Histone deacetylases as targets for treatment of multiple diseases

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Abstract

HDACs (histone deacetylases) are a group of enzymes that deacetylate histones as well as non-histone proteins. They are known as modulators of gene transcription and are associated with proliferation and differentiation of a variety of cell types and the pathogenesis of some diseases. Recently, HDACs have come to be considered crucial targets in various diseases, including cancer, interstitial fibrosis, autoimmune and inflammatory diseases, and metabolic disorders. Pharmacological inhibitors of HDACs have been used or tested to treat those diseases. In the present review, we will examine the application of HDAC inhibitors in a variety of diseases with the focus on their effects of anti-cancer, fibrosis, anti-inflammatory, immunomodulatory activity and regulating metabolic disorders.

Key words: acetylation, cancer, fibrosis, histone deacetylase, immunomodulation, inflammation, metabolic disorder

INTRODUCTION

Epigenetics refers to the regulation of gene expression via post-translational modification of protein complexes associated with DNA without alterations in the DNA sequence [1,2]. The fundamental structure of chromatin consists of the nucleosome, which is composed of 146 bp of DNA surrounding an octamer of core histones (two H2A/H2B dimers and a H3/H4 tetramer) [2]. Remodelling of chromatin between relatively ‘open’ and ‘closed’ forms has a key role in epigenetic regulation of gene expression [3]. Post-translational modifications of the N-terminal tails of histones are involved in this remodelling process, including acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, carbonylation and glycosylation [3,4].

Acetylation and deacetylation are regulated by two groups of enzymes: HATs (histone acetyltransferases) and HDACs (histone deacetylases). The reverse activities of HATs and HDACs regulate gene expression through chromatin modification [5,6]. HDACs are a class of deacetylating enzymes that remove acetyl groups from ε-amino groups of lysine residues of histones, as well as non-histone proteins, causing the condensation of chromatin structure and thereby repressing gene expression [5,6]. On the basis of their homology with respective yeast orthologues, HDACs are classified into four groups: class I HDACs (HDAC1–3 and 8), which are related to yeast Rpd3 (reduced potassium dependency 3) [7]; class II HDACs, which are divided into two subclasses, class Iia (HDAC4, 5, 7, and 9) and class Iib (HDAC6 and 10), both homologous with the yeast gene Hda1 (histone deacetylase 1) [8]; class III, which consists SIRT1–7, also known as sirtuins, are homologous with yeast Sir2 (silent information regulator 2) [9,10]; and class IV (HDAC11), which contains conserved residues in catalytic regions shared by both class I and II HDAC enzymes [11]. Class I and II are referred to as ‘classical’ HDACs.

It has been widely demonstrated that HDACs are promising targets for therapeutic interventions in cancer and other diseases. Classical HDACs are mainly involved in the development of cancer. Increased expression of class I HDACs (HDAC1–3) is associated with nodal spread and is an independent prognostic marker for gastric cancer [12]. High expression of some of the class II HDACs, such as HDAC6, is correlated with tumour invasion in breast cancer [13], and low expression of class II HDACs genes (HDAC5 and HDAC10) is associated with poor prognosis in lung cancer patients [14]. In addition to cancers, HDACs have been...
shown to be involved in other diseases, including tissue fibrosis, autoimmune and inflammatory diseases, and metabolic disorders. Since HDACs are considered as crucial targets of multiple diseases, HDACIs (HDAC inhibitors) have been evaluated in basic experiments and clinical trials. In the present review, we will evaluate the application of HDACIs in these diseases, with a focus on their effects on cancer, fibrosis, inflammation and immunomodulation and metabolic disorders (Table 1).

**HDAC Inhibitors**

HDACIs are compounds that have the ability to prevent the deacetylation of lysine residues within the N-terminal tails of histone proteins. On the basis of their chemical structure, HDACIs are categorized into the six groups: (i) hydroxamates, such as TSA (trichostatin A) and SAHA (suberoylanilide hydroxamic acid); (ii) short-chain fatty acids, such as butyric acid and valproic acid; (iii) cyclic tetrapeptides, such as CHAP31 (cyclic hydroxamic-acid-containing peptide 31) and romidepsin (FK-228); (iv) benzamides, such as entinostat (MS-275), tacedinaline (CI-994) and chidamide (CS-055); (v) electrophilic ketones, such as trifluoromethylketone; and (vi) miscellaneous compounds, such as MGCD0103. They may also be classified according to their specificity for HDACs [1]. SAHA, TSA, panobinostat, belinostat and resminostat are pan-deacetylase inhibitors. Butyrate and valproate inhibit class I and IIa HDACs, whereas romidepsin, MS-275 and mocetinostat are considered to be class I-specific [1]. Tubacin is specific to inhibit HDAC6 [15]. The ‘classical’ HDACs are specific to the Zn\(^{2+}\)-dependent class I and class II HDACs and act by binding to the Zn\(^{2+}\)-containing catalytic domain of the HDACs.

The mechanism of action of HDACIs involves inhibiting the deacetylation of histones. Hyperacetylation results in an increase in the space between the nucleosome and the DNA that is wrapped around it. The opening of chromatin structure subsequently provides the access for gene transcription. HDACIs target gene expression without changing DNA sequence. A study in colon carcinoma cells showed that 7% of genes were modulated by sodium butyrate treatment (256 genes elevated and 333 genes repressed of 8063 genes) [16]. Another study in bladder carcinoma and breast carcinoma cells demonstrated that approximately 8–10% of genes are regulated on the Affymetrix 6800 gene chips by various HDACIs [17]. Post-TSA treatment results in differential gene expression of various enzymes and transcription factors involved in apoptosis, cell-cycle regulation, extracellular matrix regulation, signal transduction, immune response and metabolism pathways [18].

Besides histones, HDACIs also have effects on non-histone proteins which include proteins involved in the regulation of gene expression, pathways of extrinsic and intrinsic apoptosis, cell-cycle progression, redox pathways, mitotic division, DNA repair, cell migration and angiogenesis [19–26]. A large number of non-histone transcription factors and transcriptional co-regulators are known to be modified by acetylation. HDACIs can alter the degree of acetylation of these molecules and, therefore, increase or repress their activity. For example, the inducible transcription factor NF-κB (nuclear factor κB) is deregulated in a large number of diseases, and application of HDACIs has been shown to repress NF-κB signalling and expression of several NF-κB target genes [27,28]. Therefore HDACIs might have an effect on immune responses, inflammation, cell survival, differentiation and proliferation. The tumour suppressor p53 is a key player in cellular signalling. HDACIs, including TSA, SAHA and MS-275, dominantly up-regulate the gene expression of p53 [17,29], which may partly be responsible for the anti-cancer effect of HDACIs. STAT3 (signal transducer and activator of transcription 3) is a transcriptional factor required for the development and progression of tissue fibrosis in multiple organs, including kidney, skin and lung. The administration of TSA can suppress transcriptional activation of STAT3 [30], which might be one of the possible mechanisms of the anti-fibrotic effects of HDACIs.

Therefore HDACIs can induce acetylation of histone, as well as non-histone proteins, which affect a variety of physiological and pathological processes, controlling apoptosis/autoapagy, cell cycle, fibrogenesis, immune response, inflammation and metabolism through its downstream molecular targets (Figure 1).

**HDACs and Cancers**

Traditionally, cancer has been considered to originate from genetic alteration, such as gene mutations, deletions, rearrangements and chromosomal abnormalities, leading to aberrant expression of tumour suppressor genes and oncogenes [31,32]. However, growing evidence suggests that epigenetic modulation also plays a crucial role in the initiation and progression of cancers [33,34]. Different from genetic defects, epigenetic changes are reversible and therefore considered as a promising new mechanistic class of anti-cancer therapy. It has been shown that a global loss of monoacetylation of histone H4 is a common hallmark of human tumour cells [35]. Changes in histone H4 acetylation occur early during the tumorigenic process [35]. The aberrant recruitment of HDACs to promoters through their physical association with oncogenic DNA-binding fusion proteins results from chromosomal translocations or overexpression of repressive transcription factors that physically interact with HDACs [3]. For example, the oncogenic PML-RARα (promyelocytic leukaemia-retinoic acid receptor α), PLZF-RARα (promyelocytic leukaemia zinc finger-retinoic acid receptor α) and AML-1 (acute myeloid leukaemia-1) transcription factors and the AML1-ETO (eight-twenty-one) corepressor fusion proteins induce leukaemogenesis by recruiting HDAC-containing repressor complexes to constitutively repress expression of specific target genes [36,37]. Inhibition of HDAC activity increased the transcriptional activity of the oncogenic fusion protein and transcription factor EWS-FLI1 by increasing its DNA binding activity in Ewing sarcoma (EWS) [38]. Individual HDACs, including HDAC1, HDAC2, HDAC3 and HDAC6, are overexpressed in a number of tumours [39–42]. siRNA (small interfering RNA)-mediated knockdown of individual HDACs in certain tumour cell lines suppresses tumour cell growth and survival [42,43], indicating the critical role of HDACs in the
<table>
<thead>
<tr>
<th>Disease model</th>
<th>HDACI</th>
<th>HDAC specificity</th>
<th>Mechanism(s)</th>
<th>Effects</th>
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<tbody>
<tr>
<td>Cancer</td>
<td>TSA, SAHA and MS-275</td>
<td>Class I and/or</td>
<td>Up-regulates tumour suppressing genes (p53, p21, pRb, tob1, HIF1α and HER2). [17,45–47]</td>
<td>Inhibits tumor growth and impairs its proliferation</td>
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<td>Class II HDACs</td>
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<td>MHY218</td>
<td>Class I/II HDACs</td>
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<td>Induces apoptosis or autophagic cell death [52]</td>
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<td>SAHA</td>
<td>Class I/II HDACs</td>
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<td>Inhibits mTOR signalling pathway; reduces Akt and ERK signalling pathways. [48]</td>
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<td>Valproic acid</td>
<td>Class I HDACs</td>
<td></td>
<td>Induces tumour cell-cycle arrest, cell differentiation, and inhibition of growth of tumour vasculature [57]</td>
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<td>Interstitial fibrosis</td>
<td>Spiruchostatin A</td>
<td>Class I HDACs</td>
<td>Inhibits the proliferation and differentiation of fibroblasts in idiopathic pulmonary fibrosis [70]</td>
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<td>TSA</td>
<td>Class I/II HDACs</td>
<td>Reduces expression of CSF-1 [65], and inhibits STAT3 activation and tubular cell apoptosis [30] in UUO</td>
<td>Attenuates macrophage infiltration, the proliferation of renal fibroblasts, the expression of α-SMA and fibronectin induced by UUO [30,65]</td>
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<td>SK-7041</td>
<td>Class I HDACs</td>
<td>Down-regulates expression of EGR-1, but the exact mechanism remain unclear [72]</td>
<td>Alleviates cardiac hypertrophy induced by chronic infusion of AngII or by aortic banding [72]</td>
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<td>Immune dysfunction</td>
<td>TSA</td>
<td>Class I/II HDACs</td>
<td>Down-regulates the expression of numerous host defence genes; impairs innate immune responses; induces expression of Mi-2β and enhances the DNA-binding activity of Mi-2/NuRD complex [80]</td>
<td>Enhances the susceptibility to bacterial and fungal infections but protects against toxic and septic shock [80]</td>
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<td></td>
<td>Tubastatin A analogues</td>
<td>HDAC 6</td>
<td>Enhances the ability of Treg-cells to inhibit the mitotic division of effector T-cells [23]</td>
<td>Enhances the immunosuppressive effects of Foxp3+ Treg-cells [23]</td>
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<td></td>
<td>FR276457</td>
<td>Class I/II HDACs</td>
<td>Inhibits the proliferation of T-cell line and suppresses mononuclear cell infiltration and vasculitis [86,87]</td>
<td>Prevents allograft rejection and prolongs allograft survival in a rat cardiac transplant model [86] and in a canine renal transplant model [87]</td>
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<td>Inflammatory</td>
<td>TSA</td>
<td>Class I/II HDACs</td>
<td>Accelerates IL-6 mRNA decay in RA fibroblast-like synoviocytes and macrophages [110]</td>
<td>Disrupts IL-6 production in RA synovial cells [110]</td>
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<td>Sodium valproate</td>
<td>Class I HDACs</td>
<td>Represses the production of IL-12 and TNF-α by LPS-induced macrophage activation, but promotes IL-10 expression [111]</td>
<td>Skews the phenotype of LPS-stimulated mouse macrophage cell line RAW264.7 and primary mouse bone marrow macrophages from M1 to M2 [111]</td>
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<td>SAHA</td>
<td>Class I/II HDACs</td>
<td>Inhibits the circulating level of pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and IFN-γ induced by LPS [112]</td>
<td>Reduces the production of pro-inflammatory cytokines in vivo and in vitro [112]</td>
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<td>Metabolic disorders</td>
<td>MC1568</td>
<td>Class II HDACs</td>
<td>Enhances expression of Pax4, a key factor required for proper β- and δ-cell development, and amplifies endocrine β- and δ-cells [127]</td>
<td>Enhances β- and δ-cell development [127]</td>
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<td>ITF2357</td>
<td>Class I/II HDACs</td>
<td>Increases islet cell viability, enhances insulin secretion, inhibits MIP-1α and MIP-2 release, reduces iNOS production and apoptosis, and inhibits the production of nitrite, TNF-α and IFN-γ [129]</td>
<td>Favours β-cell survival during inflammatory conditions [129]</td>
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<td></td>
<td>TSA</td>
<td>Class I/II HDACs</td>
<td>Down-regulates gene expression involved in the cholesterol biosynthetic pathway and fatty acid biosynthesis, and glycolysis-associated pathways [18]</td>
<td>Regulates cholesterol metabolism [18]</td>
</tr>
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Figure 1  HDACI targets and downstream effects
Inhibition of HDACs by HDACIs induces acetylation of histone proteins, as well as non-histone proteins, which leads to the alteration in various physiological and pathological processes, including apoptosis/autophagy, cell cycle, fibrogenesis, immune response, inflammation and metabolism. Therefore HDACIs may be potent therapeutic agents for anticancer, antifibrosis, anti-inflammatory and immunomodulation, and regulating metabolic disorders.

Development and progression of tumours and as anticancer therapeutic targets.

HDACIs inhibit the dynamic turnover of acetylation, resulting in hyperacetylation of target proteins [44]. This can affect a wide range of cellular functions, and promote cytostatic and cytotoxic effects in a wide range of tumour cell types, but has little effect on normal cells [44]. The molecular mechanisms underlying the anticancer effects of HDACIs remain to be fully elaborated. Genomic effects on gene transcription may be responsible for the anticancer effects of HDACIs. In several models of cancer, HDACIs, including TSA, SAHA and MS-275, up-regulate tumour suppressing genes [p53, p21, pRb (retinoblastoma protein), tob1, Hep27, Cbp (C-terminal Src kinase-binding protein)/PAG1 (phosphoprotein associated with glycosphingolipid-enriched microdomains 1), IRF [IFN (interferon) regulatory factor]-8] and down-regulate oncogenes [Src, HIF1α (hypoxia-inducible factor 1α) and HER2 (human epidermal growth factor receptor 2)] [17,45–48], therefore inhibiting the development and progression of tumours. HDACIs can cause cell-cycle arrest in a p53-independent manner due to induction of p21 and/or tob1 by HDACIs through a direct effect on the Sp1 site in the p21 promoter [49,50]. Since most cancer cells have lost p53 or pRb or both, resulting in loss of the G1/S DNA damage checkpoint, the induction of p21, p27Kip1 and/or tob1 by HDACIs produces an aberrant cell-cycle arrest (checkpoint), leading to apoptosis [17]. HDACIs can also increase the sensitivity of carcinoma cells to TRAIL [TNF (tumour necrosis factor)-related apoptosis-inducing ligand] and down-regulate c-FLIP [cellular Fas-associated death domain-like IL (interleukin)-1β-converting enzyme-inhibitory protein], resulting in activation of extrinsic apoptosis pathways and inducing apoptosis of tumour cells [51]. Besides apoptosis, HDACIs also induce caspase-independent autophagic cell death in tumour cells [52]. Furthermore, HDACIs have anti-angiogenic effects, associating with decreased expression of pro-angiogenic genes such as VEGF (vascular endothelial growth factor), bFGF (basic fibroblast growth factor), HIF1α, angiopoietin 2, TIE2 (tunica intima endothelial kinase 2), survivin and eNOS [endothelial NOS (nitric oxide synthase)] [53–57]. Inhibition of angiogenesis by HDACIs affects the nutrient supply to the primary tumour [3]. HDACIs are also reported to sensitize cancer cells to chemotherapies. TS (thymidylate synthase) is the target of the chemotherapeutic agent 5-FU (5-fluorouracil), which is associated with drug resistance. Initial gene expression studies with HDACIs recognized that TS was one of the HDACI gene targets [17]. HDACI treatment enhanced the sensitivity of cancer cells to 5-FU via down-regulation of TS expression [58,59]. In summary, HDACI-regulated gene expression can contribute to cell-cycle arrest, apoptosis and angiogenesis inhibition.

Currently, HDACIs have been successfully used as therapies for the treatment of haematological malignancies. In particular, SAHA and romidepsin (FK-228) have been approved by the United States FDA (Food and Drug Administration) for the treatment of CTCL (cutaneous T-cell lymphoma). A single dose of HDACIs demonstrated limited clinical benefit against solid tumours. However, HDACIs in combination with other anticancer agents have shown a synergistic effect. For example, SAHA enhanced sensitivity of non-small-cell lung cancer cells to 5-FU/S-1 [58]. VPA (valproic acid) can augment the antitumour
effects of 5-FU in a human pancreas cancer cell line and a cholangiocarcinoma cell line [60]. HDACs are also used as radiosensitizers in the treatment of solid tumours. The combination of an HDACI, H6CAHA, with γ-radiation completely blocked the growth of human prostate cancer xenografts over 60 days [61]. Moreover, adding SAHA to capcitabine-based chemoradiotherapy enhanced the radiosensitivity of xenografts in terms of inhibiting colorectal carcinoma growth [62]. VPN was shown to augment radiation-induced cytotoxicity in human oesophageal squamous cell carcinoma by chromatin decondensation with histone hyperacetylation and down-regulation of Rad51 [63]. Therefore HDACIs can serve as a promising therapy for cancers.

**HDACs AND INTERSTITIAL FIBROSIS**

Recent studies have shown that the HDACs play an important role in the development of multiple tissue fibrosis, including skin, kidney, liver, heart and lung. Activation of fibroblasts is critically involved in the development of interstitial fibrosis of a variety of organs. The activated fibroblast, termed a myofibroblast, demonstrates specific phenotypic changes, including the expression of α-SMA (α-smooth muscle actin) and increased production of ECM (extracellular matrix) components, including collagen and fibronectin [30]. Glenisson et al. [64] examined the role of HDACs in TGF-β1 (transforming growth factor β1)-induced myofibroblastic differentiation, a process involved in tissue fibrosis. They found that among the eight HDACs (HDAC1–HDAC8) tested, silencing of HDAC-4, HDAC-6 and HDAC-8 expression impaired TGF-β1-induced α-SMA expression. HDAC4 silencing efficiently abrogated α-SMA expression and prevented TGF-β1-mediated morphological changes. Intervention by TSA prevented α-SMA transcript and protein expression and morphological changes mediated by TGF-β1 in cultured human skin fibroblasts [64]. These findings suggest that HDACs are involved in the process of skin fibrosis and that HDAC4 is an essential epigenetic regulator of myofibroblastic differentiation.

An increase in the expression of HDAC1 and HDAC2 and a decrease in histone acetylation were observed in tubulointerstitial injury induced by UUO (unilateral ureteral obstruction) [65]. Treatment with TSA attenuated macrophage infiltration and fibrotic changes in this model. The induction of CSF-1 (colony-stimulating factor-1), a chemokine known to be involved in macrophage infiltration in tubulointerstitial injury, was reduced in the injured kidney of mice treated with TSA. TSA, valproate and the knockdown of either HDAC1 or HDAC2 also significantly reduced CSF-1 expression induced by TNF-α in renal tubular cells. These results suggest that tubular HDAC1 and HDAC2 may contribute to the production of CSF-1, macrophage infiltration and profibrotic responses in response to injury and implicates a potential use of HDAC inhibition in reducing inflammation and fibrosis in tubulointerstitial injury. Our studies have also shown that HDAC1 and HDAC2 are involved in regulating proliferation of renal interstitial fibroblasts [66]. Silencing either HDAC1 or HDAC2 with siRNA significantly inhibited cell proliferation, decreased the expression of cyclin D1 and increased the expression of p57, a negative cell-cycle regulator [66]. Furthermore, inhibition of HDAC activity with TSA blocked the proliferation and activation of renal interstitial fibroblasts in a rat model of UUO and in a rat renal interstitial fibroblast line (NRK-49F) in vitro [30]. In *in vitro* studies employing cultured NRK-49F cells, TSA treatment inhibited fibroblast proliferation as indicated by decreasing cell numbers and suppressing cyclin D1 expression. TSA also blocked fibroblast activation as shown by diminishing expression of α-SMA and fibronectin. These suggest that pharmacological HDAC inhibition may exert antifibrotic activity by inactivation of renal interstitial fibroblasts.

HSCs (hepatic stellate cells) are the major cellular sources of ECM in chronic liver diseases leading to fibrosis. In a human HSC line, sodium valproate, a class I HDACI, exerts antifibrogenic activity by blocking the TGF-β1 autocrine loop and inhibiting TGF-β1-induced collagen type 1 α1 expression [67]. Another HDACI, TSA, affects the development of the actin cytoskeleton and inhibits collagen types I and III and α-SMA in HSCs, thereby abrogating the process of HSC transdifferentiation [68,69]. These findings indicate that the antifibrogenic effect of HDACIs in the liver results from inhibiting transdifferentiation of stellate cells into myofibroblasts and the subsequent production of ECM.

In human fibroblasts from patients with idiopathic pulmonary fibrosis, Spiruchostatin A, a class I HDACI, inhibits TGF-β1-induced expression of α-SMA, collagen I and collagen III, and soluble collagen release [70]. In addition, HDAC inhibition prevents cardiac hypertrophy induced by AngII (angiotensin II) infusion and aortic banding and reverses atrial arrhythmia inducibility and fibrosis in cardiac hypertrophy independent of AngII [71,72]. HDACIs inhibit α-SMA expression and collagen synthesis and diminish DNA binding of AP-1 (activating protein-1), a key transcription factor in profibrogenic signalling in pancreatic stellate cells [73]. Collectively, these studies suggest a potential antifibrotic effect of HDACIs in a variety of organs.

There are several possible mechanisms accounting for antifibrotic effects of HDACIs. In a number of tissues, activation of STAT3 increases expression of multiple profibrotic genes and is required for activation of renal interstitial fibroblasts and the progression of renal fibrosis [74]. Administration of TSA could suppress transcriptional activation of STAT3, leading to inactivation of renal fibroblasts [30]. In addition, TSA treatment inhibits the activity of STAT-dependent signal transduction pathways in NIH 3T3 cells and sarcoma cells [75,76]. Lee et al. [77] have shown that HDACI-induced hyperacetylation of histones H3 and H4 was associated with the down-regulation of fibronectin transcription. Yoshikawa et al. [78] examined the effect of TSA on the EMT (epithelial-to-mesenchymal transition) in cultured tubular epithelial cells and found that TSA can prevent TGF-β1-induced EMT. Mechanistic studies revealed that TSA induced the expression of two inhibitory factors of TGF-β1 signals: Id2 (inhibitors of DNA binding/differentiation 2) and BMP-7 (bone morphogenetic protein-7). A ChIP (chromatin immunoprecipitation) assay confirmed that histone acetylation was involved in the down-regulation of E-cadherin and the up-regulation of Id2 and BMP-7 [78]. Overall, although the mechanisms of HDACI-exerted antifibrotic effects remain incompletely understood, transcriptional activation of repressors and acetylation of non-histone proteins may, in part, explain their antifibrotic effects [77].
**HDACs AND IMMUNOMODULATION**

Increasing evidence has implicated protein acetylation in innate and adaptive immune pathways [79]. Classical HDACs have been identified to play a key role in regulating TLR (Toll-like receptor) and IFN signalling pathways in innate immunity, as well as antigen presentation, helper T-cell polarization, lymphocyte development and function [79]. It has been demonstrated that HDACIs down-regulate the expression of numerous host defence genes, including pattern recognition receptors, kinases, transcription regulators, cytokines, chemokines, growth factors and co-stimulatory molecules, as assessed by genome-wide microarray analyses [80]. HDACIs have also been shown to induce the expression of Mi-2β and enhance the DNA-binding activity of the Mi-2/NuRD (nucleosome remodelling deacetylase) complex that acts as a transcriptional repressor of macrophage cytokine production. Furthermore, HDACIs can increase the susceptibility to bacterial and fungal infections, but confer protection against toxic and septic shock [80]. Recent studies have also shown that a tubastatin A analogue, a selective HDAC6 inhibitor, augments the immunosuppressive effect of Foxp3+ (forkhead box P3+) Treg-cells (regulatory T-cells) and inhibits the mitotic division of effector T-cells [23]. Therefore these findings suggest that HDACIs are able to regulate the expression of innate immune genes and host defences against microbial pathogens, and that HDACIs are mostly immunosuppressive. The immunosuppressive properties of HDACIs are associated with skewed dendritic cell differentiation and impaired cytokine secretion by dendritic cells [81–83]. The observed defects in dendritic cell function on exposure to HDACIs seem to reflect the obstruction of signalling through NF-κB, IRF-3 and IRF-8 [81].

On the basis of the immunosuppressive effects, HDACIs may be potent agents for decreasing autoimmunity and transplant rejection. Edens et al. [84] have shown that treatment with TSA induces antigen-specific energy in both cloned and naïve CD4+ T-cells, suggesting their potential to induce immune tolerance in organ transplantation. Tao and Hancock [85] have reported that HDAC inhibition promoted the generation of Treg-cells and enhanced their functions. In addition, administration of FR276457, a hydroxamic derivative, can inhibit T-cell proliferation and prolong allograft survival, thereby exhibiting marked immunosuppressive effects in a rat heterotopic cardiac transplant model [86] and in a canine renal transplant model [87].

Foxp3+ Treg-cells play a key part in limiting autoimmunity and maintaining peripheral tolerance, and mutations of Foxp3 lead to lethal autoimmunity in humans and mice [88–92]. Therapeutic manipulation of Foxp3 acetylation using HDACIs can promote the development and suppressive functions of Foxp3+ Treg-cells, with beneficial consequences in models of transplant rejection, colitis and arthritis [93–97]. In murine models of T-cell-dependent disease, treatment with TSA or SAHA decreased the severity of Tgβ2 (T-helper 2)-associated lung airway hypersensitivity responses [98], renal disease in MRL/lpr/lpr mice [99], colitis [100], RA (rheumatoid arthritis) [101,102], graft-versus-host disease post-bone marrow transplantation [103] and the ‘cytokine storm’ induced by the CD3 monoclonal antibody therapy used in a bone-marrow-transplant-conditioning regimen [104].

**HDACs AND INFLAMMATION**

Alterations in the balance of histone acetylation and deacetylation could affect many aspects of cellular function, including cell growth, differentiation, cell death, cell–cell and cell–matrix interactions, and the inflammatory response [105]. The inflammatory response is triggered by some stimulus-regulated transcription factors and involves a large number of differential expression genes [106,107]. Recent studies have demonstrated that HDAC3 is required for the expression of numerous inflammatory genes, including IFN-β-dependent genes [e.g. Nos2 and PtgS2 (prostaglandin-endoperoxide synthase 2)] and IFN-β-independent genes, such as IL (interleukin)-6, in macrophages in response to LPS (lipopolysaccharide) [108]. HDAC4 has also been shown to regulate vascular inflammatory responses and promote hypertension. Inhibition of HDAC4 by siRNA blocked TNF-induced monocyte adhesion, VCAM-1 (vascular cell adhesion molecule-1) expression and transcriptional activity of NF-κB in cultured rat mesenteric arterial smooth muscle cells [109]. Therefore inhibition of HDAC activity might exert anti-inflammatory effects.

In addition, HDACIs appear to be potent anti-inflammatory agents. TSA suppresses IL-6 production by accelerating IL-6 mRNA decay in RA fibroblast-like synoviocyte and macrophages [110]. Sodium valproate represses IL-12 and TNF-α production, and promotes IL-10 expression in macrophages exposed to LPS [111]. In an endotoxaemia model, SAHA exhibits dose-dependent inhibition of the circulating level of pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and IFN-γ induced by LPS [112]. In the collagen-induced arthritis mouse model, MS-275 has been shown to decrease serum IL-6 and IL-1β levels [102]. SAHA and TSA also inhibit the production of the inflammatory cytokines IL-12, IFN-γ, IL-6 and IL-10 in isolated splenocytes of MRL-lpr/lpr mice, a murine model of SLE (systemic lupus erythematosus) [99]. Moreover, HDACIs can ameliorate inflammatory bowel diseases. For example, butyrate can effectively treat Crohn’s disease [113] and ulcerative colitis [114]. It has been reported that colitis was associated with increased local expression of HDAC9. Inhibition of HDAC9 prevented colitis and reduced established colitis in mice [95]. Recent findings in humans [115] have also indicated that a novel HDAC inhibitor, ITF2357, exerts its anti-inflammatory capacity. Therefore HDACIs may be a new and promising drug class for the treatment of inflammatory diseases such as SLE, arthritis, endotoxaemia and inflammatory bowel disease.

**HDACs AND METABOLIC DISORDERS**

The aetiology of diabetes is complex and multifactorial with contributions from many genes and unknown environmental factors. There is evidence showing a genetic association between diabetes and HDACs. GWAS (genome-wide association studies) have found a significant linkage between the chromosomal region 6q21, where HDAC2 is located, and both type 1 diabetes mellitus and type 2 diabetes mellitus [116–118]. Another locus
identified in GWAS of Type 2 diabetes mellitus lies on chromosome 19q13; the HDAC Sir2 maps to this region [116,117]. In patient with diabetes, β-cell dysfunction is associated with a variable degree of insulin resistance [119]. Studies have demonstrated that regulation of the expression of insulin from β-cells is under the control of acetylation [116,119,120]. At high glucose levels, Pdx1 (pancreatic and duodenal homeobox factor 1), which is involved in glucose-stimulated insulin gene expression, interacts with the HAT p300, leading to increased acetylation of histone H4 in the insulin promoter. These events appear to be necessary for preproinsulin transcription induced by glucose [119,121–125]. Conversely, at low glucose levels, where insulin production is shut off, the acetylation of histone H4 in the insulin promoter is abolished, correlating with the recruitment of HDAC1 and HDAC2 to the insulin promoter by Pdx1 [119,121,126]. Mosley and Ozcan [119] have reported that exposure of mouse insulinoma 6 cells to high concentrations of glucose results in the hyperacetylation of histone H4 at the insulin gene promoter, which correlates with the increased level of insulin gene transcription. In addition, hyperacetylation of histone H4 in response to high concentrations of glucose also occurs at the GLUT (glucose transporter)-2 gene promoter. Recent studies demonstrated that class IIa HDAC4, HDAC5 and HDAC9 regulated the production of insulin in β-cells and somatostatin in δ-cells [127]. Treatment with MC1568, a selective class IIa HDAC1, promoted the expression of Pax4, a crucial factor required for proper β- and δ-cell differentiation, and amplifies endocrine β- and δ-cells in pancreatic explants [127]. Inhibition of HDACs has also been shown to have important functions in preventing β-cell inflammatory damage, improving insulin resistance, promoting β-cell development, proliferation, differentiation and function, and positively having an impact on late diabetic microvascular complications [121]. Both pharmacological and genetic inhibition of HDAC3 has been shown to protect β-cells against cytokine-induced apoptosis and restores glucose-stimulated insulin secretion [128]. In addition, oral administration of ITF2357, a class I and II HDAC1, improved islet function, reduced iNOS (inducible NOS) levels and apoptosis [129]. IL-1β is a key mediator of insulin resistance and β-cell failure mediated effects on isolated β-cells [130]. A novel HDAC1, THS-78-5, has been shown to protect against the IL-1β-mediated loss in β-cell viability and to attenuate IL-1β-induced iNOS expression and subsequent NO release [130], partly by inhibition of IL-1β-induced transactivation of NF-κB. HDACIs also hold promise as possible treatments for late diabetic complications, such as diabetic nephropathy [77,131] and retinal ischaemia [132]. Therefore HDACIs may prove to be novel agents for the treatment of diabetes mellitus.

In addition to the regulation of glucose, HDACs are also involved in the regulation of lipid metabolism. It has been reported that HDAC3 suppresses cytosolic PEPCK (phosphoenolpyruvate carboxykinase) transcription by inhibiting the transcriptional activators PPAR (peroxisome-proliferator-activated receptor)-γ and CREB (cAMP-response-element-binding protein) [133]. This mechanism is responsible for inhibition of glyceroneogenesis in adipocytes, which contributes to lipodystrophy in ap2-p65 transgenic mice [133]. Recent findings have also indicated that HDACIs are involved in certain crucial metabolic pathways. TSA treatment results in a clear repression of genes involved in the cholesterol biosynthetic pathway, thus down-regulating cholesterol biosynthesis, which is associated with the down-regulation of SREBP-2 (sterol-regulatory-element-binding protein-2) [18]. TSA also repress the expression of genes involved in other associated metabolic pathways, including fatty acid biosynthesis and glycolysis [18]. HDACIs may be useful as potential therapeutic entities for the control of cholesterol levels in humans.

CONCLUSIONS

Current studies have shown that HDACs are critical enzymes involved not only in the development of cancer, but also other diseases such as interstitial fibrosis, autoimmune, inflammatory diseases, and metabolic disorders. HDACIs have been tested for their therapeutic effects in treating these diseases in clinical trials and/or animal models. However, the underlying mechanism(s) by which HDACIs play a role in inhibiting cancer and other disease initiation and progression remains incompletely understood. A better understanding of the role of HDACs in these diseases will lead to the development of new drugs and specific treatment strategies. The use of HDACIs as novel therapeutic agents has shown great promise for these compounds as effective therapies in a variety of diseases, suggesting the need for further research to develop therapeutic agents for clinical trials.

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REFERENCES

HDAC-mediated pathogenesis of multiple diseases


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