltrating myeloid cells and reprogram them to eliminate antigen-negative tumor cells. In the presence of entinostat, increased IFN-γ levels in the tumor microenvironment modulate the local cytokine landscape and promote antitumor myeloid polarization (Nguyen et al., 2018). Entinostat also potentiates immunotherapy elicited by an anti-cancer vaccine designed on human carcinoembryonic antigen. In the presence of entinostat, the tumor microenvironment is marked by increased inflammation, enhanced infiltration of activated CD8+ T cells with maximal granzyme B, T-cell responses to different tumor-associated antigens, increased IFNγ and decreased of regulatory T-cells (Hicks et al., 2020). A reprogramming activity towards an immune-permissive tumor microenvironment was confirmed in another study, with beneficial therapeutic effects (Hicks et al., 2021).

These positive results in terms of therapeutic perspective have justified the phase 2 PEMDAC clinical trial. Entinostat was used in combination with the PD-1 inhibitor pembrolizumab for the treatment of metastatic uveal melanoma. This study showed that a small group of patients benefited from the combined treatment. The median overall survival of 13.4 months achieved was longer than the ten-month benchmark survival determined from historical data (Ny et al., 2021).

Mechanistically, the immunosuppressive effect of HDAC1/2 can be exploited by several mechanisms. For example, entinostat may increase the infiltration of MDSC (myeloid-derived suppressor cells) in the tumor microenvironment. In this context, HDAC2 plays the main role, independent of HDAC1. HDAC2 is modulated by CSF1 signaling and suppresses granulocytic chemokine expression in carcinoma-associated fibroblasts, limiting MDSC infiltration. (Kumar et al., 2017).

As part of a broader strategy, the CoREST complex could also be used to enhance immunotherapy. Genetic or pharmacological targeting of the CoREST complex enhances anti-tumour activity in mice (Bantscheff et al., 2011; Xiong et al., 2020).

As mentioned earlier, most cancer-related deaths are due to metastasis. There is an urgent need to develop new therapeutic interventions to treat metastasis. The creation of a favorable microenvironment contributes to tumor spread. Cancer cells at the primary site can sculpture a pre-metastatic niche by releasing soluble factors and extracellular vesicles (Liu & Cao, 2016; Peinado et al., 2017). Myeloid cells derived from bone marrow contribute to the formation of the premetastatic microenvironment. Interestingly, low-dose adjuvant epigenetic therapy (a combination of the DNA methyltransferase inhibitor 5-azacytidine and entinostat) can disrupt the premetastatic microenvironment and inhibit both metastasis formation and growth. Epigenetic switching promotes MDSC differentiation into a more interstitial, macrophage-like phenotype. A reprogramming that inhibits trafficking of MDSC through down-regulation of CCR2 and CXCR2. (Lu et al., 2020).

Entinostat was also identified as a potent inhibitor of regulatory activities responsible for immune-evasion and metastatic potential in patients with gastroenteropancreatic neuroendocrine tumors (GEP-NET). After screening with 107 different compounds, entinostat was isolated because it abrogated tumor growth, after perturbing the transcriptome in GEP-NET (Alvarez et al., 2018).

Collectively, these observations suggest that cancer cells utilize class I HDACs to create an immune-repressive environment and orchestrate immune cells activities. However, the epigenetic repression exerted is dynamic and fully reversible. Simply abrogating class I HDAC activity without any interference with other epigenetic regulators is sufficient to influence the expression of cytokines, chemokines, and other inflammatory signals that remodel immune cells activities (Hicks et al., 2020; Hicks et al., 2021; Nguyen et al., 2018).

5. HDAC3

5.1. Basic concepts

A peculiar feature of HDAC3 is its interaction with nuclear receptor co-repressor 1 (NCOR1) and silencing mediator of retinoic acid and thyroid hormone receptor (SMRT/NCOR2) (Fig. 4). These partners are fundamental for the stabilization of HDAC3 and the maturation of catalytic activity (Emmett & Lazar, 2019). Structural studies have revealed the contribution of inositol tetraphosphate ($Ins(1,4,5,6)P_4$ or IP_4) in stabilizing the interactions between NCOR2 and HDAC3. Other subunits of the complex are TBL1X and TBL1XR1 (Fig. 4). These two proteins contain WD repeats involved in histone recognition and interaction with the ubiquitylation machinery (Yoon et al., 2003). Interestingly, somatic mutations of TBL1XR1 are observed in the most aggressive B-cell lymphomas. Mutant TBL1XR1 shifts the repressive SMRT/HDAC3 complex from binding BCL6 to BACH2 TFs. This switch in partner binding results in an epigenetic reset that impairs the plasma cell differentiation program and instead impinges an immature memory B cell fate. These cells preferentially return to the germinal center to drive lymphomagenesis. (Venturutti et al., 2020).

Mice deficient for Hdac3 do not develop because of defects at the gastrulation stage (Emmett & Lazar, 2019). Interestingly, some catalytically independent activities of Hdac3 were observed in liver, a feature common to class IIa HDACs (Sun et al., 2013). The generation of tissue-specific deletions of *Hdac3* in mice has revealed the critical role of this deacetylase in several contexts (Emmett & Lazar, 2019).

5.2. HDAC3 in cancer

In pediatric rhabdomyosarcomas the HDAC3/NCOR1/NCOR2 complex acts as a differentiation brake. Its deletion reactivates the MYOD1-dependent myogenic program. HDAC3 was identified by a CRISPR/Cas9-based phenotypic screen of class I and class II HDAC genes. Interestingly, the downregulation of HDAC4, albeit to a lesser extent, also promoted the myogenic program (Phelps, Bailey, Vleeshouwer-Neumann, & Chen, 2016). In principle, selective inhibition of HDAC3 could be therapeutic in rhabdomyosarcomas. A similar effect of HDAC3 as an antagonist for the activation of enhancers involved in tumorigenesis has been observed in B-cell lymphomas (Höpken, 2017). Follicular lymphomas and diffuse large B-cell lymphomas frequently accumulate mutations in epigenetic regulators, including the HATs CREBBP and EP300 (Morin et al., 2011). CREBBP silencing in murine and human cells triggers the depletion of H3K27ac marks at enhancers and changes in gene expression that are very similar to signatures characterizing human lymphomas with CREBBP mutations. These CREBBP lymphomas strictly rely on HDAC3 to reduce H3K27ac signals. HDAC3 is recruited to critical enhancers via the BCL6/NCOR2 complex to promote lymphomagenesis (Jiang et al., 2017). A general antagonistic activity of the NCOR1/HDAC3 complex in regulating H3K27ac turnover at genomic loci under the supervision of CREBBP or EP300 was confirmed by additional studies (Basu et al., 2020). An interplay that is also engaged as adaptive response to pan-HDACIs (Minisini et al., 2022). It is important to note that these HATs can be coopted, in different cancer contexts, to sustain the transcriptional addiction required for cell fitness (Hogg et al., 2021). Tumors in which, mutations that specifically inactivate HAT enzymatic activity are the driving oncogenic lesions, should benefit from treatment with isoform specific HDAC inhibitors.

A pro-oncogenic role of HDAC3 has also been identified in leukemogenesis. In acute promyelocytic leukemia (APL), HDAC3 contributes to the repressive influence of the oncogenic fusion protein PML-RARα. Moreover, down-modulation of Hdac3 or its inhibition supported the differentiation of PML-RARα-expressing cells (Mehdipour et al., 2017).

Not surprisingly, HDAC3, similar to other HDACs and other epigenetic regulators, can exert antiproliferative effects. For example, in complex with CBX4, HDAC3 may act as a tumor suppressor. CBX4 (polycomb chromobox protein 4) is a partner of the polycomb repressive complex (PRC), PRC1. In colorectal carcinoma, CBX4 negatively regulates cancer metastasis by repressing RUNX2 transcription. HDAC3 provides H3K27 deacetylase activity at the RUNX2 promoter, where it is recruited by binding with CBX4 (Wang et al., 2016).

5.3. HDAC3 and the epigenetic-independent activities

Epigenetic independent activity of HDAC3 has been associated with breast cancer cell metastasis. HDAC3 can deacetylate the Forkhead box (FoxO) TF FoxO3, due to the bridging function of the protein Geminin. FoxO3 stimulates Dicer transcription and miRNA biogenesis. Dicer level is critical for the metastatic process of breast cancer cells. HDAC3, which promotes deacetylation of FoxO3 and downregulation of Dicer, aids metastatic potential (Zhang et al., 2017). Another non-histone target of HDAC3 that plays a role in cancer is DNA methyltransferase 1 (DNMT1). In multiple myeloma (MM), HDAC3 deacetylates DNMT1, thereby increasing its stability. In addition, DNMT1 transcription is stimulated by MYC, whose stability is reduced after inhibition by HDAC3. Therefore, HDAC3 can control DNMT1 levels in two different ways: i) directly by deacetylating it and ii) indirectly by stabilizing MYC. DNMT1 is required to maintain the proliferation of MM. Thus, inhibiting both HDAC3 and DNMT1 with their respective inhibitors will have a more potent anti-proliferative effect (Harada et al., 2017). Certainly, this dual epigenetic targeting may influence additional antiproliferative mechanisms and further enhance antitumor surveillance by the immune system (Chiappinelli et al., 2015). Another non-histone target of HDAC3 is NICD1 (Notch1 intracellular domain). As described above for DNMT1, HDAC3 deacetylates NICD1 and controls its stability to sustain Notch signaling (Ferrante et al., 2020). Since aberrant Notch signaling is critical for T-cell acute lymphoblastic leukemia (T-ALL) and chronic lymphocytic leukemia (CLL), these tumors represent other potential application of HDAC3-selective inhibitors (Fig. 3).

Akt1 is another non-histone target of HDAC3 mediated deacetylation (Long et al., 2017; Yan et al., 2018). The Speckle-type POZ protein (SPOP), the substrate-binding adaptor of the CULLIN3-RBX1 E3 ubiquitin ligase complex, is the most frequently mutated gene in human primary prostate cancer. These mutations lead to abnormal activation of the androgen receptor (AR) and AKT-mTORC1 signaling pathways (Blattner et al., 2017). HDAC3 deacetylates lysine 14 and 20 of Akt (Long et al., 2017; Yan et al., 2018). Mechanistically, HDAC3 interacts with AKT at the plasma membrane and facilitates polyubiquitylation of the lysine-63-chain and phosphorylation of AKT, by deacetylation. HDAC3 is the only class I/II HDAC that promotes AKT phosphorylation. Also in this context, the use of a relatively specific HDAC3 inhibitor (RGFP966, Fig. 3) shows promising anti-tumor activities in a preclinical model of prostate cancer (Yan et al., 2018). Fig. 6 summarizes some of the pro-oncogenic and tumor suppressive actions of HDAC3.

5.4. HDAC3 and the immunotherapy

The PD-1/ PD-L1 axis is hijacked by cancer cells to counteract immune activation, allowing the tumor to escape the immune response. PD-1 is generally expressed on tumor-infiltrating T cells, while its ligand (PD-L1) is often highly expressed on tumor to inhibit tumor-infiltrating T cell activation and cytotoxicity. (Constantinidou, Alifieris, & Trafalis, 2019). In B-cell lymphomas, HDAC3 is recruited by BCL6 as part of a complex with NCOR1/NCOR2 to the PD -L1 promoter. Consistent with this, silencing of HDAC3 promotes PD-L1 expression. More generally, upregulation of PD-L1 is a common feature of the response to HDACIs (Minisini et al., 2022). Importantly, the HDAC3-specific inhibitor RGFP966 (Fig. 3) impairs the growth of B-cell lymphomas in which PD-L1 was deleted, but not that of WT cells. This result indicates an important mechanism of resistance to HDACIs therapy in vivo in PD-L1. In the presence of PD-L1, RGFP966 acted synergistically with anti PD-L1 therapy to suppress tumor growth (Deng et al., 2019). There are further links between HDAC3 and immune-mediated elimination of cancer cells. HDAC3 represses gene programs associated with CD8 T-cells differentiation and cytotoxicity, by buffering H3K27ac levels at relative promoters (Tay et al., 2020). Hence, further efforts should be planned to isolate new potent HDAC3 inhibitors to facilitate immunotherapy in cancer.

The role of the BCL6-HDAC3 axis as an important oncogenic element has been observed in B-cell lymphomas characterized by CREBBP mutations (Jiang et al., 2017). The use of BRD4097 (Fig. 3), a new HDAC3-selective inhibitor, (Wagner et al., 2016), confirmed the oncogenic addiction for HDAC3 in these tumors. An addiction validated in primary patient-derived xenograft models of DLBCL (diffuse large B-cell lymphoma). HDAC3 is not only involved in the repression of CREBBP-target genes, but also controls the interferon response and expression of antigen presentation genes, thus restoring the immune surveillance. Practically, HDAC3 inhibition is sufficient to restore the capacity of tumor-infiltrating lymphocytes to kill DLBCL cells in a major histocompatibility complex (MHC) class I and II dependent manner, and to synergize with PD-L1 blocking antibodies (Mondello et al., 2020).

5.5. HDAC3, the DNA Damage Response (DDR) and the genome stability

As described above for HDAC1/2, there is emerging evidence for a link between DNA damage, HDACs, epigenetic remodeling, and DNA repair. Similar observations apply to HDAC3 when assembled in classical complex with NCOR1/NCOR2. Preliminary studies have shown that HDAC3 affects genome instability. The efficiency of both HR and NHEJ

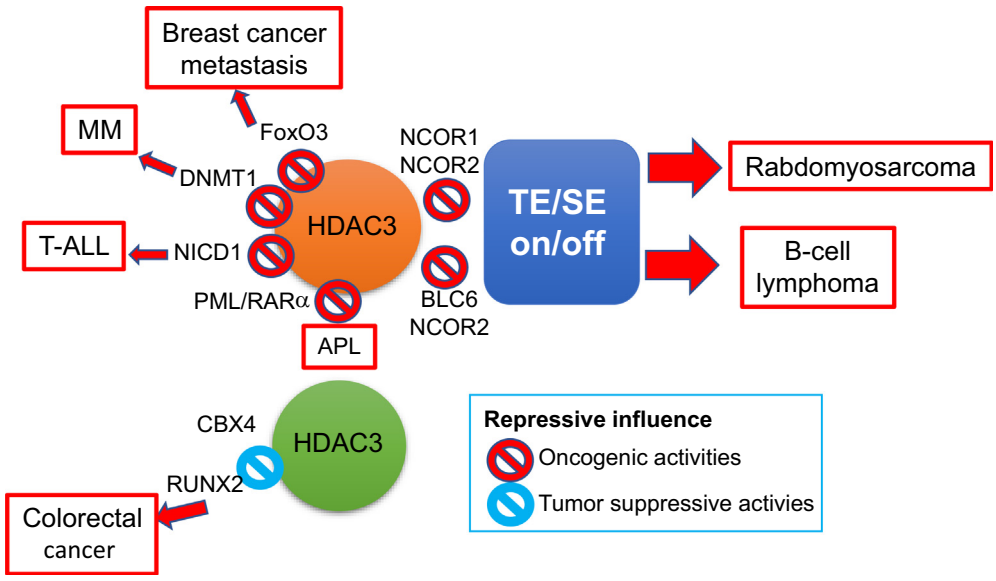


Fig. 6. Some of the pro-oncogenic and tumor suppressive activities of HDAC3.

repair pathways is reduced in the absence of Hdac3, and in these null cells the number of chromosome breaks and gaps observed in mitosis is increased. This genomic instability activates oncogenic signalling pathways and impairs p53 activities, leading to early onset of spontaneous hepatocellular carcinoma in *Hdac3*$^{-/-}$ mice. (Bhaskara et al., 2010). In the liver Hdac3 exerts a non-redundant deacetylase activity against H3K9ac, which is accompanied by an increase in H3K9me3 (Ji et al., 2019). Regulation of H3K9ac/H3K9me3 turnover is one of the first steps in the DSB repair pathway and allows binding of HAT Tip60 to DSB foci. Tip60 engagement is important to recruit other factors of the DDR (Mir et al., 2021). In general, relaxation of chromatin facilitates local recruitment of repair factors.

Not only DSBs require the intervention of HDACs for efficient repair. For example, UV irradiation induces cyclobutane-pyrimidine dimers (CPD), which are repaired via the nucleotide excision repair (NER) pathway. The dynamics of H3K14 acetylation are important for the initiation of NER (Niida et al., 2018). HDAC3, like HDAC1/2, can control this acetylation, and its depletion impairs DNA repair, leading to CDP accumulation. The dynamics of H3K14ac may be critical for the temporal recruitment of elements of the repair machinery (Nishimoto et al., 2020).

The impact of HDAC3 on genome stability can also be exploited from a therapeutic perspective. Melanomas with BRAF mutations can be treated with BRAF/MEK inhibitors. However, a consistent number of patients do not respond to or develop resistance to these therapies. Depletion of HDAC3 (but not HDAC1/2 or 6) potently cooperates with these inhibitors to kill melanoma cells. Interestingly, BRAF/MEK inhibitors in combination with entinostat (a preferred HDAC1/2/3 inhibitor) synergize to induce DNA damage and suppress expression of DDR genes involved in both HR and NHEJ (Maertens et al., 2019).

6. HDAC8

6.1. Basic concepts

HDAC8 is included in class I of HDACs but is the most divergent compared to HDAC1/2/3. HDAC8 has the shorter carboxy-terminal tail, which is phosphorylated by PKA and can utilize either Zn^{2+} or Fe^{2+} for catalysis (Gantt, Gattis, & Fierke, 2006). The HDAC8 gene is located on the X chromosome, near the X inactivation center, and encodes a 377 amino acid long protein (Hu et al., 2000; Lee, Sengupta, Villagra, Rezai-Zadeh, & Seto, 2006; Somoza et al., 2004; Van den Wyngaert et al., 2000). The HDAC8 protein is localized primarily in the nucleus, where it acts as a transcriptional repressor and shows a rather ubiquitous expression (Buggy et al., 2000; Hu et al., 2000). HDAC8 catalyzes the deacetylation of histone substrates (H3/H4) with an efficiency that can be influenced by distal protein-protein interactions or by acetyllysine chain accessibility (Castañeda et al., 2017). HDAC8 deacetylates also nonhistone proteins such as TP53 and ERR-α (Deardorff et al., 2012; Qi et al., 2015; Wilson, Tremblay, Deblois, Sylvain-Drolet, & Giguère, 2010; Yan et al., 2013). Particularly critical is the deacetylation of SMAC3, which gives HDAC8 a critical and unique role in regulating cohesin function. HDAC8 catalyzes the deacetylation of SMC3, which is required for efficient recycling of the cohesin complex (Deardorff et al., 2012). Missense mutations in HDAC8 are responsible for Cornelia de Lange Syndrome (CdLS) and associated spectrum disorders. Patients suffering for this genetic disorder are characterized by congenital anomalies which consist of distinctive facial features, upper limb abnormalities, intellectual disability, and other symptoms (Dowsett et al., 2019). In addition to dysfunctions in HDAC8, CdLS is caused by mutations in genes encoding the cohesion structural proteins SMC1A, SMC3 and RAD21 or the cohesin assembly factor NIBPL (Avagliano et al., 2020; Watrin, Kaiser, & Wendt, 2016).

The cohesin complex is organized in a ring-like structure that can hold two DNA helices together. This complex is important for the spatial organization of the genome and the segregation of chromosomes and influences several genome-related functions, from gene expression to DNA repair (Broughm et al., 2012; Haarhuis, Elbatsh, & Rowland, 2014; Singh, McKinney and Gerton, 2020; Lee, Sengupta, Villagra, Rezai-Zadeh and Seto, 2006). Dysfunction or alteration of HDAC8, as well as its targeting by specific inhibitors, could have a broader effect via control of cohesin dynamics (Dasgupta, Antony, Braithwaite, & Horsfield, 2016; Yamauchi et al., 2011). Another element of variability that should be considered is the presence of somatic mutations of the cohesin subunits in a variety of human cancers (Hill, Kim, & Waldman, 2016).

6.2. HDAC8 and cancer

HDAC8 is frequently overexpressed in different tumor types (Lehmann et al., 2014; Moreno et al., 2010; Oehme et al., 2009). In neuroblastoma, HDAC8 correlates with poor overall survival, and its knockout leads to increased cell differentiation and cell cycle arrest (Oehme

et al., 2009). More generally, HDAC8 affects cell proliferation in different tumor types (Vannini et al., 2004). Accordingly, the blockade of HDAC8 by a specific inhibitor, (PCI-34051, Fig. 3), decreases cell growth and causes apoptosis in T-cell derived tumor lines (Balasubramanian et al., 2008). HDACIs can repress the binding between HDAC8 and Yin Yang 1 (YY1), restoring the acetylation of YY1 TF that causes the suppression of mutant TP53 transcription in triple negative breast cancer (Wang et al., 2016).

Several crosses between TP53 and HDAC8 have been discovered. In AML, characterized by an inversion in chromosome 16, HDAC8 forms a complex with the fusion protein CBFB-SMMHC (CM) and TP53. CBFB is a partner of RUNX1, while SMMHC encodes a smooth muscle myosin heavy chain. In this way, HDAC8 inactivates TP53 by deacetylation. Remarkably, the CM fusion does not interact with other class I members. Importantly, deletion of HDAC8 significantly reduces the incidence of AML and treatment with a HDAC8 inhibitor increases the sensitivity of AML cells to chemotherapy in both mice and patients. (Qi et al., 2015).

The ability of HDAC8 to affect TP53 activities may also lead to some dark effects in normal cells. Hdac8 is highly expressed in long-term hematopoietic stem cells (LT-HSCs). Here, Hdac8 interacts with Tp53 and Hdac8-deficient LT-HSCs exhibit hyperacetylation and activation of TP53, causing increased apoptosis under various stress conditions. Consequently, hematopoietic progenitor cells defective in Hdac8 are impaired for long-term serial repopulation activity *in vivo* (Hua et al., 2017).

In breast cancer, TGF-β signaling correlates with stemness and metastasis via oligomerization of SMAD3/4. In this scenario HDAC8 is an important player since its inhibition suppresses cancer metastasis and chemotherapy resistance. HDAC8 can bind the SMAD3/4 complex and supplies its epigenetic influence, by creating a repressive environment at the *SIRT7* promoter. SIRT7, is an important antagonist of the TGF-β signaling, by promoting SMAD4 degradation. (Tang et al., 2020).

6.3. HDAC8 and resistance to therapy

In addition to the role of HDAC3 in melanoma described above, HDAC8 supports another mechanism of therapy resistance. The anti-BRAF/MEK therapy can promote expression of HDAC8, which in turn suppresses expression of the pro-apoptotic gene BIM and causes resistance in melanoma cell lines. Mass spectrometry-based phosphoproteomic analysis revealed that HDAC8 regulates multiple signaling pathways, of which the MAPK pathway is important for resistance. Mechanistically, HDAC8 deacetylates c-Jun to enhance its transcriptional activity. Moreover, concomitant treatment with a HDAC8 inhibitor (Fig. 3) and a BRAF inhibitor (PLX4720) restores efficacy in inhibiting tumor growth and melanoma phenotype in vivo. (Emmons et al., 2019).

Another example of possible involvement of HDAC8 in treatment resistance was recently discovered in AML. Here, HDAC8 is upregulated after treatment with quizartinib, an inhibitor of FMS-like receptor tyrosine kinase 3 (FLT3). The combination of quizartinib with the HDAC8 inhibitor 22d (Fig. 3), greatly reduces AML cell survival. This resistance depends on the deacetylase activity of HDAC8 against TP53 described above. Indeed, their interaction increases after treatment with quizartinib and leads to inactivation of TP53, which allows leukemia cell survival. On the other hand, simultaneous treatment with both inhibitors increases TP53 acetylation and activity. Upregulation of HDAC8 during FLT3 therapy is mediated by TFs FOXP2 and FOXP3 (Long et al., 2020).

7. Class IIa HDACs

7.1. Basic concepts

The class IIa subfamily includes HDAC4, HDAC5, HDAC7 and HDAC9. In contrast to all other family members, class IIa HDACs are characterized by a large molecular weight (120-135 kDa) required for the interaction with: i) various TFs, with MEF2 family members being the best characterized, ii) other co-repressors important for the repressive activity of these HDACs (Fig. 2). In vertebrates, this subfamily is characterized by limited catalytic activity, mainly due to the replacement of a tyrosine (Y) with a histidine (H) residue in the enzymatic pocket (Lahm et al., 2007). In principle, class IIa HDACs should be able to bind acetylated lysine without hydrolyzing it directly or with very low kinetics. It has been suggested that class IIa HDACs may act as readers of acetylated lysine, directing other HDAC subtypes (Fig. 4) to complete the deacetylation steps (Brancolini, Di Giorgio, Formisano, & Gagliano, 2021; Di Giorgio & Brancolini, 2016). Another important feature of these HDACs is the different levels of regulation, with 14-3-3-dependent control of nuclear cytoplasmic shuttling (Fig. 2) playing an important role in various adaptive responses and during differentiation (Di Giorgio & Brancolini, 2016). Although several studies have reported a role of class IIa HDACs in cancer, they cannot be unambiguously classified as tumor suppressors or oncogenes, and their contribution to cancer development might vary from context to context.

7.2. Class IIa HDACs and pro-oncogenic activities

Several research groups have reported a role for class IIa HDACs in regulating cancer cell proliferation. In MM, a severe malignancy with poor survival, MIR145-3p promotes apoptosis by downregulating HDAC4. Conversely, silencing of HDAC4 leads to upregulation of the pro-apoptotic BH3-only protein BCL2L11/BIM and causes inactivation of mTORC1, two actions that lead to alterations in autophagy flux and cell death (Wu et al., 2020). An activity that could be relevant in the clinic to strengthen the therapeutic efficiency of bortezomib, a MM-specific drug. The cytoplasmic pool of HDAC4 has been reported to be involved in the control of autophagy and apoptosis (Zhang, Qi, Yin and Yang, 2019). Since apoptosis and autophagy can be induced by a wide range of cellular stresses, defining the direct and indirect actions of HDAC4 is essential in this context.

There are other examples linking HDAC4 to tumorigenesis. In nasopharyngeal carcinoma (NPC), HDAC4 levels are significantly higher in neoplastic areas compared to normal tissue. High HDAC4 expression predicts poor overall survival and progression-free survival of patients. In a model of NPC cells, HDAC4 stimulates cell cycle progression and induces epithelial-to-mesenchymal transition, ultimately promoting tumor growth and metastasis *in vivo* (Cheng et al., 2021).

Dysregulations in the mechanisms controlling cell-fate decisions are at the origin of the tumorigenic process. BAP1 (breast cancer type 1 BRCA1–associated protein 1) is a tumor suppressor mutated in different human cancers and particularly in uveal melanoma. To investigate the role of Bap1 the *Xenopus* model was used. Loss of Bap1 leads to transcriptional silencing of genes regulating the transition from pluripotency-to-commitment. This repression is associated with a depletion of H3K27ac marks at the corresponding promoters. As expected, the pan-HDAC inhibitor SAHA can reverse the repressive state. Interestingly, HDAC4 is the only HDAC that is significantly upregulated by the loss of BAP1 in human uveal melanoma cells. Confirming the importance of the axis between BAP1 and HDAC4, simultaneous depletion of BAP1 and HDAC4 in the *Xenopus* model can reactivate the gene lineage commitment program and restore H3K27ac marks at the promoters of the corresponding genes. Interestingly, this activity is NCOR-independent, suggesting the contribution of other co-repressors recruited by HDAC4. Finally, HDAC4 is mainly nuclear only in BAP1-mutated uveal melanoma cells and is required for their proliferation (Kuznetsov et al., 2019). Similar pro-proliferative activities of HDAC4, with influences on H3K27ac levels, both at promoters and enhancers, have been documented in leiomyosarcomas, a rare and highly malignant tumor of mesenchymal origin (Di Giorgio et al., 2017; Di Giorgio et al., 2020).

Pro-oncogenic activities have also been documented for other class IIa HDACs members. In pancreatic cancer KRAS is the key oncogenic driver. Here, HDAC5 was identified with a screen aimed at defining the molecular basis of cancer recurrence after KRAS extinction. HDAC5 remodels the microenvironment of resistant tumors and enables a switch form neutrophil-to-macrophage infiltration (Hou et al., 2020). The influence of class IIa on infiltrating immune cells has also important therapeutic implications, as demonstrated using TMP195, a specific inhibitor of these HDACs (Fig. 3). In breast cancer, treatment with TMP195 alters the tumor microenvironment, reduces tumor burden and lung metastases by modulating macrophage phenotypes (Guerriero et al., 2017).

A pro-oncogenic influence of HDAC5 on the microenvironment was also observed in rhabdomyosarcomas. The PAX3-FOXO1 oncogenic fusion downregulates the anti-tumour cytokine IL24 with the help of HDAC5. Inhibition of PAX3-FOXO1 lowers HDAC5 levels, leading to re-expression of IL24, which in turn acts as a tumour suppressor (Lacey et al., 2018). IL24 appears to be a common target of class IIa HDACs and is suppressed by HDAC7 in breast both in normal and cancer cells (Cutano et al., 2019).

Additional studies have reported pro-oncogenic roles of HDAC7. In a mouse model of lung tumor, downregulation of HDAC7 significantly decreased the onset and burden of tumours. A pro-oncogenic activity confirmed also in human cells (Lei et al., 2017). The tumour promoting role of HDAC7 has also been demonstrated in brain tumour. Here, the Zinc-finger protein 326 up-regulates HDAC7, which in turn deacetylates β-catenin and activates the WNT pathway (Yu, Wang, Wu, Han, & Zhang, 2020). In MM like HDAC4, HDAC7 plays a pro-oncogenic role. Using a CRISPR-based screening, a role for HDAC7 in enabling immune escape has been suggested. Inhibition of HDAC7 can increase cell surface levels of B-cell maturation antigen (Ramkumar et al., 2020). Therefore, the use of class IIa HDACs inhibitors, by blocking both HDAC4 and HDAC7 could be particularly promising in MM, as they should reduce proliferative aggressiveness and also assist the anti-neoplastic action of the immune system.

There are relatively few studies on the role of HDAC9 in cancer. Oncogenic potential has been demonstrated in preclinical models. HDAC9 is highly expressed in human B-cell lymphomas, and transgenic mice overexpressing Hdac9 develop lymphoproliferations and B-cell lymphomas (Gil et al., 2016). Knock-out of HDAC9 in highly aggressive leiomyosarcomas cells reduces proliferation and increases the susceptibility to cell death. MEF2-transcriptinal activity is restored, and H3K27ac levels are augmented at specific genomic sites, frequently intergenic indicating a regulation of enhancer elements (Di Giorgio et al., 2017; Di Giorgio et al., 2020).

7.3. Class IIa HDACs and tumor suppressive activities

As described above for class I HDACs, these epigenetic regulators may also be involved in the regulation of anti-proliferative options, depending on the context. For example, mRNA levels of class IIa HDACs have been reported to be downregulated in prostate cancer, with 7% of patients having a mutation in HDAC5. Depletion of HDAC5 in the PC3 cell line leads to resistance to palbociclib, a CDK4/6 inhibitor. HDAC5 interacts with RB to provide deacetylase activity necessary for cell cycle arrest. HDAC5 is required to deacetylate H3K27 and thereby repress a subset of cell cycle-related pro-oncogenic genes (Zhou et al., 2021).

The MEF2 family TFs are important partners of class IIa HDACs. Unscheduled expression of MEF2C contributes to the development of T cells malignancies, such as acute lymphoblastic leukemia (T-ALL), and AML from myeloid cells (Di Giorgio, Hancock, & Brancolini, 2018). In AML, the serine-threonine kinases LKB1 and SIK3 (Salt-Inducible Kinase 3) are regulators of MEF2C. In particular, SIK3 phosphorylates and inactivates HDAC4, which should act as a MEF2C repressor. In this context, the activities of LKB1 and SIK3 are critical for maintaining histone acetylation (H3K27ac) at MEF2C-bound enhancer elements. Moreover, a pan-SIK inhibitor repressed MEF2C-dependent transcription in an HDAC4-dependent manner, opening new therapeutic opportunities (Tarumoto et al., 2018). The same research group demonstrated that YKL-05-099, a more specific SIK3 inhibitor, suppresses MEF2C functions by acting on HDAC4 phosphorylation and localization. In this context, blocking SIK3 activity is sufficient to accumulate HDAC4 in the nuclear compartment. Inhibition of SIK3 affected proliferation of MLL-rearranged leukemia cells and prolonged survival in mouse models of MLL-AF9 AML (Tarumoto et al., 2020). Although SIKs have multiple targets and their inhibition might have an opposite effect in other circumstances in which they act as tumor suppressors (Hollstein et al., 2019), these studies suggest an anti-proliferative role of HDAC4 or other class IIa HDACs in AML. In conclusion, a note of caution should be raised as these SIK inhibitors may also target Src family kinases (Sakamoto, Bultot, & Göransson, 2018).

Some tumor suppressive actions have also been reported for HDAC7. Argonaute2 (AGO2), a component of the miRNA processing complex, is deacetylated by HDAC7. Since AGO2 acetylation correlates with worse prognosis in lung cancer, HDAC7 could play a role in preventing lung cancer progression (Zhang et al., 2019). However, this report is in contradiction with other studies, indicating a pro-oncogenic role of HDAC7 in lung cancer (Lei et al., 2017). Finally HDAC7, as a factor involved in B-cells differentiation, plays a pivotal role in the pathogenesis of lymphoblastic leukaemia, characterized by t(4;11) translocation. In this tumor low levels of HDAC7 correlate with a poor prognosis (de Barrios et al., 2021).

7.4. The role of class IIa HDACs in treatment resistance

There is evidence for different contributions of class IIa HDACs to resistance to cancer therapies. In hepatocellular carcinoma (HCC), blockade of HDAC4 signaling enhances radiation-induced lethality. This result suggests that HDAC4 may be involved in DNA repair and therefore represents an interesting target for radiosensitization of HCC (Tsai et al., 2018). Sorafenib, a protein kinase inhibitor, was the first targeted therapy approved for the treatment of HCC, but resistance to this agent often occurs (Llovet, Montal, Sia, & Finn, 2018). Targeted inhibition of HDAC4 induces expression of SPRY4 (Sprouty RTK Signaling Antagonist 4), which in turn inhibits ERK signaling and thus sensitizes resistant HCC cells to sorafenib (Ma et al., 2021). As mentioned previously, loss of HDAC5 in prostate cancer cells leads to resistance to CDK4/6 inhibitors, suggesting a role for HDAC5 in preventing drug resistance. In contrast, HDAC5 has been described to promote tamoxifen resistance in breast cancer. HDAC5 confers resistance to tamoxifen by mediating deacetylation and nuclear localization of SOX9. In these cells, activation of MYC markedly increases HDAC5 expression (Xue et al., 2019).

HDAC7 controls the phagocytic response in lymphocytic leukaemia by modulating acetylation and phosphorylation of a non-epigenetic target: Bruton's tyrosine kinase (BTK). These findings highlight the role of HDAC7 in resistance to immunotherapy (Burgess et al., 2020). HDAC9 may also contribute to resistance to endocrine therapies in breast cancer. Estrogen receptors (ER) are transcription factors regulated by HDACs. HDAC9 acts as a suppressor of ER mRNA and protein levels in tamoxifen-sensitive MCF7 breast cancer cells and inhibits ER transcriptional activity. HDAC9 mRNA is highly overexpressed in tamoxifen-resistant MCF7 cells and in ER-negative breast tumor cell lines. In a syngeneic model, HDAC9-overexpressing cells are less sensitive to tamoxifen treatment than parental cells. Moreover, HDAC9 expression was positively associated with genes upregulated in endocrine therapy-resistant breast cancers, and high HDAC9 levels were associated with worse prognosis in patients treated with tamoxifen (Linares et al., 2019).

7.5. Class IIa HDACs and the regulation of senescence

An important epigenetic role of class IIa in favouring the initial steps of the tumorigenic process was recently demonstrated for HDAC4. This deacetylase, as well as other members of the class IIa family, are downregulated in various forms of senescence, including oncogene-induced senescence. During RAS-induced senescence, artificial maintenance of HDAC4 levels can counteract cell cycle exit and reduce the expression of CDKN1A and CDKN2A, two important senescence markers. Conversely, deprivation of HDAC4 halts proliferation and induces senescence in low-grade cancer cells. The anti-senescence role of HDAC4 is explained by competition with AP1/P300 to enforce H3K27ac status at selected enhancers and super-enhancers that orchestrate the senescence program (Di Giorgio et al., 2021). HDAC7 has also been reported to be modulated during senescence and its downregulation promotes senescence in fibroblasts (Warnon et al., 2021). Similarly, HDAC5 may also act as a senescence antagonist. SENEBLOC, a long noncoding RNA, blocks the induction of cellular senescence by inactivating CDKN1A. This effect is achieved by two distinct strategies: i) by favouring MDM2-TP53 interaction, which reduces transcriptional output at the CDKN1A promoter, ii) by controlling HDAC5 levels, which affect H3K9 and H4K5 acetylation levels at the proximal promoter of CDKN1A (Xu et al., 2020). An epigenetic regulation previously observed for others class IIa and possibly mediated by MEF2 TFs (Clocchiatti et al., 2015).

7.6. Class IIa HDACs regulate cancer metabolic pathways

NAC1 (nucleus accumbens-associated protein-1) is deregulated in various cancers. The NAC1/HDAC4/HIF-1α axis is important for the regulation of glycolysis and hypoxic adaptation in tumor cells. NAC1 can bind HDAC4 and impedes phosphorylation of deacetylase, which prevents nuclear export and causes nuclear accumulation. This leads to reduced HIF-1α acetylation, which promotes its stabilization and transcriptional activity. Therefore, HDAC4 may support the adaptive response of cells to hypoxia. A role with potential impact on tumor progression (Zhang et al., 2017).

HDAC4 is also associated with de novo lipid biosynthesis. Adaptation to the lipid requirements of cancer cells plays a critical role in the development and progression of several cancers, including breast cancer (Rohrig & Schulze, 2016). Seryl-tRNA synthetase (SerRS), a key gene for protein biosynthesis, is also involved in the control of metabolism. During evolution with vertebrates, SerRS has acquired a carboxyl-terminal domain that contains a nuclear localization signal. In the normal breast, glucose controls lipid biosynthesis by regulating nuclear import of SerRS via an acetylation switch. Once in the nuclei, SerRS can bind chromatin and repress the expression of genes involved in lipid metabolism. In contrast, in breast cancer cells, SerRS acetylation and nuclear translocation are strongly inhibited. HDAC4 and HDAC5 are the deacetylases that control the acetylation and nuclear translocation of SerRS, thus boosting lipid metabolism and cell growth (Zhao et al., 2021).

AMPK (AMP-activated protein kinase) is an important sensor of metabolic stress and the resulting adaptations (Ross, MacKintosh, & Hardie, 2016). In lung cancer, low levels of AMPK promote cell proliferation and tumour growth. A decrease in AMPK affects EMT and metastasis *in vivo*. These effects are associated with increased glycolysis, which requires upregulation of hexokinase 2 (HK2) expression. The upregulation of HK2 in cells in which AMPK is downregulated depends on HDAC4 and HDAC5 in an undefined manner (Feng et al., 2020). An opposing link between HDAC5 and glucose metabolism was reported by Hendrick et al., 2017. HDAC5-depleted cells establish a coping mechanism by reprogramming metabolic pathways to glucose and glutamine. However, when glucose and glutamine supply is disrupted in HDAC5-inhibited cancer cells, apoptotic cell death significantly increases and tumour growth is reduced *in vivo* (Hendrick et al., 2017).

In renal clear cell carcinoma loss of TCA cycle enzymes expression marks the metastatic tissues and is a peculiar feature of this type of tumour. This reduction correlates with and depends on the downregulation of the co-activator PGC-1α. TGF-β uses HDAC7 as corepressor to switch off the expression of TCA enzymes. Moreover, pharmacological inhibition of TGF-β can restore TCA cycle enzymes expression and reduce tumour growth in vivo. Altogether, these findings provide new insights into the epigenetic basis of altered mitochondrial metabolism in renal clear cell carcinoma (Nam et al., 2021).

7.7. The role of HDAC7 in breast cancer stem cells

In the past few years HDAC7 has emerged as a regulator of cancer stem cells (CSCs). In breast and ovarian cancers, HDAC1 and HDAC7 are specifically overexpressed in CSCs compared to non-stem-tumour-cells. HDAC1 and HDAC7 are necessary to maintain the properties of CSCs, and HDAC7 overexpression is sufficient to increase the CSC phenotype (Witt et al., 2017). Interestingly, HDAC7 levels are downregulated after treatment with pan-HDACIs (Minisini et al., 2022; Witt et al., 2017). HDAC1 and HDAC3 appear to be involved in maintaining the high expression of HDAC7 in an undetermined manner. The epigenomic function of HDAC7 is important in CSCs. HDAC7 binds near TSS and to SEs of oncogenes and contributes to their transcriptional regulation. This mechanism was particularly observed in stem-like breast cancer cells. HDAC7 controls the deposition of H3K27ac at transcription start sites (TSS) and super-enhancers. Paradoxically, HDAC7 is required to maintain H3K27ac levels at these regulatory elements. How this is achieved, whether it is a direct or indirect effect, deserves further work (Caslini, Hong, Ban, Chen, & Ince, 2019).

In the breast, the influence of HDAC7 on stem cell fitness was confirmed by further studies. HDAC7 is required for sculpturing the microenvironment by suppressing the expression of cytokines and other signalling regulators (Fig. 7). Moreover, in a model of RAS-induced transformation of immortalised mammary epithelial cells, HDAC7 is required for invasion and proliferation of transformed cells. (Cutano et al., 2019; Di Giorgio et al., 2021).

8. HDAC11

8.1. Basic concepts

HDAC11 is a quite unique member of the HDAC family that shares homologies with both class I and II HDACs (Gao, Cueto, Asselbergs, & Atadja, 2002). For this reason, HDAC11 alone constitutes the class IV subfamily (Núñez-Álvarez & Suelves, 2021). The HDAC11 protein has a molecular mass of 39 kDa, which is mainly occupied by the catalytic domain. It is expressed in almost all tissues and at higher levels in the brain, immune system and testis (Gao et al., 2002; Mostofa et al., 2021).

HDAC11 can deacetylate a H4-derived synthetic peptide with very low activity, and its ability to deacetylate histones by a direct mechanism remains to be demonstrated (Cao et al., 2019; Gao et al., 2002). Instead, HDAC11 shows strong defatty-acylase activity. It can remove long chain fatty-acyl groups from H3K9 peptides. As mentioned earlier, defatty-acylase activity has also been observed with other HDACs such as HDAC8, but HDAC11 is 10,000 times more effective (Cao et al., 2019). To add further mystery to HDAC11, mice *Hdac11*$^{-/-}$ show no significant abnormalities (Huang et al., 2017; Sun et al., 2018; Yue et al., 2020).

8.2. HDAC11 and cancer

As reported for other HDACs, HDAC11 expression is upregulated in multiple carcinomas compared to healthy counterparts (Deubzer et al., 2012; Gong, Zeng, Yi, & Wu, 2019; Liu, Wu, Jin, Chang, & Xu, 2020). Functional studies have shown that silencing of HDAC11 blocks the cell cycle (mainly in G2/M phase) and induces abnormal mitotic spindle formation and cell death. This evidence is more relevant in neuroblastoma cells characterized by *MYCN* amplification (Thole et al.,

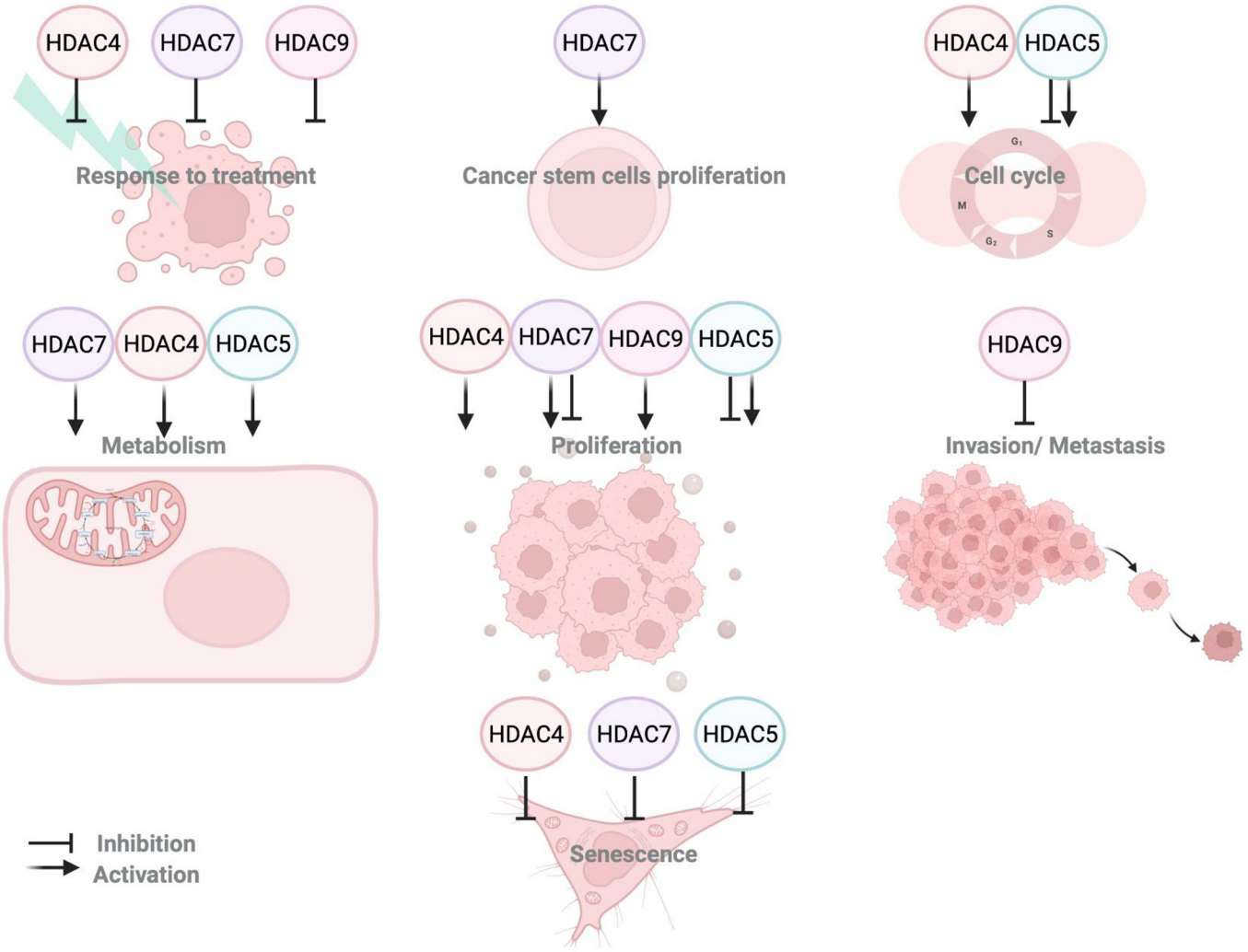


Fig. 7. Class IIa HDACs in cancer cells.
Class IIa HDACs regulate proliferation, cell cycle, metabolism, senescence, and invasion/metastasis in cancer cells. While HDAC4 is primarily thought to have a tumor-promoting role, HDAC5 and HDAC7 are associated with both pro-tumorigenic and tumor suppressive functions. HDAC7 also plays an important role in maintaining stemness properties. HDAC9 is involved in invasion/metastasis, proliferation and response to treatments.

2017). As expected, the expression of several cell cycle-related genes is altered in HDAC11-depleted cells, but it is unclear whether these genes are under the direct influence of deacetylase.

An oncogenic dependence on HDAC11 has also been observed in HCC. In a chemically induced model of liver carcinogenesis in mice, Hdac11 is essential for cancer stem cell maintenance. Hdac11 controls the expression of the tumor suppressor Lkb1 by regulating H3K9 acetylation at its promoter. Repression of Lkb1 thereby affects Ampk activation and glycolysis (Bi et al., 2021). A contribution of Hdac11 to the regulation of energy homeostasis has been observed in other studies. In high-fat fed mice, deletion of Hdac11 prevents obesity, insulin resistance and glucose intolerance by increasing energy expenditure through improved thermogenic capacity (Sun et al., 2018). In addition, loss of Hdac11 promotes brown adipose tissue accumulation and function. In contrast, these mice reduce white adipose tissue deposition. These activities require the interaction of Hdac11 with the epigenetic reader BRD2 (Bagchi et al., 2018).

HDAC11 is also involved in tumors of the hematopoietic system. In myeloproliferative neoplasms (MPN), selective HDAC11 inhibitors regulate the expression of mitotic genes, causing cell cycle arrest (Yue et al., 2020). In MM, HDAC11 regulates cell proliferation and survival by controlling IRF4 acetylation. As a result, treatment of MM cells with elevenostan, a hydroxamic acid derivative and putative selective HDAC11 inhibitor (Fig. 3), induces apoptosis similar to downregulation of HDAC11. Elevenostan exhibits activity in the nanomolar range and acts synergistically with bortezomib, an anti-myeloma drug, in inducing cell death (Mostofa et al., 2021).

The contribution of HDAC11 to the metastatic process is controversial and requires further investigations. HDAC11 is important for tumorigenesis and growth in lymph nodes, but its downregulation in the lymph nodes leads to increased migration and colonization of distal sites, resulting in distal metastasis. The effect of HDAC11 on cell proliferation depends on the repression of cell cycle inhibitors E2F7 and E2F8 (Leslie et al., 2019). In contrast, the authors proposed that the metastasis inhibitory activity is due to the repression of ribonucleotide reductase subunit M2 (RRM2). This gene is associated with poor disease-free survival in breast cancer and it can affect cancer proliferation, angiogenesis, and invasiveness (Zhang et al., 2014). According to this view, pro-metastatic behavior is coupled with a slowing of proliferative properties (Leslie et al., 2019). This study is another example of the ambivalent behavior of HDACs in cancer and another indication of adverse effects of their selective targeting in a therapeutic perspective. In the case of HDAC11, it is even more difficult to understand the reasons for these seemingly contradictory effects during cancer progression

because very little information is available about its recruitment specificity to genomic sites and whether it assembles into different multiprotein complexes (Joshi et al., 2013).

8.3. HDAC11 and the immune system

It has been known for several years that HDAC11 negatively controls IL-10 transcriptional activity in antigen-presenting cells and regulates immune tolerance (Villagra et al., 2009). Recent evidence indicates that HDAC11 levels decrease after T cell activation and mouse T cells KO for *Hdac11* exhibit an enhanced proinflammatory profile. Hdac11 binds to promoters and represses transcription of TFs Tbet and Eomes, important regulators of inflammatory cytokines and effector molecules production (Woods et al., 2017). Consequently, mice receiving T cells from Hdac11 KO donors rapidly develop graft-versus-host disease. The role of Hdac11 in regulating T cell tolerance is confirmed by its influence on anti-tumor effect of the immune system. In a B-cell lymphoma model, mice receiving T cells from Hdac11 KO donors show delayed tumor growth (Woods et al., 2017). In addition to the epigenetic activity, the effect of Hdac11 on immune tolerance can also be exploited by regulating acetylation of non-histone proteins. Hdac11 is involved in the control of Foxp3 acetylation, and its absence in Foxp3+ Treg cells results in enhanced suppressive activity due to the increased expression of some T-reg-associated genes such as *Tgf-β* and *Foxp3* (Huang et al., 2017). As suggested above, HDAC11 is also associated with innate immunity and type I IFN signaling. Through de-fatty acid acylation of SHMT2a, HDAC11 may downregulate the immune response associated with type I IFN. (Cao et al., 2019). It is plausible that the role of HDAC11, possibly in complex with other partners, in the control of the proinflammatory microenvironment and humoral immune responses is due to an amplifying effect rather than a crucial switch (Woods et al., 2017). Nevertheless, it represents an interesting target for enhancing the anti-tumor activity of the immune system.

9. Conclusions

A discussion of the importance of HDACs in cancer is a titanic task. We have focused attention on selected subfamilies (classes I, IIa, and IV) that are better understood for a contribution to epigenetic regulation. Although frequently implicated in cancer, they do not always involve epigenetic targets. In summary, the role of HDACs in cancer is critical and complex, with room for therapeutic intervention. We hope that the most promising isoform-specific inhibitors will soon clinically approved. However, we still do not understand how these enzymes can select the right substrate in a time- and context-dependent manner. Another important issue is the definition of the different complexes in which HDACs act. We have collected a lot of data about the different HDACs and their involvement in various cancer-related functions using siRNA, shRNA, or genome editing. Unfortunately, we know little about the multiprotein complexes that accompany the different HDACs in their tasks. These characterizations may also clarify some conflicting results about the biological functions of these enzymes and better justify the use of selective inhibitors in therapy. We still need to conduct research in this direction. We hope that this review will stimulate connections and new thinking among readers, and we apologize to all colleagues working on these deacetylases for not citing their manuscripts due to space limitations.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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