

## Perspectives in Pharmacology

# Histone Deacetylase: A Potential Therapeutic Target for Fibrotic Disorders

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### ABSTRACT

Histone deacetylases (HDACs) are enzymes that balance the acetylation activities of histone acetyltransferases on chromatin remodeling and play essential roles in regulating gene transcription. In the past several years, the role of HDACs in cancer initiation and progression, as well as the therapeutic effects of HDAC inhibitors in various types of cancer, has been well studied. Recent studies indicated that HDAC activity is also

associated with the development and progression of some chronic diseases characterized by fibrosis, including chronic kidney disease, cardiac hypertrophy, and idiopathic pulmonary fibrosis. Here, we review what is known about HDACs in the progression of tissue fibrosis and the potential applications of HDAC inhibitors in the treatment of disorders associated with fibroblast activation and proliferation.

### Introduction

Histone acetylation/deacetylation of the N-terminal tail is crucial in modulating gene expression. The balance between the acetylated/deacetylated states of histones is mediated by two different sets of enzymes: histone acetyltransferases (HATs) and histone deacetylases (HDACs). HATs preferentially acetylate specific lysine substrates on histones and some nonhistone proteins. Histone acetylation can lead to changes in chromatin structure and may decrease the histone-DNA interaction, promoting accessibility of the DNA for transcription activation. Acetylation of some nonhistone proteins, for example, transcriptional factors, can also affect their DNA binding properties and subsequently may regulate gene transcription (Boyes et al., 1998; Glozak et al., 2005). In addition, acetylation/deacetylation can occur in nu-

merous cytoplasmic proteins, including tubulin and heat shock protein 90 and alter their functions (Kovacs et al., 2005; Catalano et al., 2007; Choudhary et al., 2009).

HDACs are a family of enzymes that remove acetyl groups from a  $\epsilon$ -N-acetyl lysine amino acid on a histone and restore the positive charge to lysine residues (Thiagalingam et al., 2003; Acharya et al., 2005). HDAC proteins are also referred to as lysine deacetylases to more precisely describe their function rather than their targets, since they can catalyze deacetylation of many nonhistone proteins in addition to histones (Glozak et al., 2005). HDACs are classified into four groups based on their homology to yeast histone deacetylases: Class I (HDAC1, 2, 3 and 8) are related to yeast *RPD3* gene and are mostly located in the nuclei; Class II (HDAC4, 5, 6, 7, 9 and 10) are related to yeast *Hda1* gene and are primarily located in the cytoplasm but can shuttle to nucleus; Class III (SIRT1–7), also known as the sirtuins, are related to the *Sir2* gene and are virtually unaffected by the HDAC inhibitors; and Class IV (HDAC11) has a conserved domain in the catalytic regions of both Class I and Class II enzymes. Class I HDACs, such as HDAC1 and HDAC2, seem to be

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**ABBREVIATIONS:** HAT, histone acetyltransferase; HDAC, histone deacetylase; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; CSF-1, colony stimulating factor-1; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; IPF, idiopathic pulmonary fibrosis; NRK-49F, rat renal interstitial fibroblasts; NRK-52E, rat kidney tubular epithelial cells; NHLF, normal human lung fibroblast; PDGF, platelet-derived growth factor; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; PKD, polycystic kidney disease; SAHA, suberoylanilide hydroxamic acid; SK-7041, 3-(4-substituted phenyl)-N-hydroxy-2-propenamide;  $\alpha$ -SMA,  $\alpha$ -smooth muscle actin; STAT3, signal transducer and activator of transcription3; UUO, unilateral ureteral obstruction; SSc, systemic sclerosis; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; TSA, trichostatin A; 4-PBA, sodium 4-phenylbutyrate; LBH589, panobinostat; AG490, tyrphostinAG 490 [(E)-2-cyano-3-(3,4-dihydrophenyl)-N-(phenylmethyl)-2-propenamide].

important in the regulation of proliferation and survival of cancer cells (Fischle et al., 2002; Dokmanovic and Marks, 2005). Increased expression of some of the Class II HDAC enzymes (i.e., HDAC6) is linked to better survival in breast cancer and reduced expression of Class II HDAC enzymes HDAC 5 and HDAC10 is associated with poor prognosis in lung cancer patients (Osada et al., 2004). The first two groups of HDACs are considered “classical” HDACs and are the major targets of the present applications of HDAC inhibitors in therapy of cancer or other diseases.

Recent studies have shown that HDACs are critically involved in tissue fibrosis in multiple organs including kidney, heart, and lung. In this article, we review the role of HDACs in the development and progression of tissue fibrosis and discuss the potential applications of HDACs inhibitors in treatment of these disorders (Table 1).

## The Role of HDACs in Fibrosis-Related Kidney Diseases

### HDACs in Renal Interstitial Fibrosis

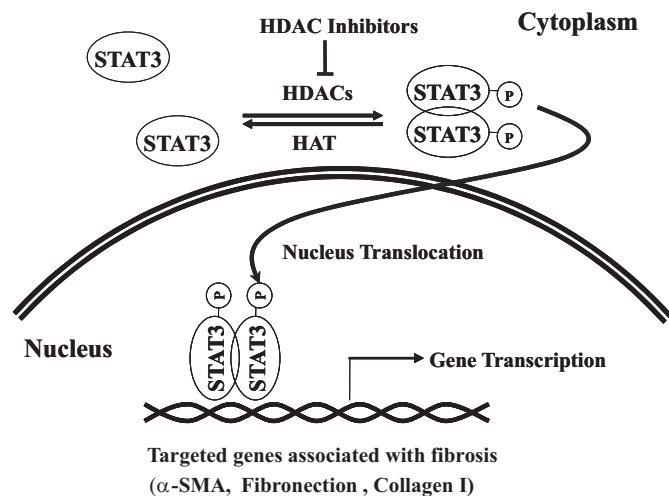
Renal interstitial fibrosis is characterized by aberrant activation and growth of renal fibroblasts. The activated fibroblast, termed myofibroblast, demonstrate specific phenotypic changes, including the expression of  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA) and increased production of extracellular matrix (ECM) components. The number of interstitial myofibroblasts directly correlates with the extent of tubulointerstitial scarring and functional outcome in clinical glomerulonephritis and IgA nephropathy (Arakawa et al., 2008).

Activation and proliferation of renal fibroblasts are stimulated by a variety of growth factors and cytokines, such as transforming growth factor, platelet-derived growth factor, fibroblast growth factor, and interleukin-6. Several intracellular signaling pathways, including the signal transducer and activator of transcription3 (STAT3) pathways, are activated in response to those growth factors/cytokines. STAT3 belongs to a family of latent cytoplasmic transcription factors. Activated STAT3 forms homodimers or heterodimers and then translocates to the nucleus, where it binds with DNA and regulates gene transcription. Numerous targeted genes of STAT3 have been identified, including cyclin D1. An abundance of active STAT3 has been observed in renal interstitial fibroblasts in the unilateral ureteral obstruction (UUO) model of renal fibrosis (Kuratsune et al., 2007; Pang et al., 2009). Regulation of STAT3 activity is critical for the modulation of its biological functions. In addition to tyrosine phosphorylation, deacetylation is involved in regulation of STAT3 activity as shown in one of our recent publications (Pang et al., 2009).

In that article, Pang et al. (2009) examined the effect of trichostatin A (TSA), a HDAC I/II inhibitor, on the activation and proliferation of renal interstitial fibroblasts *in vitro* and *in vivo*. In the *in vitro* studies employing cultured rat renal interstitial fibroblasts (NRK-49F), TSA treatment inhibited fibroblast proliferation as indicated by decreasing cell numbers and suppressing the expression of cyclin D1. TSA also blocked fibroblast activation as shown by diminishing expression of  $\alpha$ -SMA and fibronectin. STAT3 is phosphorylated when NRK-49F cells are activated and start to proliferate; this response was inhibited in the presence of TSA as well (Fig. 1).

TABLE 1  
Summary of HDAC inhibitor applications in experimental fibrotic disorders

Disease Model	HDAC Inhibitors	Selectivity	Beneficial Effects after Treatment	Mechanism	References
Renal fibrosis	TSA	HDAC I/II	Attenuates renal fibroblast proliferation, $\alpha$ -SMA expression and fibronectin deposition	Inhibits STAT3 activation induced by UUO.	Pang et al., 2009
Diabetic nephropathy	Sodium Valproate	HDAC I	Attenuates macrophage infiltration and fibrotic changes.	Reduces CSF-1 expression induced by TNF- $\alpha$ in renal tubular cells.	Marumo et al., 2009
Cardiac hypertrophy and fibrosis	TSA Valproic acid SK-7041	HDAC I/II HDAC I HDAC I/II	Decreases expressions of ECM components and prevents EMT in STZ-induced diabetic kidneys	Suppresses the ROS mediate TGF- $\beta$ 1-induced activation of HDAC-2.	Noh et al., 2009
Idiopathic pulmonary fibrosis	TSA	HDAC I/II	Blocks cardiac hypertrophy induced by angiotensin II infusion and aortic banding; reverses atrial fibrosis, connexin40 remodeling and atrial arrhythmia vulnerability	Reduces expression of early growth response gene 1 (EGR-1), but detailed mechanism remains unclear.	Kee et al., 2006
System sclerosis	TSA	HDAC I/II	Inhibits TGF- $\beta$ 1-mediated $\alpha$ -SMA type I collagen mRNA induction and contractile response in NHLFs.	Reduces AKT phosphorylation during the differentiation of NHLFs to myofibroblasts	Guo et al., 2009
Cystic fibrosis	SAHA LBH589 TSA	pan-HDAC pan-HDAC HDAC I/II	Increases PGE2 synthesis in fibroblasts from IPF patients Prevents SSc related tissue fibrosis by reducing the collagen I and fibronectin in SSc skin fibroblasts.	Restores cyclooxygenase-2 (COX-2) mRNA and protein expression that has been repressed in IPF. Inhibits the nuclear translocation and Smad binding to DNA.	Coward et al., 2009 Huber et al., 2007
	4PBA	Pan-HDAC	Increases amounts of delta F508-CFTR and corrects cellular trafficking of CFTR.	Enables a greater fraction of delta F508-CFTR to escape degradation and appear at the cell surface.	Rubenstein et al., 1997, 2000



**Fig. 1.** HDAC inhibitors attenuate STAT3-mediated fibrosis. HDAC activity is required for STAT3 phosphorylation at tyrosine 705 and dimerization (activation). The dimerized STAT3 is translocated into the nucleus, where it regulates transcription of the target genes associated with development of tissue fibrosis, such as  $\alpha$ -smooth muscle actin, fibronectin, and collagen I. HDAC inhibitors such as TSA can inhibit these actions of STAT3 and subsequently attenuate its effects in association of fibrosis.

Moreover, treatment with AG490 (tyrphostinAG 490 [(*E*)-2-cyano-3-(3,4-dihydrophenyl)-*N*-(phenylmethyl)-2-propenamide]), a STAT pathway inhibitor that is a synthetic tyrphostin derived from benzylidene malonitrile, suppressed all of the above events associated with renal fibroblast activation and proliferation, whereas a knockout of STAT3 reduced the inhibitory effect of TSA on proliferation of mouse embryonic fibroblasts. In an *in vitro* mouse model of the fibrotic kidney induced by UUO, administration of TSA also attenuated the proliferation of renal fibroblasts, expression of  $\alpha$ -SMA, and deposition of fibronectin. In addition, STAT3 phosphorylation induced in this UUO model was completely blocked by TSA. The underlying mechanism by which inhibition of HDACs alters the tyrosine phosphorylation status is not clear, but these data indicate that HDAC activity is required for STAT3 tyrosine phosphorylation and activation. The data are also consistent with the idea that HDACs are involved in proliferation and activation of renal fibroblasts as well as development of tubulointerstitial fibrosis (Fig. 1).

In a separate study, Marumo et al. (2009) also observed an increase in the expression of HDAC1 and HDAC2 and a decrease in histone acetylation in kidneys injured by ureteral obstruction. Immunohistochemical analysis revealed that HDAC1 and HDAC2 were induced in renal tubular cells. Treatment with a TSA attenuated macrophage infiltration and fibrotic changes. The induction of colony stimulating factor-1 (CSF-1), a chemokine known to be involved in macrophage infiltration in tubulointerstitial injury, was reduced in injured kidneys of mice treated with TSA. TSA, valproate, and the knockdown of either HDAC1 or HDAC2 significantly reduced CSF-1 expression induced by tumor necrosis factor- $\alpha$  in renal tubular cells. These results suggest that increased expression of HDAC1 and HDAC2 contribute to the production of CSF-1, macrophage infiltration, and other profibrotic responses in response to injury and implicate a potential of HDAC inhibition in reducing inflammation and fibrosis in tubulointerstitial injury.

## HDACs in Diabetic Nephropathy

Progressive accumulation of ECM in glomerular mesangium and tubulointerstitium is the hallmark of diabetic nephropathy (Mauer et al., 1984). It has been reported that myofibroblasts can be derived from tubular epithelial cells by epithelial to mesenchymal transition (EMT), a process that is involved in the loss of epithelial cell adhesion, expression of  $\alpha$ -SMA and reorganization of actin, disruption of tubular basement membrane, and enhancement of cell migration and invasion. Recently, Noh et al. (2009) demonstrated that TSA decreased the expressions of ECM components and prevented EMT in streptozotocin-induced diabetic kidneys as well as rat kidney tubular epithelial cells (NRK-52E) exposed to transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1). Similar effects were also observed in NRK-52E cells treated with another HDAC I/II inhibitor, valproic acid, or HDAC I-selective HDAC inhibitor 3-(4-substituted phenyl)-*N*-hydroxy-2-propenamide (SK-7041). This suggests that Class I HDACs play an essential role in initiation of EMT. Among the six HDACs tested (HDAC-1–5 and HDAC-8), HDAC-2 activity was significantly increased in the kidneys of streptozotocin-induced diabetic rats, *db/db* mice, and TGF- $\beta$ 1-treated NRK-52E cells. Knockdown of HDAC-2 with its specific siRNA decreased expression of fibronectin and  $\alpha$ -SMA in NRK-52E cells. These findings suggest the importance of HDAC-2 in mediating accumulation of ECM and development of EMT (Noh et al., 2009). Further studies are needed to validate the role of HDACs in *in vivo* models of diabetic kidney.

## HDACs in Polycystic Kidney Disease

Polycystic kidney disease (PKD) is a common human genetic disease with mutation of the cilia-localized polycystin proteins 1 and 2 (PKD1 and PKD2) responsible for the significant majority of PKD patients. Pugacheva et al. (2007) demonstrated that activation of HDAC6, a tubulin deacetylase, promotes ciliary disassembly, whereas treatment with either TSA, a HDAC pan inhibitor, or tubacin, an inhibitor specifically targeting HDAC6, completely blocked serum-induced ciliary disassembly. Cao et al. (2009) recently demonstrated that inhibiting Class I HDACs, either by valproic acid or by knocking down HDAC1, suppressed kidney cyst formation and body curvature caused by PKD2 deficiency. In addition, they showed that valproic acid was effective in attenuating the progression of cyst formation and slowing the decline of kidney function in a mouse autosomal dominant PKD model. Taken together, these studies suggest that HDACs also play a critical role in the pathogenesis of PKD.

## The Role of HDACs in Cardiac Hypertrophy and Fibrosis

Cardiac fibrosis is a classical feature of hypertrophy and is characterized by the expansion of the extracellular matrix due to the accumulation of collagen, particularly collagen types I and III (Manabe et al., 2002; Zhang et al., 2002). Treatment of cultured cardiac myocytes with HDAC inhibitors prevented pressure overload-induced hypertrophy after constriction of the thoracic aorta (sarcomere organization and activation of the fetal gene program normally evoked by hypertrophic agonists), suggesting that HDACs play dual roles as repressors and activators of cardiac hypertrophy (Antos et al., 2003). Because both TSA and SK-7041, two



Class I HDAC-selective inhibitors, can block the development of cardiac hypertrophy, this suggests that Class I HDACs are prohypertrophic factors in cardiomyocyte (Kee et al., 2006). Besides, Class II HDACs may also be involved in the pathogenesis of cardiac hypertrophy. In a study investigating the action of natriuretic peptide receptor-A-induced cardiac hypertrophy and fibrosis, Ellmers et al. (2007) demonstrated that HDAC 7a mRNA expression was increased in natriuretic peptide receptor-A knockout mice at a more advanced stage, followed by increased TGF  $\beta$ 1 and other structural molecules associated with cardiac fibrosis such as collagen I.

Atrial interstitial fibrosis is a significant factor driving arrhythmia genesis and is highly prevalent in heart failure and cardiac hypertrophy (Verheule et al., 2004). Atrial fibrosis influences the development of atrial fibrillation, particularly in the setting of structural heart disease where angiotensin-inhibition is partially effective in reducing atrial fibrosis and atrial fibrillation. Previous studies showed the involvement of the renin-angiotensin-aldosterone system in the development of atrial fibrosis (Sun et al., 1997; McEwan et al., 1998). Recent studies demonstrated that HDAC overactivation also causes atrial fibrosis, connexin 40 down-regulation, and atrial arrhythmia susceptibility in transgenic mice and that pharmacologic inhibition of HDAC suppresses deleterious atrial remodeling. In mice overexpressing homeodomain-only protein, TSA treatment reduced atrial arrhythmia duration and atrial fibrosis and normalized the expression and size distribution of connexin 40 gap junctions. These results clearly indicate that HDAC inhibition reverses atrial fibrosis, connexin 40 remodeling, and atrial arrhythmia vulnerability (Liu et al., 2008).

### The Role of HDACs in Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is a progressive and lethal fibrotic lung disorder characterized by inflammatory injury and irreversible fibrosis of the lung parenchyma (Gross and Hunninghake, 2001). Among the identified fibrotic mediators, the cytokine TGF- $\beta$ 1 and the lipid mediator prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) have been identified as potent profibrotic and antifibrotic mediators, respectively, and therefore are critical in IPF pathogenesis (Atamas and White, 2003).

To investigate the molecular link between TGF- $\beta$ 1-mediated myofibroblast differentiation and HDAC activity, normal human lung fibroblasts (NHLFs) were treated with TSA. TSA inhibited TGF- $\beta$ 1-mediated expression of  $\alpha$ -SMA and collagen I. Furthermore, inhibition of  $\alpha$ -SMA by TSA decreased phosphorylation of AKT, a critical mediator in epithelial-mesenchymal transition (Lien et al., 2006; Guo et al., 2009). Additional studies identified HDAC4 as a HDAC that mediates those processes in TGF- $\beta$ 1-treated NHLFs. Taken together, these data suggest that differentiation of NHLFs to myofibroblasts is mediated by HDAC4-dependent activation of AKT signaling pathway (Guo et al., 2009).

PGE<sub>2</sub> synthesis is associated with cyclooxygenase-2 activation, and decreased cyclooxygenase-2 activity contributes to a lower production of prostaglandin E<sub>2</sub>. A recent study showed that COX-2 expression declined in the lung of the patients with IPF, whereas treatment with HDAC inhibitors

suberoylanilide hydroxamic acid (SAHA) and LBH589 (panobinostat) was effective in the restoration of cyclooxygenase-2 expression that had been repressed in IPF. The mechanism by which HDAC regulates expression of cyclooxygenase-2 remains unclear but may be associated with epigenetic abnormality caused by histone hypoacetylation in IPF (Wilborn et al., 1995; Coward et al., 2009). In another recent study, Wang et al. (2009) showed that SAHA abrogated TGF- $\beta$ 1-induced transdifferentiation of lung fibroblasts into myofibroblasts and inhibited serum-induced fibroblast proliferation, suggesting a therapeutic potential of HDAC inhibitors in pulmonary fibrosis.

### The Role of HDACs in Other Diseases Involving Fibrotic Injuries

#### Systemic Sclerosis

Systemic sclerosis (SSc) is characterized by severe fibrosis in the skin and various other organs (Derk and Jimenez, 2003). Several profibrotic cytokines, including TGF- $\beta$ 1, interleukin-4, and platelet-derived growth factor (Gay et al., 1989; Higley et al., 1994; Blobe et al., 2000; Distler et al., 2006), as well as the downstream signaling cascades of TGF- $\beta$ 1, have been implicated in the pathogenesis of SSc (Mori et al., 2003; Ishida et al., 2006). It appears that HDAC activity is also required for initiation and development of SSc. This is evident by the fact that inhibition of HDAC activity by TSA attenuated expression of collagen I and fibronectin in both normal and SSc skin fibroblasts and reduced the accumulation of total collagen proteins in SSc skin fibroblasts in response to various cytokines (Huber et al., 2007). Moreover, in a mouse model of skin fibrosis, Hemmatazad et al. (2009) showed that TSA treatment prevented the dermal accumulation of extracellular matrix.

#### Primary Myelofibrosis

Primary myelofibrosis is a chronic malignant hematological disorder with fibrotic pathogenic changes (Tefferi, 2005). Wang et al. (2008) reported that some Class I/II isoforms of HDACs (HDAC1, 2, 4, 5, 6, 8, 10) and all Class III HDACs were significantly elevated in patients with primary myelofibrosis relative to those with other myeloproliferative neoplasms and normal volunteers, suggesting that HDACs may be involved in the pathogenesis of primary myelofibrosis. Further studies are needed to identify the role of individual HDACs involved in this process.

HDACs are able to increase the stability of hypoxia-inducible factor-1 $\alpha$  through deacetylation (Jeong et al., 2002), suggesting the possibility that HDACs are indirectly involved in increased angiogenesis occurring in primary myelofibrosis (Arora et al., 2004). HDACs are also found to interact with the nuclear factor- $\kappa$ B (NF- $\kappa$ B) pathway by acetylating and deacetylating NF- $\kappa$ B, a transcriptional factor that is associated with the pathogenesis of primary myelofibrosis (Komura et al., 2005). HDAC1 and HDAC3 bind to the inhibitor of NF- $\kappa$ B $\alpha$ , I $\kappa$ B $\alpha$ , and as a result, NF- $\kappa$ B expression levels are increased (Kiernan et al., 2003; Viatour et al., 2003). This study has laid the groundwork for suggesting a role for HDAC inhibitors in primary myelofibrosis treatment (Wang et al., 2008). A clinical trial for treatment of primary myelofibrosis with HDAC inhibitors is underway (Hemavathy and Wang, 2009).

## Cystic Fibrosis

Cystic fibrosis (CF) is a common hereditary disease most commonly characterized by infection-induced inflammation followed by pulmonary fibrosis. It stems from mutations in a chloride channel responsible for transepithelial salt and water transport, the cystic fibrosis transmembrane conductance regulator (CFTR) (Dörk et al., 1991; Jensen et al., 1995). The mutant CFTR is retained in the endoplasmic reticulum and degraded, resulting in the loss of CFTR in patients with CF (Dörk et al., 1991; Jensen et al., 1995).

However, in the presence of sodium 4-phenylbutyrate (4-PBA), a HDAC inhibitor, a greater fraction of  $\Delta F508$ -CFTR escapes from degradation and appears at the cell surface of the primary cultures of nasal polyp epithelia from CF patients and the CF bronchial epithelial cell line IB3-1. These data suggest that inhibition of HDAC may be able to correct the CF phenotype in patients carrying mutated CFTR (Rubenstein et al., 1997). In addition, 4-PBA treatment was reported to correct cellular trafficking of CFTR (Rubenstein and Zeitlin, 2000). Mechanistic studies have shown that the activation of intestinal CFTR by histone acetylation was mediated by transcription factors hepatic nuclear factor 1 $\alpha$ , Cdx2, and Tcf4, which converge to modify chromatin architecture. These studies suggest a therapeutic potential for inhibition of HDAC activity in increasing CFTR expression (Paul et al., 2007).

## HDAC Inhibitors and Their Applications

HDAC inhibitors are a new group of agents that can regulate gene expression, induce apoptosis, and arrest cell cycle of cancer cells by altering the acetylation status of chromatin and other nonhistone proteins. They are divided into four categories based on their structures (Table 2): hydroxamates, cyclic peptides, aliphatic acids, and benzamides (Miller et al., 2003; Marks et al., 2004; Dokmanovic and Marks, 2005; Marks and Xu, 2009). The major mechanism of these HDAC inhibitors is to bind a critical Zn<sup>2+</sup> ion required for catalytic function of the HDAC enzymes (Finnin et al., 1999).

Genetic deletion of the Class I genes HDAC1 or HDAC2 results in embryonic or perinatal lethality, respectively (Lagger et al., 2002; Trivedi et al., 2007). In many tumor cell lines, inhibition or down-regulation of HDACs also leads to cell-cycle arrest by up-regulation of cell cycle gene p21, and blockade of the cyclin D/cyclin-dependent kinase 4 complex

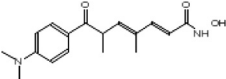
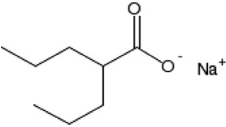
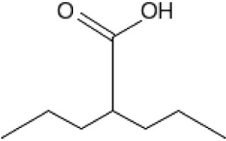
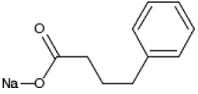
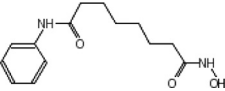
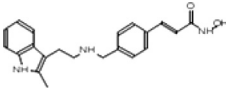
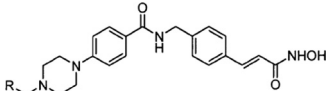
(Richon et al., 2000; Sandor et al., 2000). In addition, HDAC inhibitors can suppress the tumor cells survival by accelerating degradation of proangiogenic transcription factor, hypoxia-inducible factor 1 $\alpha$ , decreasing the expression of vascular endothelial growth factor receptor, or/and increasing generation of intracellular reactive oxygen species (Deroanne et al., 2002; Jeong et al., 2002; Carew et al., 2008).

As described above, numerous investigations have been conducted to address the mechanisms of anti-tumor actions of HDAC inhibitors. Preclinical and early clinical trials of HDAC inhibitors have achieved variable efficacy (Lane and Chabner, 2009; Ma et al., 2009; Marks and Xu, 2009), and SAHA (vorinostat) and romidepsin have recently been approved for treatment of refractory cutaneous T-cell lymphoma (Monneret, 2007; Grant et al., 2010). Although treatment of cancer has been the primary target for the clinical development of HDAC inhibitors, administration of HDAC inhibitors has also shown beneficial effects in some noncancer disorders, such as sickle cell anemia, muscular dystrophy, neurodegenerative diseases, and inflammatory disorders (Wiech et al., 2009). In addition, an inhibitory effect of TSA on hepatic fibrosis has been reported by Niki et al. (1999). Recently, we reported that inhibition of the HDAC activity with TSA also decreased activation of myofibroblast and excessive expression of ECM components such as fibronectin (Pang et al., 2009). In addition, increased HDAC activity was required for TGF- $\beta$ 1-induced myofibroblastic differentiation (Glenisson et al., 2007; Guo et al., 2009). Thus, understanding the molecular events responsible for activation and proliferation of renal fibroblasts may lead to new approaches for slowing the progression of chronic kidney disease and chronic diseases associated with fibrosis in other organs.

## Conclusion and Future Directions

Recent studies have demonstrated that treatment with HDAC inhibitors inhibits activation and proliferation of cultured fibroblasts and attenuates fibrosis in multiple organs in vivo animal models. This suggests that HDAC activity is required for the development and progression of tissue fibrosis. The mechanisms of HDAC-mediated profibrotic actions remain largely unknown but may be associated with expression of some fibrosis-related genes and activation of some cellular molecules that mediate tissue fibrosis. For example, HDAC inhibition can decrease transcription of profibrotic

TABLE 2  
Chemical structure of HDAC inhibitors

TSA	Sodium Valproate	Valproic Acid (Depakote)	4-PBA
			
SAHA (Vorinostat)	LBH589 (Panobinostat)	SK-7041	
			

TSA (Taunton et al., 1996); sodium valproate (Gottlicher et al., 2004); valproic acid (Depakote) (Zelent et al., 2005); 4-PBA (Wiech et al., 2009); SAHA (vorinostat) (Marks and Breslow, 2007); LBH589 (panobinostat) (Atadja et al., 2009); SK-7041 (Kim et al., 2003).

genes such as CSF-1 (Marumo et al., 2009) and reduce activation of some transcriptional factors, such as STAT3 and NF- $\kappa$ B (Hu and Colburn, 2005; Pang et al., 2009). In addition, HDAC inhibitors have been reported to suppress TGF- $\beta$ , a major profibrotic cytokine involved in induced activation and proliferation of fibroblasts and deposition of ECM, suggesting the involvement of HDACs in regulating TGF-triggered fibrotic signaling; however, the targeted proteins have not been identified. Currently, the profile of HDAC-modulated proteins in the setting of fibrosis is not clear. A global analysis of protein lysine acetylation in response to HDAC inhibition using proteomic approach would resolve this issue. This knowledge is not only important for further understanding of the mechanism by which HDACs induce tissue fibrosis but also helpful to develop HDAC inhibition as a novel therapeutic strategy for diseases with fibrosis as their pathogenesis.

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