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REVIEW

Histone deacetylase inhibitors in hematological malignancies and solid tumors

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Abstract Histone deacetylase (HDAC) inhibitors are emerging as promising anticancer drugs. Because aberrant activity and expression of HDACs have been implicated in various cancer types, a wide range of HDAC inhibitors are being investigated as anticancer agents. Furthermore, due to the demonstrable anticancer activity in both in vitro and in vivo studies, numerous HDAC inhibitors have undergone a rapid phase of clinical development in various cancer types, either as a monotherapy or in combination with other anticancer agents. Although preclinical trials show that HDAC inhibitors have a variety of biological effects across multiple pathways, including regulation of gene expression, inducing apoptosis and cell cycle arrest, inhibiting angiogenesis, and regulation of DNA damage and repair, the mechanism by which the clinical activity is mediated remains unclear. Understanding the mechanisms of anticancer activity of HDAC inhibitors is essential not only for rational drug design for targeted therapies, but for the design of optimized clinical protocols. This paper describes the links between HDACs and cancer, and the underlying mechanisms of action of HDAC inhibitors against hematological malignancies and solid tumors. Further, this review presents the clinical outcomes of vorinostat, romidepsin, and belinostat, which are approved by the United States Food and Drug Administration for the treatment of lymphomas.

Keywords Histone deacetylase inhibitors · Cancer · Apoptosis · Cell cycle arrest · Angiogenesis · DNA damage and repair

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Introduction

Histone deacetylase (HDAC) inhibitors are a class of compounds that regulate acetylation states of histone proteins and other non-histone proteins by inhibiting the activity of HDAC. Because of the demonstrable antitumor activity in both in vitro and in vivo studies, HDAC inhibitors have undergone a rapid phase of clinical development in a wide range of cancer types, either as a monotherapy or in combination with other anticancer agents (Younes et al. 2014; Seo 2012; El-Khoury et al. 2014; Ogura et al. 2014).

To date, three HDAC inhibitors are approved by the United States Food and Drug Administration (U.S. FDA) based on the good clinical activity and favorable toxicity profile: vorinostat, also called suberoylanilide hydroxamic acid (Zolinza®, Merck and Co., Inc.) for treating cutaneous T cell lymphoma (CTCL) in October 2006; romidepsin, also known as depsipeptide and FK228 (Istodax®, Gloucester Pharmaceuticals—a subsidiary of Celgene Corp) for the treatment of CTCL in November 2009 and for peripheral T-cell lymphoma (PTCL) in May 2011; belinostat, also called PXD101 (Beleodaq®, Spectrum Pharms, Inc.) for the treatment of patients with relapsed or refractory PTCL in July 2014 (Drugs@FDA).

HDACs play a critical role towards the transcription regulation, removing the acetyl group from the ε-amino groups of the lysine residues on histones. While acetylation correlates with nucleosome remodeling and is generally associated with elevated gene transcription, deacetylation of histone tails induces transcriptional repression through chromatin condensation. This may be explained by the fact that acetylation neutralizes the positive charge of lysine residues and leads to relaxation of the chromatin structure, facilitating the accessibility of a variety of transcription factors to DNA (Norton et al. 1989; Grunstein 1997;

Ropero and Esteller 2007). Besides regulating histone modification, HDACs also regulate the post-translational acetylation status of many non-histone proteins. HDAC-mediated deacetylation alters the transcriptional activity of nuclear transcription factors, E2F1, GATA1, GATA2, MyoD, nuclear factor κB (NF-κB), p53, runt-related transcription factor 3 (RUNX3), and Ying Yang 1 (YY1). In addition, proteins that regulate cellular cytoskeleton and protein stabilization are also being regulated by HDAC. HDAC6 deacetylates α-tubulin and aids in the remodeling of the synapse, which regulates the organization of adhesion and signaling molecules. Moreover, HDAC6 has been shown to regulate acetylation of Hsp90 that plays a major role in the proper wrapping and stabilization of several oncoproteins. It has also been shown that HDAC6 interacts with short heterodimer partner (SHP) and contributes to the intrinsic transrepressive activity of SHP (Table 1). These findings imply that antitumor activity of HDAC inhibitors includes effects on non-histone proteins as well as effects on chromatin modification. Through hyperacetylation of histone and non-histone targets, HDAC inhibitors can induce diverse biological effects, including regulation of gene expression, inducing apoptosis and cell cycle arrest, inhibiting angiogenesis, and regulation of DNA damage and repair pathway (Richon et al. 2000; Kim et al. 2014; Konstantinopoulos et al. 2014; Zhou et al. 2014). Therefore, HDAC inhibitors have great potential as anticancer drugs.

The present review describes the underlying mechanisms of anticancer activity of HDAC inhibitors, focusing on the agents that could have implications for the future use in cancer treatment.

The classification of HDACs

In humans, 18 HDAC enzymes have been identified and classified into four classes according to their homology to yeast HDACs (yHdas), their subcellular location, their tissue specificity and their enzymatic activity. Among them, the 11 enzymes, which require Zn^{2+} as a cofactor for their deacetylase activity, belong to classes I, II, and IV whereas the sirtuins (SIRT1-7) belong to class III HDACs and they are Zn^{2+}-independent (de Ruijter et al. 2003; Gregoretti et al. 2004). The class III HDACs will not be discussed in this paper due to two reasons. Firstly, the role of some sirtuins in

Table 1 Functional consequences of deacetylation of non-histone proteins and HDACs implicated

HDAC	Proteins	Categories	Consequence of deacetylation	References
HDAC1	AR	Steroid receptor	Repressed the activity of AR	Gaughan et al. (2002)
HDAC1	E2F1	Transcription factor	Repressed the activity of E2F1	Martínez-Balbás et al. (2000)
HDAC1	ERa	Steroid receptor	Reduced the protein levels of ERa and increased cell proliferation	Kawai et al. (2003)
HDAC3, 4, 5	GATA1	Transcription factor	Repressed the activity of GATA1	Watamoto et al. (2003)
HDAC3, 5	GATA2	Transcription factor	Repressed the activity of GATA2	Ozawa et al. (2001)
HDAC6	Hsp90	Chaperone protein	Repressed Hsp90 function	Bali et al. (2005) and Kovacs et al. (2005)
HDAC1	MyoD	Transcription factor	Inhibition of MyoD deacetylation of MyoD repressed myoblast proliferation	Mal et al. (2001) and Mal and Harter (2003)
HDAC3	NF-κB	Transcription factor	Termination of the NF-κB transcriptional response. Contribution to the replenishment of latent NF-κB-IκBo	Chen et al. (2001)
HDAC1	p53	Transcription factor	Reduced the level of apoptosis	Luo et al. (2000)
HDAC7	PLAG1, PLAGL2	Adenoma gene	Repressed activity of PLAG1 and PLAGL2	Zheng and Yang (2005)
HDAC4, 5	RUNX3	Transcription factor	Degradation of RUNX3	Jin et al. (2004)
HDAC1, 3, 6	SHP	Nuclear receptor	Repressed transcription activity of SHP	Gobinet et al. (2005)
HDAC8	SMC3	Cohesion complex component gene	Increased cohesion	Decroos et al. (2014)
HDAC1, 2, 3	STAT3	Signaling mediator	Inhibited transcription of STAT3 target genes	Yuan et al. (2005)
HDAC6	α-Tubulin	Structural protein	Translocation of the microtubule-organizing center. Impaired IL-2 production	Serrador et al. (2004)
HDAC1, 2, 3	YY1	Transcription factor	Repressed transcription	Yang et al. (1997)

AR, androgen receptor; ERα, estrogen receptor α; NF-κB, nuclear factor-kB; PLAG1, pleomorphic adenoma gene 1; PLAGL2, PLAG-like 2; RUNX3, runt-related transcription factor 3; SHP, short heterodimer partner; SMC3, structural maintenance of chromosomes 3; STAT3, signal transducer and activator of transcription 3; YY1, Ying Yang 1

tumorigenesis is still controversial. For example, SIRT1 is expressed at a higher level in cancerous cells and promotes oncogenesis through deacetylation of lysine 382 in Burkitt lymphoma cells (Heltweg et al. 2006). However, in a colon cancer mouse model, increased SIRT1 expression suppressed cell proliferation and tumor formation (Firestein et al. 2008). The second reason is that a wide range of structures have been identified to be able to inhibit the activity of classes I, II, and IV HDACs, but not the NAD^+-dependent class III enzymes (Marks et al. 2001; Johnstone 2002).

The class I HDACs are homologous to yeast (*Saccharomyces cerevisiae*) transcriptional regulator RPD3 and include HDAC1, -2, -3, and -8. They are usually located in the nucleus and ubiquitously expressed in various human tissues (Bertrand 2010). The class II HDACs have homology to yeast HDAC 1 (yHda1), and are further subdivided into two subclasses, IIa and IIb, based on sequence homology and domain organization. The class IIa HDACs, HDAC4, -5, -7, and -9, contain a highly conserved C-terminal deacetylase catalytic domain ($\sim$420 amino acids) homologous to yHda1 and share an N-terminal domain ($\sim$450–600 amino acids) with no similarity to HDACs in other classes. The N-terminal domains mediate interactions with myocyte enhancer factor 2 family of transcription factors, transcriptional corepressor C-terminal binding protein, and others. The class IIa HDACs are shuttled between the cytoplasm and the nucleus, and their expression is tissue-specific. The class IIb HDACs, HDAC6 and -10, are characterized by the presence of two catalytic HDAC domains arranged in tandem. The class IIb HDACs are mainly located in cytoplasm and are expressed in a restricted number of cell types (Bertos et al. 2001; Fischle et al. 2001; Verdin et al. 2003). HDAC11 is the sole member of class IV and resides in the nucleus; however, in activity assays, HDAC11 co-precipitates with the cytoplasmically localized HDAC6 (Bertos et al. 2001; Gao et al. 2002).

HDACs and cancer

HDACs regulate expression and activity of numerous proteins involved in both cancer initiation and cancer progression. Recruitment of HDACs to promoters causes a closed chromatin conformation that is inaccessible to transcription machinery or its mediators, resulting in transcriptional repression. Alterations in histone acetylation status have been involved in the development of cancer. In particular, a global loss of monoacetylation of lysine 16 and trimethylation of lysine 20 on histone H4 (H4K16ac and H4K20me3) has been found to be a common hallmark of human cancer (Fraga et al. 2005).

Aberrant expression and mutation of HDACs have been implicated in a variety of tumor types, making them attractive targets for anticancer drugs and therapies (Table 2). Overexpression of individual HDACs has been found to be significantly associated with poor disease-free, event-free, and overall survival (Weichert et al. 2008c; Oehme et al. 2009; Milde et al. 2010; Moreno et al. 2010; Minamiya et al. 2011; Quint et al. 2011). Furthermore, overexpressed HDACs have been correlated with aggressiveness, invasion and migration of cancer and have been found to be a poor prognosis indicator that is independent from other factors such as tumor type, age, sex, or comorbid condition (Song et al. 2005; Weichert et al. 2008a, b; Hayashi et al. 2010; Park et al. 2011; Wang et al. 2011; Müller et al. 2013; Li et al. 2014).

However, overexpression of HDACs is not always a poor prognostic marker. Interestingly, overexpressed HDAC1 and -6 have been associated with favorable outcome in Hodgkin's lymphoma and in chronic lymphocytic leukemia, respectively (Marquard et al. 2008; Adams et al. 2010). What's more, in non-small cell lung cancer, lower level of gene expression has been related with poorer prognosis (Osada et al. 2004). On top of that, overexpression of HDACs can serve as a molecular biomarker: up-regulation of HDAC2 involves in early events of colorectal carcinogenesis (Stypula-Cyrus et al. 2013) while high HDAC7 expression has been able to discriminate pancreatic adenocarcinomas from other pancreatic tumors (Ouaïssi et al. 2008). Overexpression of HDAC1 and -2 has been observed in many cancer types and in some cases it is significantly related to tumor cell growth with corresponding decrease in p21 expression (Halkidou et al. 2004; Xie et al. 2012; Jung et al. 2012). Indeed, the cyclin-dependent kinase (CDK) inhibitor p21 is a crucial target for HDAC inhibitors. In many cancer cell lines, HDAC inhibitors causes the transcriptional upregulation of this antiproliferative gene p21 and subsequently block the cyclin/CDK complexes, leading to cell G1 cycle arrest (Sandor et al. 2000). Moreover, in non-small cell lung cancer, HDAC2 inactivation resulted in regression of tumor cell growth via inductions of p53 and Bax expression and simultaneously suppressed Bcl-2 expression (Jung et al. 2012). Mutations of HDACs also contribute to tumorigenesis: the presence of the HDAC2 frame shift mutation renders cancer cells resistant to the anti-proliferative and proapoptotic effects of HDAC inhibitors in patients with hereditary nonpolyposis colorectal cancer (Ropero et al. 2006). Recently, overexpression of HDAC5 has been demonstrated in human hepatocellular carcinoma cell lines, which promotes tumor cell proliferation through up-regulation of Six1 (Feng et al. 2014).

In addition to altered expression of HDACs, the aberrant recruitment of HDACs to certain target genes through binding to oncogenic fusion proteins has been proposed as an important mechanism of tumorigenesis. For example, promyelocytic leukemia–retinoic acid receptor α (PML–

Table 2 Clinical or preclinical effects of HDAC dysregulation in cancer

Cancer types	Implicated HDAC and expression	Clinical/preclinical effects	References
ALL	↑ HDAC2, 3, 6, 7, 8	Overexpression of HDAC3, -7, and -9 was associated with poor EFS	Moreno et al. (2010)
CLL	↑ HDAC1, 3, 6, 7, 9, 10	Higher expression levels were associated with higher levels of prognostic factors	Wang et al. (2011)
Gastric	↑ HDAC2	Overexpression of HDAC2 was associated with tumor aggressiveness	Song et al. (2005)
	↑ HDAC1, 2	Overexpression of HDAC1 and -2 was independent prognosis indicator	Weichert et al. (2008a)
Breast	↑ HDAC1, 2, 3	Overexpression of HDAC2 and -3 was associated with cancer progression	Müller et al. (2013)
	↑ HDAC4, 6, 8	Overexpression of HDAC6 and -8 increased invasion	Park et al. (2011)
	↑ HDAC11	Depletion of HDAC11 inhibited metabolic activity of cancer cell	Deubzer et al. (2013)
Colon	Truncating mutation in HDAC2	Cells became more resistant to antiproliferative/proapoptotic effects of HDAC inhibitor	Ropero et al. (2006)
	↑ HDAC1, 2, 3	Overexpression of HDAC2 was an independent prognosis indicator	Weichert et al. (2008b)
	↑ HDAC1, 2, 3, 5, 7	Overexpression of HDAC2 may serve as a biomarker of colorectal carcinogenesis	Stypula-Cyrus et al. (2013)
Liver	↑ HDAC1	Sustained suppression of HDAC1 regressed tumor cell growth	Xie et al. (2012)
	↑ HDAC1, 2, 3 (HCC)	Overexpression of HDAC2 was associated with poor survival	Quint et al. (2011)
	↑ HDAC5 (HCC)	Overexpression of HDAC5 promoted cell proliferation by up-regulation of Six1	Feng et al. (2014)
Medulloblastoma	↑ HDAC5, 9	Overexpression of HDAC5 and -9 were associated with poor OS	Milde et al. (2010)
NSCLC	↑ HDAC1	Overexpression of HDAC1 was associated with poor DFS	Minamiya et al. (2011)
	↑ HDAC2	Sustained suppression of HDAC2 regressed tumor cell growth	Jung et al. (2012)
	↓ HDAC5, 7	Lower level of gene expression was associated with poorer prognosis	Osada et al. (2004)
Lymphoma	↑ HDAC1, 2, 6 (CTCL)	Overexpression of HDAC6 was associated with favorable outcome	Marquard et al. (2008)
	↑ HDAC1, 2, 3 (HL)	Overexpression of HDAC1 woo associated with favorable outcome	Adams et al. (2010)
Neuroblastoma	↑ HDAC8	Overexpression of HDAC8 was associated with poor EFS	Oehme et al. (2009)
Ovarian	↑ HDAC1, 2, 3	Overexpression of HDAC1 was associated with a poor outcome	Hayashi et al. (2010)
Pancreatic	↑ HDAC6	HDAC6 stimulated the migration of pancreatic cancer cells	Li et al. (2014)
	↑ HDAC7 (PA)	Overexpression of HDAC7 discriminated PA from other pancreatic tumors	Ouaïssi et al. (2008)
Prostate	↑ HDAC1	Overexpression of HDAC1 enhanced cell proliferation	Halkidou et al. (2004)
	↑ HDAC1, 2, 3	Overexpression of HDAC2 was associated with poor PSA-relapse-FS	Weichert et al. (2008c)
Renal	↑ HDAC1, 2	None of the HDACs was significantly associated with the patient survival time	Fritzsche et al. (2008)

↑, Overexpression; ↓, underexpression; DFS, disease-free survival; EFS, event-free survival; OS overall survival; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; HCC, hepatocellular carcinoma; HL, Hodgkin's lymphoma; NSLC, non-small cell lung cancer; PA, pancreatic adenocarcinomas; PSA, prostate specific antigen

RARα) and PML zinc finger (PLZF)–RARα, mutant forms of RARα produced by chromosomal translocations with either PML gene or PLZF gene, gain corepressor activity upon aberrant recruitment of HDAC complexes containing nuclear receptor corepressor (NCoR), and obtain subsequent leukemogenic potential upon aberrant recruitment of mSin3a and silencing mediator for retinoid and thyroid receptors (Hörlein et al. 1995; Lin et al. 1998; Kouzarides 1999). Similar phenomena have been described for acute myeloid leukemia 1-eight twenty-one (AML1–ETO), a fusion of the AML1 and ETO proteins (Gelmetti et al. 1998). Atsumi et al. (2006) have demonstrated that, in acute PML cells, HDAC3 is recruited to target promoters by PML–RARα, a component of the NCoR repressor complex, to repress transcription whereas Amann et al. (2001) have shown that the AML1–ETO fusion protein recruits HDAC1, -2, and -3 via ETO to repress transcription of leukemic cells.

Mechanisms of action of HDAC inhibitors

A large number of structurally diverse HDAC inhibitors have been identified from natural sources and developed synthetically, and many of them are undergoing clinical trials (Fig. 1). Numerous studies have shown that certain HDAC inhibitors selectively inhibit different HDACs (Table 3). Furthermore, the finding that normal cells are relatively resistant to HDAC inhibitor-induced cell death compared to tumor cells (Ungerstedt et al. 2005) is fundamental to the success in clinical application of HDAC inhibitors.

By inducing acetylation of histones and non-histone proteins, HDAC inhibitors are able to elicit a wide range of biological effects (Fig. 2).

Selective alterations of gene expression

There is accumulating evidence that anticancer activity of HDAC inhibitors is linked to their ability to regulate the expression of specific proliferative and/or apoptotic genes. After treatment with SAHA, in bladder carcinoma cells, a significant increase in p21 mRNA and concurrent accumulation of acetylated histones H3 and H4 were observed independently of p53. In addition, SAHA caused a marked decrease in HDAC1 in the protein complex bound to the p21 promoter in multiple myeloma (MM) cells. However, the expression of p27 gene that is actively expressed in MM cells was not altered (Richon et al. 2000; Gui et al. 2004). Further evidence of the effects of HDAC inhibitors on gene expression has been demonstrated. In breast cancer cells, FK228 caused downregulation of cyclin D1 and upregulation of CDK inhibitor p21, resulting in dephosphorylation of the tumor suppressor retinoblastoma (Rb) and growth arrest in the early G1 phase (Sandor et al. 2000). Moreover, by SAHA and FK228, multiple genes within the Myc, transforming growth factor-β1, cyclin/CDK, tumor-necrosis factor (TNF), Bcl-2, and caspase pathways were up- or down-regulated, inducing apoptosis and inhibiting cellular proliferation. Although, a substantial number of genes were regulated in common by both SAHA and FK228, some genes, including apoptosis-regulatory BNip family members BNip1 and BNip3L, and NF-κB-pathway genes IκB were differentially regulated (Peart et al. 2005).

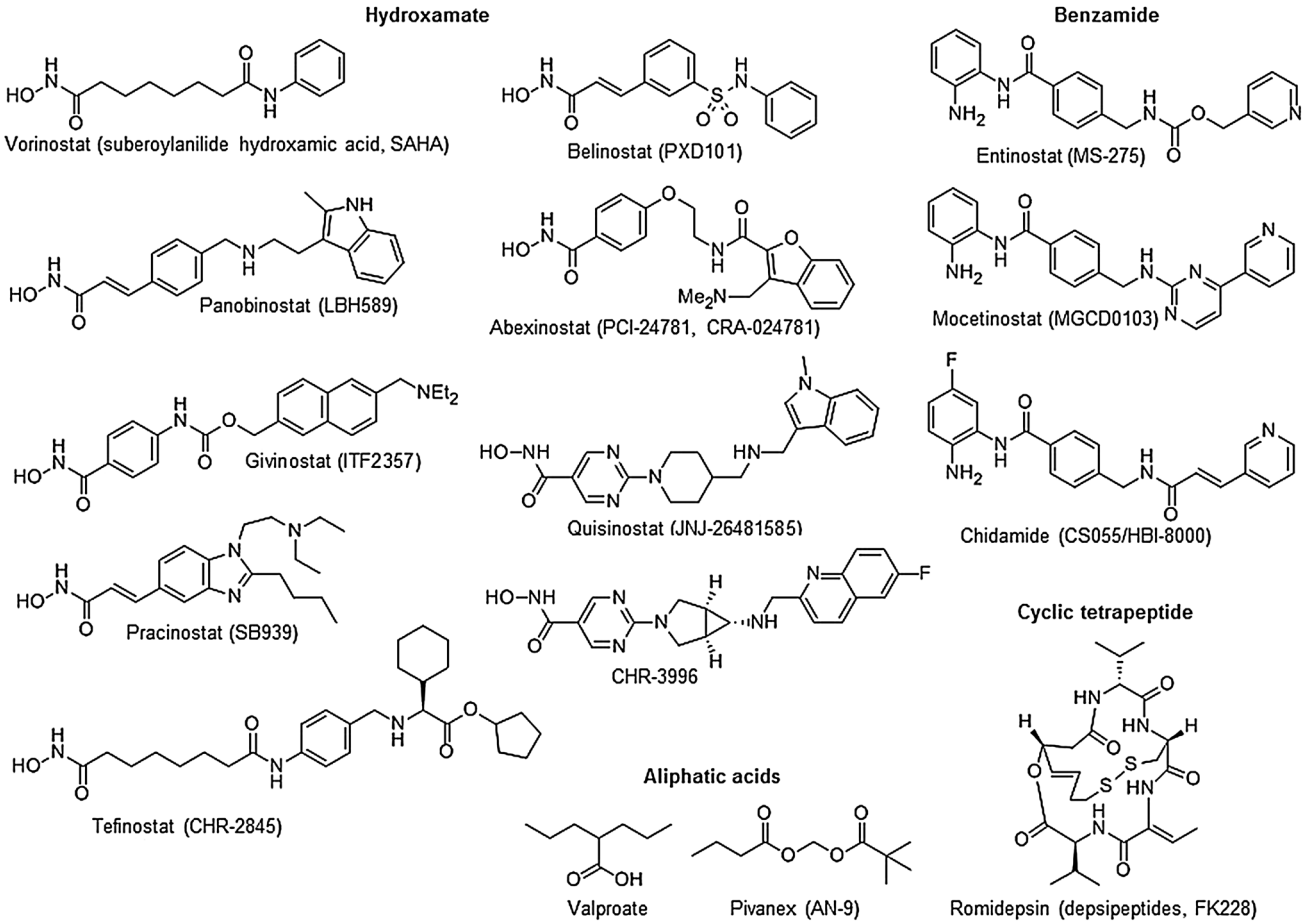

Fig. 1 Four main chemical structures of HDAC inhibitors currently undergoing clinical trials

Table 3 The spectrum of inhibitory activity of HDAC inhibitors against different HDAC enzymes

HDAC inhibitors	HDAC enzymes											References
	Class I				Class IIa				Class IIb		Class IV	
	HDAC1	HDAC2	HDAC3	HDAC8	HDAC4	HDAC5	HDAC7	HDAC9	HDAC 6	HDAC10	HDAC11	
Belinostat	↓	↓	↓	↔	–	–	–	–	↓	↔	–	Bantscheff et al. (2011)
	↓	↓	↓	↓	↓	–	↓	↓	↓	–	–	Khan et al. (2008)
Panobinostat	↓	↓	↓	↓	↓	–	↓	↓	↓	–	–	Khan et al. (2008)
Vorinostat	↓	↓	↓	↔	–	–	–	–	↓	↓	–	Bantscheff et al. (2011)
	↓	↓	↓	–	↔	↔	↔	↔	↓	–	–	Bradner et al. (2010)
	↓	↓	↓	↓	↓	–	↓	↓	↓	–	–	Khan et al. (2008)
Entinostat	↓	–	↓	↔	–	–	–	–	–	–	–	Hu et al. (2003)
	↓	↓	↓	↔	↔	–	↔	↓	↔	–	–	Khan et al. (2008)
Givinostat	↓	↓	↓	–	↓	–	↓	–	↓	–	–	Khan et al. (2008)
Mocetinostst	↓	↓	↓	↔	↔	–	↔	–	↔	–	–	Khan et al. (2008)
	↓	↓	↓	↔	↔	↔	↔	–	–	–	↓	Fournel et al. (2008)
Romidepsin	↓	↓	↓	↔	–	–	–	–	↔	↔	–	Bantscheff et al. (2011)
	↓	↓	–	–	↔	–	–	–	↔	–	–	Furumai et al. (2002)
Valproic acid	↓	↓	↓	↓	–	–	–	–	↔	↔	–	Bantscheff et al. (2011)
	↓	↓	↓	↓	–	–	↔	↔	↔	–	–	Khan et al. (2008)

↓ Inhibition, ↔ no inhibition, – not determined

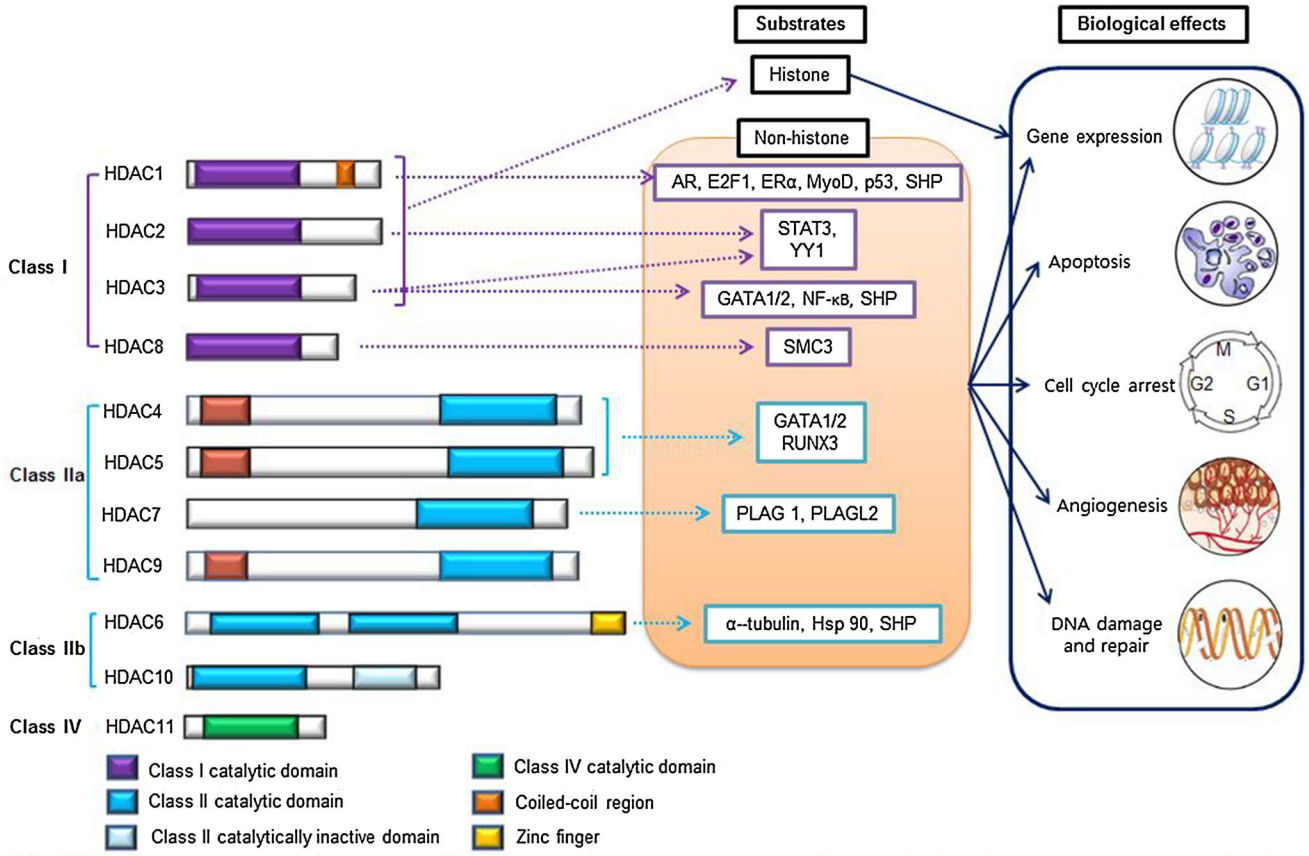

Fig. 2 The molecular targets of HDACs and the biological effects of HDAC inhibition. HDAC inhibitors induce acetylation of histones and non-histone proteins, leading to a wide range of biological effects, including regulation of gene expression, including apoptosis and cell cycle arrest, inhibiting angiogenesis, and regulation of DNA damage and repair pathway. AR, androgen receptor; ERα, estrogen receptor α; NF-κB, nuclear factor-κB; PLAG1, pleomorphic adenoma gene 1; PLAGL2, PLAG-like 2; RUNX3, runt-related transcription factor 3; SHP, short heterodimer partner; SMC3, structural maintenance of chromosomes 3; STAT3, signal transducer and activator of transcription 3; YY1, Ying Yang 1

The findings described above explain, at least in part, the selective effect of HDAC inhibitors in altering gene expression.

HDAC inhibitor-induced antitumor pathways

Activation of apoptotic pathways

The apoptotic process is accompanied by major changes in chromatin structure and gene expression. HDAC inhibitors induce apoptosis via both transcription-dependent and transcription-independent mechanisms in solid and hematological malignancies (Bolden et al. 2006). There are numerous studies reporting HDAC inhibitor-induced apoptosis via both the extrinsic and intrinsic pathways.

The extrinsic pathway of apoptosis is initiated upon binding of death ligands, the TNF superfamily receptors, including TNF-related apoptosis-inducing ligand (TRAIL), TNF-α, and Fas ligand (FasL), to their cognate death receptors, resulting in activation of caspase-8 and -10. The activated caspase-8 and -10 subsequently activate proteases caspase-3, -6, and -7, leading to apoptotic cell death (Ashkenazi 2002; Bolden et al. 2006).

By valproic acid, the TNF family ligands and receptors, including TRAIL, DR5, Fas and FasL, are transcriptionally activated, leading to initiation of the extrinsic apoptosis pathways in leukemic cells, but not in normal cells. That effect correlates with activation of caspase-8 and -3 (Insinga et al. 2005). Treatment of leukemia cells with FK228 also caused upregulation of TNF-α via hyperacetylation of histones H3 and H4 in its promoter region and induced activation of caspase-8 and -10, resulting in apoptotic cell death (Sutheesophon et al. 2005).

On the other hand, the intrinsic pathway, involving the mitochondria, is activated in response to lethal stimuli from inside the cell, such as DNA damage, oxidative stress, hypoxia, or chemotherapeutic drugs. Activation of mitochondrial apoptotic pathway causes release of pro-apoptotic proteins, including cytochrome *c*, from the intermembrane space into the cytosol. Cytochrome *c* can then

bind Apaf-1 forming the apoptosome and activating caspase-9. Once activated, caspase-9 cleaves and activates the same set of caspases that are activated through the extrinsic pathway by caspase-8 and -10 (Ashkenazi 2002; Burz et al. 2009). In response to suberoyl bis-hydroxamic acid, the expression of pro-apoptotic proteins including Bim, Bak, Bax, and caspase-3 increased while the expression of anti-apoptotic proteins including Bcl-2, Bcl-X_L, Mcl-1, and X-linked inhibitor of apoptosis decreased in melanoma cells (Zhang et al. 2004).

Recently, we have demonstrated that MHY218, a hydroxamic acid derivative, induces apoptosis in colon cancer cells. By MHY218, Bax was markedly upregulated while Bcl-2 was downregulated, leading to a significant increase in Bax/Bcl-2 ratio in a concentration-dependent manner. In addition, the activity of caspase-3, -8 and -9 was significantly increased, suggesting MHY218 induces apoptosis via both the internal and external pathway in colon cancer cells (Kim et al. 2014).

Further evidence demonstrates that HDAC inhibitors alter the factors that mediate or regulate the intrinsic apoptosis pathway. SAHA caused apoptosis by promoting recruitment of E2F1 to the Bim promoter and inducing upregulation of Bim in colon cancer cells lacking p53 (Zhao et al. 2005). In addition, by SAHA, mitochondrial disruption was achieved by the cleavage of the BH3-only pro-apoptotic Bcl-2 family member Bid in leukemia cells, which was not blocked by caspase inhibitors or the overexpression of Bcl-2 (Ruefli et al. 2001). Moreover, SAHA treatment increased levels of Noxa and Puma in leukemia cells (Pérez-Perarnau et al. 2011).

In addition to SAHA, FK228 induced Bmf expression, concomitant with hyperacetylation of histones H3 and H4 at Bmf promoter region in squamous carcinoma cells (Zhang et al. 2006) while sodium butyrate increased the expression of the Bad protein in glioma cells with no changes in the levels of Bcl-2, Bcl-X_L, Bax, and Fas (Sawa et al. 2001).

HDAC inhibitor-mediated apoptosis can be cell-type-dependent. Furthermore, it seems that different effects of HDAC inhibitors in the same cell type may be attributed to the structural features of diverse HDAC inhibitors.

Induction of cell cycle arrest

HDAC inhibitors have been found to induce cell cycle arrest at G1 via upregulation of the CDK inhibitor p21 (Richon et al. 1996; Qiu et al. 2000). In addition, HDAC inhibitors reduce CDK activity through downregulation of cyclins, causing dephosphorylation of Rb and subsequently inhibiting E2F activities in the transcription of genes for G1 progression and G1/S transition (Rosato and Grant 2005; Zhao et al. 2005). In our study, MHY218, a hydroxamic acid derivative, induced G2/M phase arrest by p53-independent upregulation of p21 in colon cancer cells (Kim et al. 2014). SAHA caused predominantly G1 arrest at low concentration while at higher concentrations, both G1 and G2/M arrests were induced (Richon et al. 2000). The cytostatic activity of HDAC inhibitors at low doses is not restricted to tumour cells, since G1 arrest is also observed in normal cells. At higher doses, HDAC inhibitors are selectively cytotoxic, killing a wide range of cancer cells and transformed cells but not normal cells (Qiu et al. 2000; Burgess et al. 2004).

Although, HDAC inhibitor-induced arrest is largely associated with induction of p21, several reports have suggested the existence of a p21-independent pathway of growth arrest by HDAC inhibitors. Trichostatin A (TSA) activates the $p15^{Ink4b}$ gene, a member of INK4 family proteins, and induces cell growth inhibition of colon cancer cells lacking p21 (Hitomi et al. 2003). Recently it has been demonstrated that HDAC inhibitors can interfere with the interaction between HDACs and Aurora kinases involved in alteration of the G2–M cell cycle transition. In renal cancer cells, LBH589-mediated down-regulation of Aurora A and B induced G2/M cell cycle arrest through inhibition of HDAC3 and -6 (Cha et al. 2009). In the case of TSA, it increased levels of acetylated histones H3 and H4 in hepatoma cells, leading to G0/G1 phase arrest (Yamashita et al. 2003). What's more, TSA increased levels of p21 gene and reduced levels of CyclinB1, Plk1, and Survivin, resulting in delay at the G2/M transition (Noh et al. 2009).

Collectively, HDAC inhibitors can affect cell cycle by interacting with cell cycle regulators, resulting in cell cycle arrest at certain phases.

Inhibition of angiogenesis

There is growing evidence that HDAC inhibitors can target tumor angiogenesis. The anti-angiogenic effect of HDAC inhibitors is associated with suppression of pro-angiogenic factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor, hypoxia-inducible factor-1α (HIF-1α), chemokine (C-X-C motif) receptor 4 (CXCR4), angiopoietin, tunica intima endothelial kinase 2, and endothelial nitric oxide synthase (eNOS).

HIF-1α plays a key role in the cellular adaptations to hypoxic microenvironment, which is critical for survival of tumor cells. Repression of HIF-1α activity by HDAC inhibitors has been reported by various mechanisms. The observation that TSA inhibits hypoxia-induced angiogenesis through upregulation of p53 and von Hippel–Lindau, and concurrent suppression of HIF-1α and VEGF suggests that HDAC modulation can be closely involved in hypoxia-induced angiogenesis (Kim et al. 2001). In addition, anti-angiogenic activity of FK228 has been demonstrated in

Lewis lung carcinoma model. FK228 effectively inhibited the DNA binding activity of HIF-1α and the expression of VEGF mRNA under hypoxia (Lee et al. 2003). The reduced HIF-1α DNA-binding activity and decreased level of VEGF protein were also observed with butyrate. In colon cancer cells, HIF-1α nuclear sequestration was repressed through inhibition of nuclear translocation, which could be responsible for decreased VEGF expression and anti-angiogenic effects (Zgouras et al. 2003).

Furthermore, the activity of HIF-1α is regulated upon binding to the transcriptional co-activator cAMP-response element-binding protein-binding protein (CBP)/p300. HIF-1αCAD, the carboxyl-terminal transactivation domain of HIF-1α, provides the major transactivation activity. TSA induced hyperacetylation of p300 and repressed the HIF-1αp300 complex independently of direct acetylation of HIF-α (Fath et al. 2006).

Besides, HDAC inhibitors induce hyperacetylation of chaperone protein Hsp90 via inhibition of HDAC6, leading to increased affinity to HIF-1α. As a result, HIF-1α disrupts Hsp90 chaperone function and exposes HIF-1α to proteasomal degradation by Hsp70 (Kong et al. 2006).

Many studies have shown that the classes I and II HDACs are associated with HIF-1α activity, which are inhibited by different HDAC inhibitors: inhibition of HDAC1 and -3 by butyrate and valproic acid; inhibition of HDAC4 and -6 by LBH589 and valproic acid; inhibition of HDAC9 by SAHA (Qian et al. 2006; Kim et al. 2007; Hutt et al. 2014). HDAC inhibitors that inhibit HDAC7 may also contribute to anti-angiogenesis. Under hypoxic conditions, HDAC7 moves into the nucleus and increases transcriptional activity of HIF-1α through the formation of a complex with HIF-1α, HDAC7, and p300. HDAC inhibitors reduce the HIF-1α activity by inhibiting HDAC7 (Kato et al. 2004).

Taken together, HDAC inhibitors repress neovascularization by inhibiting positive factors of angiogenesis or altering angiogenesis signaling pathway.

Induction of DNA damage and inhibition of DNA repair

Changes in chromatin structure induced by HDAC inhibitors directly activate the DNA-damage pathway despite the fact that HDAC inhibitors alone do not induce double-strand breaks (DSBs; Bakkenist and Kastan 2003).

It has been well established that HDAC inhibitors can synergize with ionizing radiation (IR) and DNA-damaging agents to cause delay in tumor growth. The number of studies reporting the additive effect has grown exponentially. The combination of radiation and LBH589, SAHA, and butyrate increased the duration of γ-H2AX foci in irradiated cells (Geng et al. 2006; Munshi et al. 2005; Koprinarova et al. 2011). This finding suggests that HDAC inhibitors suppress DSB repair and/or render DNA more susceptible to IR-induced damage.

In addition to their action on chromatin structure during DSB repair, HDAC inhibitors have been shown to affect the expression of DNA repair proteins. Treatments with SAHA and MS-275 led to hyperacetylation of Ku70, a key non-homologous end joining component, concomitant with reduced DNA-binding affinity (Chen et al. 2007). HDAC inhibitors can also affect the expression of genes encoding homologous recombination (HR) components. SAHA induced the coordinated down-regulation of HR pathway genes, including RAD51 and BRCA1 in ovarian cancer cells (Konstantinopoulos et al. 2014).

A number of studies have reported that after treatment with HDAC inhibitors, reactive oxygen species (ROS) are accumulated, leading to DNA damage and oxidative stress. HDAC inhibition by SAHA and MS-275 caused accumulation of ROS and increased sensitivity to cell death induced by those agents in transformed but not normal cells (Ungerstedt et al. 2005).

HDAC inhibitors and clinical outcomes

HDAC inhibitors are undergoing extensive clinical evaluation as single agent or in combination with other agents. Among them, vorinostat, romidepsin, and belinostat have received approval from the U.S. FDA for the treatment of lymphoma.

Vorinostat

Vorinostat, also known as SAHA, has received approval from the U.S. FDA for the treatment of CTCL in patients with progressive, persistent, or recurrent disease on or following two systemic therapies. The major trial supporting approval was a phase II trial that recruited 74 patients with at least stage IB CTCL (61 had stage IIB or higher) who had failed two systemic therapies. The patients received 400 mg of oral vorinostat once daily as a single agent. The objective response rate (ORR) was 29.7 and 32 % of patients had pruritus relief. The common drug-related adverse events were diarrhea (49 %), fatigue (46 %), nausea (43 %), and anorexia (26 %) and most were grade 2 or lower. The 400 mg dose of vorinostat was generally well-tolerated (Olsen et al. 2007; Mann et al. 2007).

Vorinostat has also been investigated for other hematological malignancies and solid tumors. Recently, two phase II studies have reported favorable responses in relapsed/refractory indolent follicular lymphoma with acceptable safety profiles (Kirschbaum et al. 2011; Ogura et al. 2014). Despite the demonstrated effect in lymphomas,

unfortunately, only a moderate effect was observed in solid tumors: squamous cell carcinoma of the head and neck (Blumenschein Jr et al. 2008), breast, colorectal, and non-small-cell lung cancer (Luu et al. 2008; Vansteenkiste et al. 2008; Traynor et al. 2009), ovarian and peritoneal (Modesitt et al. 2008), and prostate (Bradley et al. 2009). Although in the study by Bradley et al. (2009), 41 % of the patients discontinued therapy because of grade 2/3 toxicity including fatigue, nausea, and anorexia, oral vorinostat was generally well-tolerated at a dose of 400 mg daily.

Romidepsin

Romidepsin, also known as FK228 and depsipeptide, serves as a prodrug. Upon reduction of its disulfide bond, one of the sulfhydryl groups interacts with the zinc in the active site of the HDACs, preventing access of substrate. Romidepsin possesses stronger activity against HDAC1 and -2 than against HDAC4 and -6 (Furumai et al. 2002). In 2009, the U.S. FDA has granted approval to romidepsin for treating CTCL patients who have failed at least one prior systemic therapy based on the efficacy and safety evaluated in two phase II trials (U.S. FDA Drugs. Romidepsin; Piekarz et al. 2009; Whittaker et al. 2010). In those two studies, 71 and 96 patients with CTCL were included and treated with intravenous romidepsin at a dose of 14 mg/m^2/day on days 1, 8, and 15 of a 28-day cycle. The ORRs were 35 and 34 % in each study and the complete response (CR) rate was 6 % in both studies. The most common drug related adverse events were nausea and fatigue being grade 2 or 3 in both studies. The serious adverse events reported in >2 % of the patients were leukopenia, lymphopenia, and granulocytopenia in the study by Piekarz et al. (2009), and nausea, asthenic conditions including fatigue, and anemia in the study by Whittaker et al. (2010).

Romidepsin was also approved by the U.S. FDA in 2011 for the treatment of relapsed or refractory PTCL. The approval was based on the results of a multinational phase II trial conducted in 130 patients who were refractory to at least one prior systemic therapy or for whom at least one prior systemic therapy failed. The patients received intravenous romidepsin at a dose of 14 mg/m^2/day on days 1, 8, and 15 of a 28-day cycle. The ORR was 25 %, including 15 % with CR. The most common grade ≥3 drug-related adverse events were thrombocytopenia (23 %), neutropenia (18 %), and infections (6 %, Coiffier et al. 2012).

A series of phase II trials of romidepsin have also been conducted in patients with solid tumors, however, the single agent activity of romidepsin is disappointingly very low: renal cancer (Stadler et al. 2006), colorectal cancer (Whitehead et al. 2009), castration-resistant prostate cancer (Molife et al. 2010), small-cell lung cancer (Otterson et al. 2010), anaplastic glioma and glioblastoma multiforme (Iwamoto et al. 2011), and squamous cell carcinoma of the head and neck (Haigentz Jr et al. 2012). When romidepsin was administered at the dose of 14 mg/m^2/day, the most common adverse events were GI disturbances, hematologic abnormalities, asthenic conditions, and infections.

Belinostat

Belinostat, also known as PXD101, is a hydroxamate pan-HDAC inhibitor that was approved by the U.S. FDA for the treatment of patients with relapsed or refractory PTCL. The approval was based on the result of the phase II trial conducted in 120 patients with PTCL that was refractory or had relapsed after prior treatment, including PTCL patients with baseline platelets <100,000/ml. Belinostat was administered by intravenous infusion at a dose of 1,000 mg/m^2 once daily on days 1–5 of a 21-day cycle. The ORR was 25.8 % and median progression-free survival was 1.6 months. The most common grade ≥3 adverse events were thrombocytopenia (15 %), neutropenia (13 %), leukopenia (13 %), and anemia (12 %, ClinicalTrials.gov. BELIEF study; U.S. FDA Drugs. Belinostat).

Belinostat has also been investigated for other hematological malignancies and solid tumors. Recently, a phase II trial has reported favorable responses in recurrent/refractory PTCL and CTCL with acceptable safety profiles (Foss et al. 2014). Despite the demonstrated effect in lymphomas, unfortunately, only a moderate effect was observed in solid tumors: malignant pleural mesothelioma (Ramalingam et al. 2009), ovarian cancer (Mackay et al. 2010), and thymoma and thymic carcinoma (Giaccone et al. 2011).

The most common adverse events were anemia, nausea, QTc prolongation, and thrombocytopenia. Most of the toxicities were grades 2–3. In general, belinostat was well-tolerated at a dose of 1,000 mg/m^2/day.

Safety issues with the use of HDAC inhibitors

Although toxicities are favorable and largely manageable, in some instances cardiotoxicities as well as hematologic and gastrointestinal adverse effects can be dose limiting. The most common dose-limiting toxicities related with vorinostat, romidepsin, and belinostat were constitutional and gastrointestinal effects, including anorexia, asthenia, diarrhea, fatigue, nausea, and vomiting, and hematologic effects, such as anemia, leucopenia, lymphopenia, neutropenia, and thrombocytopenia (Duvic et al. 2006; Garcia-Manero et al. 2008; Vansteenkiste et al. 2008; Steele et al. 2008; Lassen et al. 2010; Mwakwari et al. 2010).

As various HDAC inhibitors are being studied in clinical trials against cancers, accumulating evidence shows that use of HDAC inhibitors is associated with cardiotoxicity

such as T-wave flattening, ST segment depression and QTc-prolongation (Piekarz et al. 2006). QT interval prolongation has been to date the most severe cardiac event in patients treated with HDAC inhibitors due to the risks for potentially life-threatening arrhythmia (Wolbrette 2004; Straus et al. 2006). HDAC inhibitors have been variably associated with QT prolongation. Romidepsin, previously called as FK228 and depsipeptide, has been associated with relatively frequent electrocardiogram (ECG) changes and QT prolongation, and rare sudden cardiac death (Bates et al. 2006; Shah et al. 2006; Mwakwari et al. 2010). The use of belinostat (PXD101) was also associated with QTc prolongation in patients treated with it (Lassen et al. 2010; Cashen et al. 2012). In case of vorinostat (SAHA), no QTc prolongation was reported in clinical trials (Kelly et al. 2005; Duvic et al. 2006; Bradley et al. 2009). However, recently, one case of torsade de pointes has been reported, drawing attention to the need for periodic monitoring of ECGs in patients using vorinostat (Lynch Jr et al. 2012).

Interference on human ether-a-go–go-related gene potassium ion (HERG K^+) channels seems to be a common mechanism for these drugs (Strevel et al. 2007; Bagnes et al. 2010). The plausible hypothesis is that these HDAC inhibitors uniquely interact with HERG K^+ channel, resulting in QTc prolongation. The activation of the HERG K^+ channel leads to ventricular repolarization, thus blocking of this channel may result in QTc prolongation (Curran et al. 1995). In addition, mutations of HERG are presumed to be linked to QTc prolongation. In fact, increasing evidence suggests that QTc prolongation associated with HDAC inhibitors may be the result of altered gene expression and probably inhibition of specific HDAC isoforms (Montgomery et al. 2007). Therefore, changes in HERG expression or those of the coregulators of HERG activity may serve as another mechanism of QTc prolongation.

Collectively, although ECG abnormalities observed with the administration of HDAC inhibitors have not been associated with myocardial damage or altered left ventricular ejection fraction, the potential effect of heart rate-corrected QT interval prolongation remains under study.

Conclusion

HDAC inhibitors have shown good anticancer activity both in preclinical and clinical trials, with relatively little effect on normal tissues and favorable toxicity profile. Although preclinical trials show that HDAC inhibitors have a variety of biological effects across multiple pathways, the mechanism by which the clinical activity is mediated remains unclear. Accumulating evidence demonstrates the selective effect of HDAC inhibitors in altering gene expression and apoptosis, however, it has not yet been determined which inhibitor will be more therapeutically-effective against specific cancer types. What's worse, comparing the anticancer activity and toxicity profile of pan-HDAC inhibitors, such as vorinostat and belinostat, and class I-selective HDAC inhibitors such as romidepsin, there are no significant differences between them in terms of inhibitory potency and adverse events. Nevertheless, certain biomarkers may indicate the potential for greater effect of HDAC inhibitors. Like other cancer therapies, a targeted therapy may be successful. The therapeutic response of HDAC inhibitors could be greater in those patients who strongly express HDACs in their cancer cells.

Positive therapeutic responses with some HDAC inhibitors have been shown consistently in hematologic malignancies, but the clinical outcomes in solid tumors are disappointing when used as single agents. Many clinical trials have examined combination therapies of HDAC inhibitors with chemotherapeutic and biotherapeutic agents in both solid and hematologic cancers. Even though none of these combinations is yet approved by the U.S. FDA to treat cancer, combination therapy with other medicines will yield improved clinical outcomes over those seen with single agents.

To conclude, understanding mechanisms of action of HDAC inhibitors is essential not only for intelligent drug design for targeted therapies in cancer, but for finding of predictive biomarkers in cancer initiation and progression, and further for the design of optimized clinical protocols.

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