Cell Cycle 14:12, 1779–1785; June 15, 2015; © 2015 Taylor & Francis Group, LLC

Histone deacetylases 1 and 2 regulate DNA replication and DNA repair: potential targets for genome stability-mechanism-based therapeutics for a subset of cancers

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Histone deacetylases 1 and 2 (HDAC1,2) belong to the class I HDAC family, which are targeted by the FDA-approved small molecule HDAC inhibitors currently used in cancer therapy. HDAC1,2 are recruited to DNA break sites during DNA repair and to chromatin around forks during DNA replication. Cancer cells use DNA repair and DNA replication as survival mechanisms and to evade chemotherapy-induced cytotoxicity. Hence, it is vital to understand how HDAC1,2 function during the genome maintenance processes (DNA replication and DNA repair) in order to gain insights into the mode-of-action of HDAC inhibitors in cancer therapeutics. The first-in-class HDAC1,2-selective inhibitors and *Hdac1,2* conditional knockout systems greatly facilitated dissecting the precise mechanisms by which HDAC1,2 control genome stability in normal and cancer cells. In this perspective, I summarize the findings on the mechanistic functions of class I HDACs, specifically, HDAC1,2 in genome maintenance, unanswered questions for future investigations and views on how this knowledge could be harnessed for better-targeted cancer therapeutics for a subset of cancers.

Keywords: DNA repair, DNA replication, Genome stability, HDAC1,2, mechanism-based cancer therapy

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Submitted: 02/24/2015

Accepted: 04/13/2015

http://dx.doi.org/10.1080/15384101.2015.1042634

Histone Deacetylases (HDACs) and Histone Deacetylase Inhibitors (HDIs)

Histone deacetylases remove acetyl groups from histone and non-histone proteins;[1,2] they are properly termed as lysine deacetylases or KDACs, but for historical reasons remain better known as HDACs. Eighteen HDACs have been identified so far in mammalian cells, which are divided into four classes: Class I HDACs include HDACs 1, 2, 3 and 8, and are homologous to yeast Rpd3. Class II consists of HDACs 4, 5, 6, 7, 9 and 10, and they have high similarity to yeast Hda1. Additionally, Class IIa enzymes (HDACs 4, 5, 7, 9) depend on Class I HDACs for their activity. Sirtuins, similar to yeast Sir2, form Class III HDACs and their activity is dependent on NAD. HDAC11 is the only Class IV HDAC with features similar to Class I and Class II HDACs.[2,3]

Histone deacetylase inhibitors (HDIs), small molecules that inhibit HDAC activities, are potent anti-proliferative agents that selectively kill cancer cells. Currently available HDIs belong to four classes based on their chemical structure: hydroxamate, cyclic peptide, aliphatic acids and benzamide.[4] When used as a monotherapy agent, HDIs have shown a very positive response in patients with hematologic malignancies, but they have been less effective on solid tumors.[5] Two broad-spectrum or pan−HDIs, SAHA or Vorinostat (a hydroxamate class inhibitor) and Depsipeptide or Romidepsin (a cyclic peptide class inhibitor), are currently approved by the FDA for the treatment of refractory cutaneous T-cell lymphoma (CTCL).[6,7] This approval has spurred the creation and testing of several novel HDIs and at least 20 of them are currently in preclinical or clinical trials.[8] An effective

HDI to treat B-cell malignancies is still not available in the clinic and several phase I studies using SAHA/vorinostat are currently underway for lymphomas.

While SAHA and Depsipeptide are effective anti-neoplastic agents, the broad-spectrum mechanism of action of these FDA-approved HDIs was not fully understood. HDACs and HDIs have been studied mainly in the context of gene transcription and not genome stability in cancers. For instance, acute myeloid leukemia (AML) is associated with misregulated gene expression due to recruitment of HDACs to ectopic loci by mutant transcription factors.[9,10] HDI treatment is therefore thought to kill these AML cells by reversing the repressive effects of aberrantly targeted HDACs.[11] To develop and use specific selective inhibitors to individual HDACs, which might lead to a safer and more effective HDAC inhibitors with less side-effects, it is imperative to understand the transcription-independent functions of individual HDACs in normal and cancer cells. This knowledge is required for not only better design, but also for the better understanding of the mode-of-action of selective HDAC inhibitors.

Genetic analyses of Class I HDACs using conditional knockout mouse models and knockdown systems reveal their important functions in the genome maintenance

Several HDAC inhibitors are currently being used in clinical trials for B-cell lymphomas and two of these inhibitors are FDA-approved drugs for the treatment of refractory cutaneous T-cell lymphoma. However, these FDA-approved broad-spectrum HDAC inhibitors inhibit ten different HDACs, and therefore have several adverse side effects in cancer patients, which include cardiac toxicity, thrombocytopenia and gastrointestinal toxicity. Hence, using selective inhibitors against specific class I will cause maximum cytotoxicity in cancer cells, but at the same time minimize the side effects caused by the use of pan-HDAC inhibitors. Before, we use selective HDAC inhibitors in the clinic; it is imperative to understand biological functions of individual HDACs. Class I HDACs consist of HDAC1, 2, 3 and 8. Our previous studies uncovered novel functions for HDAC3 in genome maintenance processes (DNA repair and DNA replication).[12-14]

Targeted deletion of *Hdac3* in the germ line led to embryonic lethality.[12] Using conditional *Hdac3* knockout mice, we showed that the deletion of *Hdac3* leads to S-phase-dependent DNA double-strand breaks in cycling cells and not in quiescent cells, which could provide a therapeutic window.[12] Cell cycle analysis of *Hdac3-null* cells revealed a delay in the progression of cells through the S-phase, accumulation of S-phase dependent DNA damage and activation of the S-phase cell cycle checkpoint response.[12] Moreover, *HDAC3*-null cells displayed a reduction in both homologous recombination and non-homologous recombination pathways,[14] suggesting that the absence of HDAC3 impairs double-strand DNA break repair. Even though short-term loss of *Hdac3* causes cell death in primary mouse embryo fibroblasts, long-term deletion of *Hdac3* in livers leads to hepatocellular carcinoma in mice.[14] Thus, genetic knockout studies provide not only insights into the biological functions of HDACs, but also provide valuable information regarding the efficacy of HDIs in cancer therapy when used for a short duration and the demerits of their sustained long-term use.[12-14] HDAC3 activity is also required for the removal of H3K4 acetylation (H3K4ac) at centromeres and loss of HDAC3 leads to dissociation of sister chromatids in HeLa cells.[15] The increase in H3K4ac in *HDAC3*-depleted cells occurs before the entry of cells into mitosis,[15] which is consistent with our findings that HDAC3 dissociates from chromatin before the metaphase stage.[14] Hence, in addition to DNA repair and DNA replication, HDAC3 maintains genome stability by controlling centromeric functions.[15] Recent intriguing discoveries demonstrate that HDAC3 could regulate transcription independent of its catalytic activity in mouse livers.[16] In this study, the interaction of HDAC3 with its stable partners NCoR and SMRT was found to be important for the deacetylase-independent functions of HDAC3 during transcription.[16] Interestingly, we previously found that knockdown of both *NCoR* and *SMRT* decreased the total cellular levels of HDAC3 and activated the DNA damage response, demonstrating the importance of NCoR-SMRT-HDAC3 nexus in regulating genome stability.[14] Hence, targeting *NCOR* and *SMRT*, in addition to targeting *Hdac3*, would be another strategy to trigger DNA damage response in cancer cells.

A great deal of knowledge gained from the elegant studies using conditional single gene or double gene knockout mouse models in various cell lineages or tissues have together shed light on the vital, redundant and non-redundant biological functions of HDAC1 and HDAC2, which are two highly similar enzymes. Targeted deletion of *Hdac1* led to embryonic lethality.[17] Distinct phenotypes in *Hdac2*-null (*Hdac2*$^{-/-}$) mice were observed. In one study, *Hdac2*$^{-/-}$ pups died within a month, due to cardiac defects and abnormalities in myocyte proliferation.[18] In another study, 50 percent of *Hdac2*$^{-/-}$ pups died perinatally, whereas the remaining littermates survived.[19] Other studies indicate that *Hdac2*$^{-/-}$ mice are viable.[20,21] However, mice that survived had smaller hearts when compared to the control littermates. The reason behind different phenotypes in *Hdac2*$^{-/-}$ mice could be attributed to the different strategies used to make these knockout mice and (or) due to different mouse strains. In the vast majority of cell types (including those made during hematopoiesis), targeted deletion of either *Hdac1* or *Hdac2* has minimal effects on proliferation and the cell cycle, likely due to compensation for one by the other.[22,23] However, combined deletion of both *Hdac1* and *Hdac2* (*Hdac1,2*) dramatically impairs proliferation in multiple cell types by blocking cells at the G1 to S phase stage.[23] Simultaneous deletion of *Hdac1* and *Hdac2* in early B-cell progenitors leads to a dramatic block in B-cell development and apoptosis. However, mature non-dividing terminally differentiated B-cells can tolerate loss of both *Hdac1* and *Hdac2*, but their deletion affects proliferation upon exogenous mitogenic stimulation.[23] Loss of HDAC1,2 function in embryonic stem cells led to chromatin bridges and mitotic instability.[24] Similarly, loss of HDAC1,2 function in fibrosarcoma cells led to mitotic catastrophy.[25]

HDAC1,2 dissociate from mitotic chromosomes during mitosis and reappear in the daughter nuclei.[26] Interestingly, loss of HDAC1 function in tumor cells led to defective entry into mitosis, which in turn leads to apoptosis.[27] These findings together reveal that HDAC1,2 play a crucial role when cells progress through the cell cycle.

In mice with *Hdac2* alleles but without the *Hdac1* alleles (i.e., *Hdac1-null* mice), immature thymocytes accumulation and lymphoblastic lymphomas were observed.[28] Similarly, mice with *Hdac1* and no *Hdac2* alleles (i.e., *Hdac2-null* mice) also developed lymphomas.[28] However, lymphamogenesis was not observed when both HDAC1 and HDAC2 activities are lost in T-cells.[28] Instead, deletion of *Hdac1* and *Hdac2* led to a block in thymocyte development. A similar dosage dependent effect upon loss of either *Hdac1* or *Hdac2* was observed on epidermal proliferation and differentiation.[29] In this elegant study, deletion of a single *Hdac2* allele in *Hdac1*-knockout mice caused severe defects in the development of epidermal lineages and spontaneous tumor formation.[29] Therefore, the use of genetic knockout mouse models in parallel with the isotype selective inhibitors of HDACs is required to compare and assess the mode-of-action, specificity, benefits, and pitfalls of complete and partial inhibition of individual HDACs.

The deletion of *Hdac8*, another class I HDAC, in mice led to death within 4–6 h following birth.[30] Conditional deletion of *Hdac8* specifically in the brain caused loss of cranial neural crest cells and instability of the skull in mice.[30] Hence, HDAC8 function is vital for skull development. HDAC8 is a deacetylase for SMC3 (Sister Maintenance of Chromosome protein 3, a core cohesion component), and mutations in *HDAC8* that disrupt SMC3 deacetylation result in an improper renewal of cohesin components and inadequate recycling of the cohesin components in the next cell cycle. This results in decreased cohesin occupancy in the genome leading to clinical features of Cornelia de Lange syndrome (CdLS), which is characterized by the congenital malformation disorder.[31]

Hence, all four class I HDACs are required for genome maintenance in mammalian cells. However, HDAC1,2 are recruited to sites of DNA replication and DNA damage break sites, suggesting a direct role for these two enzymes during DNA replication and double-strand break (DSB) repair.[32-34] HDAC3 associates with nascent chromatin during DNA replication.[33] Even though loss of HDAC3 impairs DSB repair,[14] it is not localized to DSB DNA damage sites.[14,32] Hence, the repair defects observed in *Hdac3-null* cells is likely due to the defective chromatin structure. Direct functions for HDAC8 at the replication fork and at DNA damage sites remain to be investigated.

HDAC1,2 maintain genome stability during DNA replication - the engine that drives rapid proliferation of cycling cells

Defective DNA repair and/or DNA replication are major causes of genome instability, which can trigger cell death. A single unrepaired double strand break is sufficient to cause cell death. Hence, it is crucial to understand functions for HDACs in genome maintenance pathways. HDAC1,2 interact with PCNA[35] and localize to sites of replication.[33,34] Deletion of both *HDAC1* and *HDAC2* using conditional knockout system results in the arrest of cells in the G1 phase,[23] and hence it was difficult to study functions of HDAC1,2 in the S-phase progression. Selective inhibitors make an excellent tool to study biological functions of HDAC1,2 as we could transiently inhibit their functions for a short duration of time when there is an impact on DNA replication and repair and before cells arrest in G1 phase.

Using selective inhibitors, we examined whether HDAC1,2 activities are required for efficient DNA replication in mammalian cells. A decrease in replication fork velocity was observed upon HDAC1,2 inhibition, demonstrating that HDAC1,2 activities are required for efficient replication fork elongation.[34] If stalled forks are not restarted in a timely fashion, it can result in fork collapse, formation of DSBs and activation of the DNA damage response.[36] Increased γH2AX (phosphorylated form of H2AX and a marker of double-strand breaks) was observed in cells treated with HDAC1,2 inhibitor or following knockdown of *Hdac1,2*. RNA-seq analysis of S-phase cells treated with HDAC1,2 selective inhibitor showed no change in the expression of genes involved in DNA replication or DNA repair.[34] Hence, our findings showed a direct role for HDAC1,2 in ensuring the proper progression of the replication fork. Activation of DNA damage response in S-phase cells due to obstruction of DNA fork progression provided the mechanistic basis for how HDAC1,2 selective inhibitors might be able to directly target regulators of genome stability in order to kill the rapidly cycling cancer cells.

Upon dissection of the mechanism behind these replication defects, we found that these enzymes control chromatin structure at and around replicating regions by targeting histone acetylation. We developed a technique termed modified BrdU-ChIP assay to examine histone modifications and replication proteins on nascent DNA.[34] This technique is a complement to the elegant iPOND technique that permits one to follow the dynamics of replication proteins associated with nascent DNA.[33] Using the modified BrdU-ChIP approach, we showed that inhibition of HDAC1,2 activity increases H4K16ac present on newly synthesized DNA and at replicating origins.[34] Hence, HDAC1,2 regulate H4K16ac associated with nascent chromatin during DNA replication. H4K16ac is known to block inter-nucleosomal interactions and disrupt chromatin packaging.[37] Therefore, we asked whether HDAC1,2 regulate nascent chromatin packaging around replication forks by targeting H4K16ac? An increase in the release of BrdU-labeled nascent DNA associated with di- and tri-nucleosomes was observed following micrococcal nuclease digestion in HDAC1,2 inhibitor-treated cells, confirming the role for HDAC1,2 activities in chromatin compaction during DNA replication.[34] SMARCA5 is a ISWI-family ATP-dependent chromatin remodeler, whose activity is inhibited by H4K16ac and H4K12ac marks,[38] and both of these marks are targets of HDAC1,2.[34] Hence, we asked is there a connection between SMARCA5 and HDAC1,2 functions at the

replication fork? We found that SMARCA5 is present on nascent DNA and importantly, loss of SMARCA5 also reduced fork velocity similar to the loss of HDAC1,2 activities.[34] We found that the level of SMARCA5 associated with replication origins increases whereas the level of H4K16ac at replication origins decreases when cells enter the S-phase.[34] Thus, HDAC1,2 activity appears to be required for the deacetylation of H4K16ac to facilitate SMARCA5-mediated remodeling of chromatin around the replication fork during S-phase. Overall, our studies demonstrated the functional interplay between a chromatin remodeler (SMARCA5), regulatory histone modifications (H4K12ac and H4K16ac) and histone deacetylases (HDAC1,2 that target H4K12ac and H4K16ac) at the replication fork. Moreover, these studies highlight the mechanism by which selective inhibition of HDAC1,2 is able to directly target chromatin structure and the chromatin remodeling around replication forks in order to obstruct the progression of DNA replication, trigger DNA damage response and selectively kill the rapidly cycling cancer cells.

HDAC1,2 is involved in DSB repair pathways that contribute to chemoresistance

Diffuse large B-cell lymphoma (DLBCL), a type of non-Hodgkin's lymphoma, is the most common lymphoid malignancy in the United States accounting for 40% of adult lymphomas.[39] A major advancement has been made in treating DLBCL with the addition of rituximab (an anti-CD20 monoclonal antibody) to the standard chemotherapy regimen CHOP (vincistrine, doxorubicin, cyclophosphamide, prednisone).[40,41] Despite overall improvement in treating DLBCL using this cocktail regimen, one-third of the patients fail standard therapy and have a poor outcome.[40,41] A vast majority of B-cell lymphomas are derived from the germinal centers of lymphomas.[42] These lymphoma cells constitutively express *BCL6* oncogene, due to translocations or mutations that result in deregulated *BCL6* expression.[42] BCL6 oncoprotein acts as a key transcriptional repressor of the ATM/ATR/p53 DNA damage-signaling pathway and facilitates hyperproliferation to provide a survival advantage to lymphoma cells.[43,44] The transcriptional repression is mediated through recruitment of HDACs 1, 2 and 3, via recruitment of SMRT, NCoR and BCoR to the BCL6-regulated genes.[45,46] Hence, small molecule inhibitors targeting the BCL6 oncoprotein or HDACs (specifically, HDAC1, 2 or HDAC3) would have therapeutic benefits in this subset of lymphoma.

Cancer cells in general, and refractory cancer cells in particular, utilize DNA repair activities as a survival mechanism to overcome the DNA damage caused by many chemotherapy drugs. Hence, inhibiting specific DNA repair pathways could induce cytotoxicity selectively in cancer cells. HDAC1,2 localize to double-strand damage sites and facilitate DNA repair.[32] Can we use this property of HDAC1,2 to target specific cancers resulting from increased DNA repair activities due to mutations in genes that code for proteins involved in DNA repair?

Sequencing of DLBCL lymph node biopsy samples has identified somatic mutations in *EZH2* (enhancer of zeste homolog 2).[47] EZH2 is the enzymatic component of the polycomb repression complex 2 (PRC2) and catalyzes trimethylation of H3 at the K27 residue (H3K27me3).[48] In about 22% of germinal center derived lymphomas, gain-of-function mutations in the tyrosine residue (Y641) within the EZH2 catalytic SET domain is observed.[47] This gain-of-function mutation in EZH2 ($EZH2^{GOF}$) results in high levels of H3K27me3 levels in DLBCL cells and has been implicated to promote lymphomagenesis.[49] In fact, a positive relationship has been observed between increased H3K27me3 and chemoresistance in ovarian cancer.[50] GSK126, a potent inhibitor of EZH2 activity, decreases H3K27me3 and promotes death in $EZH2^{GOF}$ DLBCL cells.[51] Thus, inhibiting EZH2 activity using small molecules to decrease the aberrant H3K27me3 is one potential strategy to overcome lymphomagenesis and/or chemoresistance in these refractory EZH2-activating mutant DLBCL cells.

H3K27me3 is also associated with DSB repair,[52] in addition to transcription. Hence, one could postulate that increased levels of H3K27me3 at break sites would protect these $EZH2^{GOF}$ DLBCL cells from DNA damage due to increased DNA repair. Hence, inhibiting EZH2-specific DNA repair pathway could cause cytotoxicity and DNA damage selectively in DLBCL cells that harbor the $EZH2^{GOF}$ mutation.[51] Our results showed that selective inhibition of HDAC1,2 increases global H3K27ac without decreasing pre-existing H3K27me3, but it decreases H3K27me3 specifically at DNA break sites, and causes cytotoxicity in the $EZH2^{GOF}$DLBCL cells.[53] Hence, our results further indicate a new mechanism whereby HDAC1,2 inhibition induces cytotoxicity in the $EZH2^{GOF}$ DLBCL cells by just altering the H3K27ac/H3K27me3 ratio and the level of H3K27me3 specifically at DSB sites.[53] In the future, we will further decipher the cross talk between HDAC1,2 and EZH2-mediated repair signaling in DLBCL cells to nail down the precise mechanism by which HDAC1,2 inhibition overcomes the H3K27me3-mediated chemoresistance in DLBCL cells.

We have also found that $EZH2^{GOF}$ DLBCL cells overexpress BBAP or DTX3L (Deltex (DTX)-3-like E3 histone ubiquitin ligase),[53] a chromatin-modifying enzyme that has a demonstrated role in chemoresistance.[54] BBAP enzyme catalyzes monoubiquitination of H4K91[55] and hence the activity of BBAP is not counteracted by the EZH2 inhibitor, GSK126. Therefore, in addition to overcoming EZH2 and H3K27me3-mediated chemoresistance, it is also important to overcome the chemoresistance mediated by BBAP. Importantly, BBAP catalyzes H4K91 monoubiquitylation (H4K91ub1),[55,56] that facilitates DNA repair signaling mediated by 53BP1.[55] We have found that selective inhibition of HDAC1,2 results in an increase in H4K91ac, decreases H4K91ub1 levels during DNA repair following treatment with doxorubicin (a chemotherapy agent) and thereby sensitizes the refractory $EZH2^{GOF}$DLBCL cells to doxorubicin.[53] Hence, inhibition of HDAC1,2 activities is able to impair the DNA repair processes by altering the H3K27ac-H3K27me3 switch and the H4K91ac-H4K91ubiquityl switch during

DNA repair mediated by EZH2 and BBAP enzymes, respectively, in order to overcome chemoresistance in the refractory EZH2^{GOF}DLBCL cells. Previously, knockdown of *HDAC1,2* was reported to impair 53BP1 recruitment to DNA damage sites and this phenotype was attributed to the increase in H4K16ac.[57] Hence, increased H4K91ac and H4K16ac is likely to contribute to the observed 53BP1-mediated DSB repair defects upon inhibition of HDAC1,2. H4K91 is also linked to chromatin assembly during DNA repair in yeast,[58] in addition to 53BP1 signaling during DNA repair in mammalian cells. It is possible that increased H4K91ac following HDAC1,2 inhibition could prevent H2A-H2B deposition onto the H3-H4 tetramer and disrupt nucleosome assembly, which in turn might result in an unstable nucleosome/chromatin that is prone to DNA damage in cancer cells, thus providing another mechanism for HDAC1,2 inhibitor action. Overall, selective inhibition of HDAC1,2 activity using small molecules could therefore provide a novel DNA repair mechanism-based therapeutic approach in the EZH2-^{GOF}DLBCL cells by simultaneously negating two parallel and important pathways of DSB repair mediated by EZH2- and BBAP. These findings along with our DNA replication studies reveal that HDIs, more specifically selective HDAC1,2 inhibitors, act as genome-stability mechanism based cancer therapeutics.

Summary and Perspectives

In our recent publications (Bhaskara et al. (2013) and Johnson et al. (2015)),[34,53] we showed novel functions for HDAC1,2 during DNA replication and DNA repair that contributes to chemoresistance in cancer cells. These studies provide the basis to design a novel genome stability-targeted HDAC inhibitor therapy for a subset of cancers. HDAC1,2 inhibition causes DNA damage activation as a result of replication stress response and defective nascent chromatin structure in rapidly cycling cancer cells.[34] At the same time, defective DSB repair results in the failure of cancer cells to repair DNA damage resulting from collapsed replication forks and from treatment of cancer cells with chemotherapy agents, such as, doxorubicin. We are currently testing whether this mechanism based strategy would be applicable for other cancers with perturbed specific repair pathways that involve HDAC1,2. Can HDAC1,2 inhibition then provide therapeutic benefits for all cancers with increased DSB DNA repair? The answer is probably not. There are 18 HDACs in mammalian cells[59] and class III Sirtuin family HDACs have been associated with DSB repair.[60-63] Hence, one could speculate that repair defects due to loss of HDAC1,2 activity might be compensated by other HDACs or the Sirtuin family members that are also involved in DSB repair. However, HDAC1,2 inhibition might still be a efficient way to target specific repair pathways that are addicted to HDAC1,2 and do not require Sirtuin functions. Hence, it is critical to dissect out the repair mechanisms specifically targeted by HDAC1,2 and not compensated by other HDAC family members. Moreover, chromosomal translocations that recruit HDAC1,2 as well as HDAC3 in certain cancers, cannot be targeted by inhibiting HDAC1,2 or HDAC3 alone. In these cases, combined action of HDAC1,2 and HDAC3 selective inhibitors may be necessary to achieve maximum clinical potency. Hence, mechanistic studies are going to be critical in order to avoid using selective HDAC inhibitors as a pan-cancer therapy, which might lead to unwanted toxicity with little clinical effectiveness. Moreover, Mi-2/NuRD complex that contains HDAC1,2 is required for proper heterochromatin formation and for S-phase progression.[64] NuRD complex also localizes to double strand break sites.[52] Do HDAC1,2 regulate the chromatin during DNA replication and DNA repair via the NuRD complex needs to be investigated in future. While transient inhibition of HDACs causes cytotoxicity in cancer cells, conditional deletion of *Hdac3* specifically in the liver leads to hepatomegaly[65] and hepatocellular carcinoma.[14] Therefore, while long-term loss of HDAC function may result in secondary tumors, short-term inhibition of HDAC activity is a viable option for treating cancers. Overall, the comprehensive knowledge of how class I HDACs regulate genome stability will help us in the innovative design of monotherapy or combination therapies with inhibitors against other chromatin-modifying enzymes for certain cancers. The use of new mechanism-based strategies will augment our ongoing efforts to understand cancer epigenetics as well as provide a potential novel effective cancer therapeutic strategy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

I sincerely thank my colleague in the HDAC field, Christian Seiser (Max F. Perutz Laboratories, Vienna Biocenter, Medical University of Vienna) for his critical reading of this review and valuable suggestions. I also thank Mahesh Chandrasekharan (Huntsman Cancer Institute, University of Utah) for his valuable comments on the review. I thank all the members of my lab for their valuable contributions to the work described on HDAC1,2.

Funding

Funding for the work described here was provided by development funds to S.B. from the Huntsman Cancer Institute and the Department of Radiation Oncology.

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