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Characterization of the Regulation and Function of Zinc-Dependent Histone Deacetylases During Mouse Liver Regeneration

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Abstract

The studies reported here were undertaken to define the regulation and functional importance of zinc-dependent histone deacetylase (Zn-HDAC) activity during liver regeneration using the mouse partial hepatectomy (PH) model. The results showed that hepatic HDAC activity was significantly increased in nuclear and cytoplasmic fractions following PH. Further analyses showed isoform-specific effects of PH on HDAC mRNA and protein expression, with increased expression of the class I HDACs, 1 and 8, and class II HDAC4 in regenerating liver. Hepatic expression of (class II) HDAC5 was unchanged after PH; however HDAC5 exhibited transient nuclear accumulation in regenerating liver. These changes in hepatic HDAC expression, subcellular localization, and activity coincided with diminished histone acetylation in regenerating liver. The significance of these events was investigated by determining the effects of suberoylanilide hydroxyamic acid (SAHA, a specific inhibitor of Zn-HDAC activity) on hepatic regeneration. The results showed that SAHA-treatment suppressed the effects of PH on histone deacetylation and hepatocellular BrdU incorporation. Further examination showed that SAHA blunted hepatic expression and activation of cell cycle signals downstream of induction of cyclin D1 expression in mice subjected to PH.

Conclusion—The data reported here demonstrate isoform-specific regulation of Zn-HDAC expression, subcellular localization, and activity in regenerating liver. These studies also indicate that HDAC activity promotes liver regeneration by regulating hepatocellular cell cycle progression at a step downstream of cyclin D1 induction.

The liver has remarkable capacity to regenerate from injury. Studies using the rodent partial hepatectomy (PH) model show that this regenerative response is highly regulated, and results in precise restoration of the pre-resection liver-to-body mass ratio. Such studies have also identified many signaling events that are regulated during and necessary for normal regeneration (reviewed in (1-4)). Nevertheless, the earliest signals that initiate hepatic regeneration remain incompletely defined.

Mice subjected to PH develop hypoglycemia (5), followed by increased catabolism of body mass (6), and hepatic accumulation of lipid (7) and other metabolites (8). Further more, nutritional, pharmacological, and genetic strategies that disrupt this metabolic response to hepatic insufficiency impair the ensuing regenerative response. For example, both dextrose-supplemented- and adipose-deficient-mice exhibit impaired liver regeneration (5;6). These findings implicate the metabolic changes that occur following liver injury as essential for

appropriate regulation of liver regeneration. However, the molecular mechanisms that link altered metabolism to regenerative hepatocellular proliferation remain unknown.

Recent observations suggest that modifications in protein acetylation could provide the mechanistic link between metabolism and liver regeneration. For example, glucose-supplementation augments protein acetylation in cell culture (9), and glucose-derived acetyl-CoA is an important determinant of chromatin remodeling (10;11). Changes in protein acetylation are determined in part by the action of zinc-dependent histone deacetylases (Zn-HDACs), which, despite their designation, act on both histone- (12;13) and non-histone (14-16) proteins. The activities of these HDACs influence interactions between histone proteins, transcriptional regulators, and genomic DNA, and also affect non-histone protein-protein interactions and stability. Moreover, changes in protein acetylation impact cell survival and differentiation, cell cycle progression, and tumorigenesis (17;18). Higher organisms have evolved considerable complexity in the HDAC family, with 11 zinc-dependent mammalian enzymes divided into 3 sub-families (classes I, II, and IV) and 7 NAD-dependent enzymes in a fourth (sirtuin) sub-family (class III, (12;18)). Recently, the subcellular localization and activity of the class II HDACs (4, 5, and 7) were shown to affect glucose homeostasis (19). Further more, the class I enzyme HDAC1 was reported to modulate differential effects of CAATT/Enhancer Binding Protein on liver regeneration in young and old mice (20;21). These data raise the possibility that Zn-HDAC activity is regulated by the metabolic response to hepatic insufficiency, and that such regulation is essential for the subsequent initiation of liver regeneration. Based on these considerations, the studies described here were conducted to define the regulation and functional significance of Zn-HDAC expression and activity during liver regeneration in the mouse PH model.

Experimental Procedures

Animal Husbandry and Surgery

PH was performed on mice as described in Supporting Information and previously (5-7;22;23). Some mice were treated with the Zn-HDAC inhibitor suberoylanilide hydroxyamic acid (SAHA) or vehicle as described in Supporting Information. All experiments were approved by the Washington University Animal Studies Committee and conducted in accordance with institutional guidelines and the criteria outlined in the "Guide for Care and Use of Laboratory Animals" (NIH publication 86-23).

Liver Tissue Extracts and Lysates

Whole liver lysates were made as described previously (5-7;22;23). Hepatic nuclear and cytoplasmic extracts were made using a Nuclear Extract Kit (Active Motif) according to the manufacturer's instructions. Acid extracts of liver were generated as described in Supporting Information.

Histology, Immunohistochemistry, Gene and Protein Expression, HDAC Activity, and Statistics

Analyses were conducted as described in Supporting Information and previously (5-7;22;23).

Acetyl-Histone Chromatin-Immunoprecipitation-Polymerase-Chain-Reaction (ChIP-PCR) Analyses

Acetylation of histone H3 on lysine residue 9 (Ac-H3K9), an epigenetic mark of transcriptional activation (24), was assessed in promoters of genes of interest by ChIP-PCR as described in Supporting Information.

Results

Hepatic Zn-HDAC Activity is Induced in Regenerating Liver

To begin to characterize HDAC regulation during liver regeneration, Zn-HDAC activity was quantified in lysates from whole liver harvested at serial times after PH. This analysis showed significantly increased activity 36-72 hours after PH compared to that seen at earlier timepoints after PH or in un-operated and sham-operated control mice (Figure 1A). This timeframe of increased HDAC activity encompasses the hepatocellular proliferative response to PH (5;6;23), with both HDAC activity (Figure 1A) and hepatocellular proliferation (data not shown) returning to pre-hepatectomy baseline levels by 7 days after surgery. Addition of trichostatin A (TSA), which is a pan-inhibitor of Zn-HDACs (12;25), to the lysates quenched the majority of HDAC activity at all timepoints (Figure 1A). Zn-HDACs are categorized into subfamilies based on sequence homology (12;18). Amongst the class I HDACs (1, 2, 3, and 8), HDACs 1 and 2 are exclusively localized in the nucleus (12). In contrast, subcellular localization of the class II HDACs 4, 5, and 7 is regulated by phosphorylation-dependent shuttling between the cytoplasmic and nuclear compartments (12). Therefore, to further define HDAC regulation during liver regeneration, changes in hepatic nuclear and cytoplasmic activity after PH were determined. This experiment showed increased HDAC activity after liver resection in both cytoplasmic and nuclear compartments (Figure 1B-C), with kinetics similar to that observed in the whole tissue analysis (Figure 1A). As seen with the whole liver lysates, the majority of compartment-specific activity was inhibitable by TSA (Figure 1B-C). These data show that Zn-HDAC activity is up-regulated in cytoplasmic and nuclear compartments of regenerating liver.

Zn-HDACs Exhibit Isoform Specific Regulation of Expression during Liver Regeneration

To elucidate the mechanisms responsible for induction of hepatic HDAC activity after PH, the expression of each of the known zinc-dependent enzymes was determined. Gene expression was examined first. The results showed significant induction of expression of HDACs 1, 8 (class I) and 4 (class II) after PH, prior to onset of hepatocellular proliferation compared to sham- and un-operated liver (Figure 1D-F). In contrast, expression of HDACs 2 and 3 (class I), 5, 6, 7, 9, and 10 (class II), and 11 (class IV) were not increased after PH, and, in fact, HDACs 5, 9 and 11 were significantly down-regulated prior to onset of hepatocellular proliferation in regenerating liver (Supplementary Figure 1A-H).

Next, HDAC protein expression in lysates of regenerating- and sham operated-liver was characterized. Consistent with the mRNA expression analyses, HDACs 1 and 8 increased and HDAC 4 rose after an initial decline while HDACs 2, 3, 5, and 7 did not increase in regenerating vs. control liver (Figure 2A-B). As previously reported, HDACs 1 and 2 were exclusively detectable in the nuclear compartment (12) while HDACs 3 and 8 were present in both nuclear and cytoplasmic fractions (Figure 3A-B). The class II HDACs shuttle between nucleus and cytoplasm in response to stimuli (12). Nevertheless, cytoplasmic levels of HDACs 4, 5, and 7 were virtually undetectable in quiescent and regenerating livers (Figure 3A-B), perhaps because of experimental sensitivity limitations. However, these studies did reveal transient accumulation of HDAC5 in nuclear fractions recovered from early regenerating liver (Figure 3A-B), despite no increase in the level of hepatic HDAC5 mRNA (Supplementary Figure 1C) or protein expression in whole liver lysate (Figure 2A-B) throughout regeneration. Thus, these data demonstrate PH-induced nuclear translocation of this HDAC isoform. Such translocation is reported to be dynamically regulated by dephosphorylation of cytoplasmic HDAC5 (12), and phosphorylated HDAC5 levels were also diminished in whole tissue lysates of regenerating liver harvested 12-24 hours after PH (Figure 2A-B). These findings illustrate isoform-specific regulation of zinc-dependent HDAC expression and localization during liver regeneration.

Histone Acetylation Declines during Liver Regeneration

Next, the impact of PH-induced changes in hepatic HDAC activity, expression, and subcellular localization on global histone and non-histone protein acetylation in regenerating liver was investigated. First, the pattern of acetyl-protein abundance in whole tissue lysate was assessed by immunoblot analysis with an acetyl-lysine-specific antibody. Multiple immunoreactive protein bands were detectable in quiescent and regenerating liver; however, this analysis did not identify specific acetyl-proteins in regenerating liver whose levels were reduced at times coincident with increased HDAC activity (Figure 4A). Next, the level of acetylated-histones in acid-extracted liver tissue harvested at serial times after PH or sham surgery was quantified using both acetyl-lysine- and histone-specific antibodies. This experiment showed greater reduction in hepatic levels of acetylated histones H3 (Ac-H3) and H4 (Ac-H4) after PH compared to liver from sham- and unoperated animals (Figure 4B-C). Histone H3 protein acetylated on lysine residue 9 (Ac-H3K9), an epigenetic mark associated with transcriptional activation (12;24;26), was also reduced in regenerating liver compared to controls (Figure 4B-C). In each case, the decline in acetyl-histone abundance (Figure 4C) began prior to the time of peak PH-induced hepatocellular proliferation (which occurs 36 hours after surgery, (5;6;23;27)).

Suberoylanilide Hydroxamic Acid Impairs Liver Regeneration

To examine the functional significance of increased hepatic HDAC expression and activity during liver regeneration, the effect of suberoylanilide hydroxyamic acid (SAHA), which (like TSA) is a specific inhibitor of Zn-HDAC activity (12;25), on the regenerative response was determined. Mice were administered SAHA using a regimen modified from one that suppresses hepatocellular carcinoma xenograft growth in mice (28). First, the effect of SAHA on histone acetylation in regenerating liver was assessed. The results showed increased hepatic acetyl-histone-H3 and -H4 abundance in SAHA- vs. vehicle-treated animals 12-24 hours after PH (Figure 5A-B), consistent with SAHA-dependent inhibition of Zn-HDAC activity. However, hepatic acetyl-H3 abundance was diminished in both SAHA- and vehicle-treated mice at 36 hours after PH compared to earlier timepoints (Figure 5A-B), suggesting either incomplete inhibition of zinc-dependent deacetylase activity, increased SAHA-resistant deacetylase (*e.g.* sirtuin) activity, or decreased histone acetyltransferase activity in regenerating liver at this timepoint. Next, the influence of SAHA on hepatocellular proliferation was examined. The results showed that hepatocellular BrdU incorporation was significantly reduced in SAHA-treated animals 36 hours after PH (Figure 5C-D), which is the timepoint corresponding to peak proliferation during normal liver regeneration (5;6;23;27). Hepatocellular BrdU incorporation and mitotic frequency were comparable in SAHA- and vehicle-treated mice 48 hours after PH (Figure 5C-F). These data show that SAHA delays PH-induced histone deacetylation and hepatocellular proliferation.

SAHA administration did not suppress the development of hypoglycemia or accumulation of hepatic fat in mice subjected to PH (Supplementary Figure 2A-B), indicating that the signals that regulate the PH-induced metabolic response to hepatic insufficiency are not downstream, but could be upstream, of the target of SAHA's anti-regenerative activity. Moreover, SAHA did not lead to a reduction in recovery of liver-to-body-mass 36 hours after PH, perhaps because of compensatory hepatocellular hyperplasia (as suggested by modestly diminished hepatocellular nuclear density in SAHA- vs. vehicle-treated mice; Supplementary Figure 2C-D). The anti-regenerative effect of SAHA did not appear to be due to hepatic or systemic drug toxicity, based on comparable body mass, normal liver histology, and no evidence of injury-induced hepatocellular BrdU incorporation in sham-operated mice treated with SAHA vs. vehicle (Supplementary Figure 3A-B).

SAHA Augments Expression and Promoter-Associated Histone Acetylation of p19^{INK4d}

To investigate the molecular mechanisms by which SAHA delays liver regeneration, hepatocellular cell cycle progression in SAHA-treated mice subjected to PH was further examined. Surprisingly, SAHA treatment did not alter the induction of hepatic cyclin D1 mRNA (Figure 6A) or protein (Figure 6E-F) expression, indicating that quiescent hepatocytes enter G1 of the cell cycle and up-regulate cyclin D1 normally in SAHA-treated mice subjected to PH. However, cyclin E1 showed a trend of reduced expression 36 hours after PH in regenerating liver from SAHA-treated mice (Figure 6B, $p=0.06$ for 36 hours vehicle vs. SAHA), and expression of cyclins A2 and B1 were significantly reduced by the HDAC inhibitor at this timepoint (Figure 6C-F). These cyclins direct cell cycle progression downstream of cyclin D1 expression, including transitions from G1 to S, through S, and from G2 to M, respectively (29). Consistent with this finding, SAHA also suppressed the induction of FoxM1 in regenerating liver (Supplementary Figure 4A). FoxM1 is induced in S phase, activates the expression of G2/M phase-specific genes, and is essential for normal liver regeneration (30). These studies demonstrate that SAHA impairs regeneration by delaying hepatocellular cell cycle progression at a step distal to cyclin D1 induction, suggesting that Zn-HDAC activity regulates hepatocellular progression through a downstream G1 checkpoint in regenerating liver. Cyclin dependent kinase inhibitors (CDKIs), which include members of the Cip-Kip and INK4 families, regulate such cell cycle progression (31;32). Moreover, Zn-HDAC inhibition stimulates the transcription of many CDKIs (33-40). Therefore the influence of SAHA on CDKI expression in regenerating liver was examined. The results showed that SAHA administration significantly augmented hepatic mRNA expression of p19^{INK4d} 12 hours after PH (Figure 7A). p19^{INK4d} protein also showed a trend of increased expression in SAHA- vs. vehicle-treated mice in both nuclear ($p=0.1$) and cytosolic ($p=0.2$) fractions of regenerating liver at this timepoint (Figure 7C-D). In contrast, p21^{Cip1}, p27^{Kip1}, p57^{Kip2}, and p18^{INK4c} expression were not augmented by SAHA (Supplementary Figure 4B-F). Zn-HDAC inhibition has previously been reported to augment the expression of p19^{INK4d} and other CDKIs (33-40), raising the possibility that PH-induced Zn-HDAC activity might promote normal liver regeneration by suppressing hepatic p19^{INK4d} expression. Therefore, hepatic p19^{INK4d} expression was also quantified in untreated mice subjected to PH or sham surgery. The results showed that this CDKI is not transcriptionally suppressed during early regeneration, but its mRNA is induced during later regeneration (Figure 7B). Interestingly, nuclear p19^{INK4d} protein, which is detectable in quiescent liver, declined in response to PH but not sham surgery (Figure 7C-D).

Next, the effect of SAHA on hepatic p19^{INK4d} promoter-associated histone acetylation was investigated using chromatin immunoprecipitation (ChIP) with an antibody to histone H3 acetylated on lysine residue 9 (Ac-H3K9), followed by qRT-PCR analysis (ChIP-PCR) of immunoprecipitated chromatin for p19^{INK4d} promoter sequence. The results showed that p19^{INK4d}-promoter-associated histone acetylation is augmented in livers of SAHA- vs. vehicle-treated mice 12 hours after PH (Figure 7E), *i.e.* the timepoint when hepatic p19^{INK4d} mRNA expression and nuclear protein accumulation are augmented in SAHA-treated mice. These data suggest that SAHA directly induces p19^{INK4d} expression in regenerating liver by increasing p19^{INK4d} promoter-associated histone acetylation, and they implicate this CDKI as mediating delayed regeneration in SAHA-treated mice. To examine the specificity of SAHA's effect on the p19^{INK4d} promoter, cyclin A2 was similarly analyzed. Hepatic cyclin A2 mRNA expression was suppressed by SAHA 36 hours after PH (Figure 6C), and the acetylation of corresponding promoter-associated histones was not augmented by treatment with the Zn-HDAC inhibitor at this timepoint (Figure 7F). Thus, SAHA exerts promoter-specific effects on histone acetylation during liver regeneration.

Discussion

The studies reported here were conducted to characterize the expression, activity, and functional significance of Zn-HDACs during liver regeneration. Our interest in this question was born out of our prior work implicating the metabolic response to hepatic insufficiency as a promoter of hepatic regeneration (4-7) and recent reports demonstrating the influence of metabolism on protein acetylation (9-11). Together, those observations suggested the possibility that HDAC-dependent protein deacetylation could link altered metabolism in response to hepatic insufficiency to hepatocellular proliferation during normal liver regeneration. The results reported here show that HDAC activity and histone deacetylation are induced in regenerating liver, and that such regulation occurs through effects on both HDAC expression and subcellular localization.

We previously reported the inhibitory effect of enteral dextrose-supplementation on normal liver regeneration (5). Recently reported data shows that glucose supplementation augments histone and non-histone protein-acetylation in cell culture (9-11). Together with the data described here showing diminished histone acetylation in regenerating liver (Fig 5B) at a time coincident with PH-induced hypoglycemia, these observations support the hypothesis that metabolic alterations in response to hepatic insufficiency could regulate liver regeneration via effects on protein acetylation. Although these findings do not establish the causal link between such metabolic changes and HDAC-dependent promotion of liver regeneration, our observation that hepatic HDAC5 undergoes PH-induced nuclear translocation during early regeneration (Figure 3A-B) is particularly intriguing in light of recent data implicating nuclear translocation of HDAC5 as a modifier of glycemic regulation (19). Based on these considerations, we investigated whether enteral dextrose-supplementation inhibits hepatic HDAC5 dephosphorylation or nuclear accumulation in mice subjected to PH. No such inhibition was identified (data not shown). However, it is important to note that the enteral dextrose supplementation regimen used for this experiment (and in our previous study (5)) does not prevent hypoglycemia and suppresses but does not completely abrogate hepatocellular proliferation in response to PH. Thus, development of an experimental paradigm with which euglycemia is maintained after PH will be necessary for rigorous examination of the influence of hypoglycemia on HDAC activity, expression, and localization and histone and non-histone protein acetylation during liver regeneration.

The analyses described here also show that SAHA, which specifically inhibits Zn-HDAC activity (25), delays liver regeneration by impairing hepatocellular cell cycle progression downstream of cyclin D1 in mice subjected to PH. Increased histone acetylation, as occurs with Zn-HDAC inhibition, typically augments the expression of nearby genes. Thus, our data showing that PH-induced hepatic expression of cyclins E1, A2, and B1 is inhibited by SAHA-administration is likely secondary to indirect effects of the Zn-HDAC inhibitor on such expression. This could occur, for example, by SAHA-stimulated expression of an inhibitor of cell cycle progression. Indeed, our data showing that SAHA augments hepatic p19^{INK4d} promoter-associated histone acetylation, mRNA expression, and nuclear protein accumulation in mice subjected to PH provide support for this consideration and suggest a model in which induction of p19^{INK4d}, which inhibits cyclin D-dependent cell cycle progression through G1, mediates SAHA-dependent inhibition of liver regeneration (Figure 8). These studies leave open the possibility that PH-induced, HDAC-dependent deacetylation of other specific histone (or non-histone) proteins is also essential for progression of normal liver regeneration (Figure 8). Although beyond the scope of this work, future efforts to combine acetyl-histone ChIP with next-generation DNA sequencing technology (ChIP-Seq) could identify additional genetic targets that contribute to the anti-regenerative activity of SAHA.

Finally, although these studies showed that SAHA significantly delays PH-induced liver regeneration, such regeneration was not completely abrogated. This finding could be the result of either incomplete inhibition of Zn-HDAC activity or compensatory changes in other (non-Zn-dependent) HDAC activities in SAHA-treated mice. Our data showing that PH-induced histone deacetylation is delayed but not entirely prevented by SAHA (Figure 5A-B) is consistent with either of these considerations. Thus, future investigations should examine the effects of alternative pharmacological Zn-HDAC inhibition strategies and of isoform-specific genetic disruption of Zn-HDAC activity on liver regeneration. Such studies should also consider the impact of PH-induced alterations in metabolism on NAD-dependent (class III) HDACs (*i.e.* sirtuins), which have also been implicated in the modulation of liver regeneration (41).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

BrdU	bromodeoxyuridine
Ac-H3K9	histone H3 acetylated on lysine 9
Zn-HDAC	zinc-dependent histone deacetylase
PH	partial hepatectomy
qRT-PCR	semi-quantitative real-time reverse-transcription polymerase-chain-reaction
SAHA	suberoylanilide hydroxyamic acid
TSA	trichostatin A

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Figure 1. Hepatic Zn-HDAC Activity and mRNA Expression after PH

(A-C) Hepatic Zn-HDAC activity (relative units) at serial times after PH or sham surgery in (A) whole liver lysates (* $p < 0.01$ vs. 12 hours PH; $^{\wedge}p < 0.001$ vs. 0 and 12-24 hours PH; $^{+}p < 0.01$ vs. 0 and 12-24 hours PH and $p < 0.03$ vs. 72 hours sham); hepatic HDAC enzyme specific activity in 0 hour replicates: 149 ± 25 pmol/mg/min); (B) cytoplasmic lysate preparations (* $p < 0.04$ vs. 0 hours; specific activity in 0 hour replicates: 217 ± 7 pmol/mg/min); and (C) nuclear lysate preparations ($p < 0.04$ between groups; specific activity in 0 hour replicates: 440 ± 24 pmol/mg/min). TSA-resistant activity in all samples is also shown. (D-F) Hepatic mRNA expression of (D) HDAC1 (* $p < 0.001$ vs. 0 and 36 hours sham), (E) HDAC8 (* $p < 0.02$ vs. sham), and (F) HDAC4 (* $p < 0.03$ vs. sham) at serial times after PH or sham surgery. Data standardized to expression of 2-microglobulin.

Figure 2. Hepatic Zn-HDAC Protein Expression during Liver Regeneration
 (A) Representative protein immunoblot analyses for class I (1, 2, 3, and 8), and class II (4, 5, and 7) HDACs in pooled replicate lysates of quiescent (0 hour, 0h), regenerating (12 hours (12h) to 7 day (7d)), and sham-operated (12h to 7d) livers. HeLa cell nuclear extract is shown as positive control and β -actin and GAPDH expression as loading controls. (B) Quantification of HDAC protein levels standardized to GAPDH.

Figure 3. Subcellular Compartment-Specific Hepatic Zn-HDAC Expression during Liver Regeneration

(A) Representative protein immunoblot analyses for class I and class II HDACs in pooled replicate nuclear and cytoplasmic lysates of quiescent (0h) and regenerating (12h to 7d) livers. β -actin and GAPDH expression shown as loading controls. (B) Quantification of HDAC protein levels standardized to GAPDH (cytoplasmic) or β -actin (nuclear).

Figure 4. Hepatic Acetyl-Protein Abundance after PH

Representative immunoblot analyses for (A) acetyl-lysine-containing proteins in pooled replicate whole liver lysates and (B) acetyl-lysine-containing histones, histone H3 acetylated on Lys9 (Ac-H3K9), and total histone H3 in pooled replicate acid extracts of whole liver harvested at serial times after PH or sham surgery. (C) Quantification of acetyl-histones standardized to total histone H3.

Figure 5. The Effect of SAHA on Histone Acetylation and Hepatocellular Proliferation after PH (A) Representative immunoblot analyses of acetyl-lysine containing histone H3 (Ac-H3) and H4 (Ac-H4) and total H3 in pooled replicate acid extracts of whole liver from vehicle- and SAHA-treated mice harvested 12-48 hours after PH. (B) Quantification of acetyl-histone standardized to total histone H3. (C) Immunohistochemical analysis (36 hours after PH; 100 micron bar shown) and (D) quantification (24-48 hours after PH) of hepatocellular BrdU incorporation in vehicle- and SAHA-treated mice (*p < 0.05 vs. vehicle). (E) Hematoxylin and eosin staining and (F) quantification of hepatocellular mitotic frequency (per 10 high-powered fields) 48 hours after PH in vehicle- and SAHA-treated mice.

Figure 6. The Effect of SAHA on Hepatic Expression of Cell Cycle Regulators after PH
Hepatic mRNA expression of (A) cyclin D1, (B) cyclin E1, (C) cyclin A2 (* $p < 0.001$ vs. vehicle), and (D) cyclin B1 (* $p < 0.001$ vs. vehicle) in un-operated (control) and vehicle- or SAHA-treated mice 12-36 hours after PH. Data standardized to expression of 2-microglobulin. (E) Representative immunoblot analyses of cyclin D1 and cyclin B1 in pooled replicate whole liver lysates from vehicle- and SAHA-treated mice 12-48 hours after PH. GAPDH shown as loading control. (F) Quantification of cyclin expression standardized to GAPDH.

Figure 7. The Effect of SAHA on Hepatic p19^{INK4d} Expression and Promoter Acetylation in Regenerating Liver

Hepatic mRNA expression of p19^{INK4d} (A) in un-operated (control) and vehicle- or SAHA-treated mice 12-36 hours after PH and (B) in untreated mice at serial times after PH or sham surgery (*p<0.001 vs. 0 hour and sham). Data standardized to expression of 2-microglobulin. (C) Representative immunoblot expression analyses and (D) quantification of hepatic p19^{INK4d} expression after PH or sham surgery in pooled replicate nuclear and cytoplasmic fractions from untreated mice or 12 hours after PH in vehicle- and SAHA-treated mice. Ac-H3K9 (or normal rabbit IgG as control) ChIP-PCR of (E) p19^{INK4d} (*p=0.01 vs. vehicle) and (F) cyclin A2 in liver from vehicle- and SAHA-treated mice.

PH induces quiescent (G₀) hepatocytes to re-enter the cell cycle (G₁). As reported here, PH also induces hepatic Zn-HDAC activity and SAHA, which inhibits such activity, impairs PH-stimulated hepatocellular cell cycle progression downstream of cyclin D1 induction and augments hepatic p19^{INK4d} expression. These data suggest that Zn-HDAC activity promotes hepatocellular G₁ cell cycle progression during normal liver regeneration and that p19^{INK4d} mediates the inhibitory effect of SAHA on such regeneration. (Solid lines indicate positive (—) and negative (—) regulation demonstrated by data reported here. Dashed lines represent hypothesized regulation based on these data and discussed in the text.)