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Identification of an epigenetic signature of early mouse liver regeneration that is disrupted by Zn-HDAC inhibition

Jiansheng Huang[1], Andrew E Schriefer[2], Wei Yang[2], Paul F Cliften[2], and David A Rudnick[1,3,*]

[1]Department of Pediatrics; Washington University School of Medicine; St. Louis, MO USA; [2]Department of Pediatrics; Department of Genetics; Developmental Biology; Washington University School of Medicine; St. Louis, MO USA; [3]Department of Developmental Biology; Washington University School of Medicine; St. Louis, MO USA

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Abbreviations: Ac-H3K9, histone H3 acetylated on lysine 9; CDKI, cyclin dependent kinase inhibitor; ChIP-Seq, chromatin immunoprecipitation-next generation DNA sequencing; GO, gene ontology; PH, partial hepatectomy; qRT-PCR, semi-quantitative real-time reverse-transcription polymerase-chain-reaction; SAHA, suberoylanilide hydroxamic acid; TSS, transcription start sites; Zn-HDAC, zinc-dependent histone deacetylase.

Liver regeneration has been well studied with hope of discovering strategies to improve liver disease outcomes. Nevertheless, the signals that initiate such regeneration remain incompletely defined, and translation of mechanism-based pro-regenerative interventions into new treatments for hepatic diseases has not yet been achieved. We previously reported the isoform-specific regulation and essential function of zinc-dependent histone deacetylases (Zn-HDACs) during mouse liver regeneration. Those data suggest that epigenetically regulated anti-proliferative genes are deacetylated and transcriptionally suppressed by Zn-HDAC activity or that pro-regenerative factors are acetylated and induced by such activity in response to partial hepatectomy (PH). To investigate these possibilities, we conducted genome-wide interrogation of the liver histone acetylome during early PH-induced liver regeneration in mice using acetyL-histone chromatin immunoprecipitation and next generation DNA sequencing. We also compared the findings of that study to those seen during the impaired regenerative response that occurs with Zn-HDAC inhibition. The results reveal an epigenetic signature of early liver regeneration that includes both hyperacetylation of pro-regenerative factors and deacetylation of anti-proliferative and pro-apoptotic genes. Our data also show that administration of an anti-regenerative regimen of the Zn-HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) not only disrupts gene-specific pro-regenerative changes in liver histone deacetylation but also reverses PH-induced effects on histone hyperacetylation. Taken together, these studies offer new insight into and suggest novel hypotheses about the epigenetic mechanisms that regulate liver regeneration.

Introduction

Recovery from all liver injuries depends on the ability of the liver to regenerate. Such regeneration has been extensively studied with hope of discovering new therapeutic strategies that improve human liver disease outcomes. Mouse two-thirds partial hepatectomy (PH) has been the paradigm most commonly used to study the regulation of liver regeneration.[1] Experiments using this model show that partial liver resection induces a characteristic hepatocellular proliferative response regulated by specific cytokines, growth and transcription factors and intracellular signaling events. This response restores normal hepatic mass and function, after which hepatocytes return to their pre-regenerative state of proliferative inactivity. Nevertheless, the earliest events that initiate hepatic regeneration remain incompletely defined, and translation of mechanism-based, pro-regenerative interventions into new treatments for liver diseases has not yet been achieved.

We recently reported the isoform-specific regulation and essential function of zinc-dependent histone deacetylases (Zn-HDACs) during mouse liver regeneration.[2] Those studies showed that total Zn-HDAC activity increases and the global abundance of histone H3 acetylated on lysine residue 9 (Ac-H3K9, an epigenetic mark of transcriptional activation) coincidentally declines in regenerating liver. We also discovered that some Zn-HDACs (e.g., HDACs 1, 4, and 8) exhibit increased hepatic expression, others (HDACs 9 and 11) show decreased expression, and HDAC5 undergoes nuclear translocation in early regenerating liver. Finally, we evaluated Zn-HDAC regenerative function using suberoylanilide hydroxamic acid (SAHA),

*Correspondence to: David A Rudnick; Email: rudnick_d@kids.wustl.edu
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a broad inhibitor of these enzymes, and showed that SAHA suppresses PH-induced hepatocellular proliferation. Those data suggest a model of liver regeneration in which epigenetically-regulated anti-proliferative factors are deacetylated and transcriptionally suppressed by direct Zn-HDAC-dependent histone deacetylation in response to PH. Consistent with that idea, the cyclin dependent kinase inhibitor (CDKI) *Cdkn2d* (i.e., $p19^{Ink4d}$) is concordantly hyperacetylated and transcriptionally-induced by SAHA in early regenerating liver.[2] Alternatively, Zn-HDAC inhibition might also suppress regeneration by indirectly promoting the deacetylation and suppressing the expression of pro-regenerative factors; however, such regulation has not yet been described in any model of liver regeneration. Based on these considerations, we undertook genome-wide interrogation of the liver histone acetylome during early regeneration in the absence and presence of Zn-HDAC inhibition, using Ac-H3K9 chromatin immunoprecipitation combined with next-generation DNA sequencing (ChIP-Seq), to elucidate the epigenetic regulation of liver regeneration. The results of these analyses are reported here.

Results

Identification of PH-induced changes in liver histone acetylation

AcetyL-histone H3K9 ChIP-Seq was performed on liver tissue harvested 12 hours after PH or sham surgery from 3 replicate animals in each surgical group. The sequence data were first examined to characterize the abundance and distribution of acetylated sequences with respect to gene transcription start sites (TSS). This analysis showed accumulation of acetylated sequence in close proximity to TSS, with 76.6% of sequences immunoprecipitated from regenerating liver and 78.3% of those recovered from sham-operated liver within ±2000 base pairs (bp) of such sites (Supplementary **Fig. 1A**).

Next, the sequence data were analyzed to define and compare the heights of individual sequence peaks between experimental groups. The specific genes containing or proximate to sequence peaks identified as differentially abundant in regenerating vs. sham-operated liver were also determined. Gene sequences over-represented in DNA immunoprecipitated from regenerating (vs. sham-operated) liver correspond to loci that are hyperacetylated in response to PH, while under-represented sequences represent genomic sites that are deacetylated during regeneration. Using a (Benjamini-Hochberg) false discovery rate threshold of $q < 0.1$ (with which <10% of genes identified as differentially acetylated are expected to be false positives[3]), this analysis identified 454 gene sequences with decreased acetylation and 480 with increased acetylation in regenerating (vs. sham-operated) liver (**Table 1** and Supplementary **Table 1**). These sequences corresponded to 392 and 410 unique genes, respectively (i.e., several genes are represented by multiple distinct sequences in this dataset). These data establish the methodology for and feasibility of using acetyL-histone ChIP-Seq to define genome-wide patterns of histone acetylation during liver regeneration.

Functional classification of regenerative changes in liver histone acetylation

Next, to characterize functional patterns of change in liver histone acetylation during early regeneration, gene ontology (GO) and similar classification schema were used to categorize genes identified as differentially acetylated in liver after PH vs. sham surgery. First, we identified gene category terms that were significantly enriched (using $q < 0.1$) for genes that are significantly hyper- or de-acetylated by PH (also using $q < 0.1$). The results showed enrichment, among hyperacetylated genes, for those associated with 'regulation of cell cycle' (**Table 1**). Genes linked to development- and metabolism-related categories were also enriched in this group. Of note, the total numbers of hyper- and de-acetylated genes identified in early regenerating liver were comparable (**Table 1** and Supplementary **Table 1**). Nevertheless, in contrast to the analysis of hyperacetylated genes, functional classification of the deacetylated genes did not show enrichment in any cell proliferation-associated term but did demonstrate these genes to be enriched in categories associated with regulation of cell death (**Table 1**). The deacetylated genes, like those that are hyperacetylated, were also enriched in metabolism-related categories.

Hyperacetylated genes

Based on the findings described above, functional annotation tools were subsequently used to more comprehensively identify all differentially-acetylated genes associated with any cell proliferation- or cell death-related gene category term (whether or not those gene categories were significantly enriched for such differentially acetylated genes). Using this approach, 36 of the 410 unique, hyperacetylated genes in regenerating liver were linked to terms associated with regulation of cell cycle and 8 were linked to cell death terms (**Tables 1 and 2**). Many of these hyperacetylated genes are known to be induced during, to positively regulate, or to interact with factors that promote liver regeneration. For example, *Ccnd1* (i.e., cyclin d1), *Cdkn1a* (i.e., p21), *Gadd45g*, *Lcn2*, and *Myc* are induced in early regenerating liver [4-7]; *Ccnd1*, *Egfr*, and *Prlr* promote liver regeneration [8-11]; and *Tcf7l2* (also known as *Tcf4*) binds to β-catenin, whose signaling is activated during and promotes regeneration.[12]

Deacetylated genes

Similar examination of genes deacetylated in response to PH showed 37 of 392 associated with cell cycle- and 31 linked to cell death-related gene category terms (**Tables 1 and 2**). Unlike the hyperacetylated genes, this list included genes linked to 'cell cycle arrest' and 'negative regulation of cell proliferation' (**Table 2**). Thus, many more genes deacetylated by PH are associated with anti-proliferative and/or pro-apoptotic activity compared to those hyperacetylated by PH. Several of these deacetylated genes, including *Cebpa*, *Foxo3*, *Gas1*, and *Lats2*, are also known to be suppressed during early liver regeneration [4,13-15] and *Cebpa* was recently shown to be essential for termination of regeneration.[16] Nevertheless, the regenerative regulation and function of many deacetylated genes in **Table 2** is not yet well-characterized.

Table 1. Summary of differentially acetylated genes in liver 12 hr after PH vs. Sham Surgery (FDR<0.1)

Effect of PH	Total (unique) genes[1]	GO terms enriched for differentially acetylated genes (FDR<0.1)[2]	Number of genes linked to[3]: Cell proliferation[4]	Cell Death[4]
Decreased Acetylation	454 (392)	cellular ketone metabolic process, **regulation of cell death**, lipid metabolic process, **regulation of programmed cell death**, sulfur amino acid metabolic process, organic acid metabolic process	37	31
Increased Acetylation	480 (410)	gland development, cellular process, system development, anatomical structure development, organ development, multicellular organismal development, lactation, developmental process, cellular metabolic process, **regulation of cell cycle**, vasculature development	36	8

[1]In PH vs. Sham replicates.
[2]Identified using DAVID bioinformatics database (see references 11–12) from GOTERM_BP_ALL, PANTHER_BP_ALL, KEGG_PATHWAY, PANTHER_PATHWAY on significantly de- or hyper-acetylated genes, respectively.
[3]Based on examination of all terms into which DAVID sorted differentially acetylated genes.
[4]See Table 2 for specific genes.

Together, these data define stereotypical, early regenerative changes in liver histone acetylation, and show that those patterns include both hyperacetylation of pro-regenerative and pro-proliferative genes and deacetylation of anti-proliferative and pro-apoptotic genes. Specific examples of histone acetylation patterns in regenerating and sham-operated liver for several differentially acetylated genes listed in **Table 2** are shown in **Figure 1**.

Next, we examined biological interactions between genes identified as differentially acetylated after PH. The goal of this *interactome* analysis was to assess whether differences in patterns of functional connectivity between hyper- vs. de-acetylated genes could be identified, and, if so, to determine the specific genes with the greatest levels of connectivity to other hyper- and de-acetylated genes. Such genetic "nodes" are candidate drivers of the biological functions associated with hyper- vs. de-acetylated genes (e.g., in this case regenerative function), and also might be useful for distinguishing regenerative patterns of connectivity between such genes. This analysis showed that several genes linked to cell proliferation- and cell death-associated gene category terms (**Table 2**) were also among the most highly connected nodes within genes hyper- or de-acetylated, respectively, by PH (**Figs. 2A, B**). For example, *Myc*, *Egfr*, *Ccnd1*, *Tcf7l2*, *Pik3r1*, and *Rarg* were identified as over-connected, hyperacetylated genes (**Table 2** and **Fig. 2A**) and *Nr3c1* (i.e., glucocorticoid receptor α), *Cebpa*, *Foxo1*, and *Foxo3* were highly connected among deacetylated genes (**Table 2** and **Fig. 2B**) in early regenerating liver. Several of these over-connected genes are also known to be transcriptional regulators, and many of the genes to which they are connected are targets of their regulation. Thus, these data show that epigenetic regulation of liver regeneration is characterized by coincident, concordant alterations in the histone acetylation of specific transcription factors and their targets.

The influence of SAHA on regenerative changes in liver histone acetylation

Anti-proliferative genes that are deacetylated (and transcriptionally suppressed) by Zn-HDACs during normal liver regeneration could mediate the anti-regenerative activity of Zn-HDAC inhibition, and discovery of such genes should elucidate the epigenetic mechanisms that control regeneration. We previously identified *Cdkn2d* (i.e., $p19^{Ink4d}$) as one such candidate.[2] Notably, the analysis here demonstrated that *Cdkn2d* is deacetylated after PH (with $q = 0.11$; Supplementary **Table 1** and Supplementary **Fig. 2**). To identify other gene candidates whose SAHA-dependent reversal of PH-induced histone deacetylation might mediate the anti-regenerative effects of Zn-HDAC inhibition, ChIP-Seq was used to compare acetylation patterns in regenerating liver harvested 12 h after PH from replicates of SAHA- vs. vehicle-treated mice ($n = 4$ each). This analysis identified 1198 gene sequences with decreased acetylation and 1257 with increased acetylation (corresponding to 1162 and 1229 unique genes respectively) in SAHA- (vs. vehicle-) treated liver (for $q<0.1$; **Table 3** and Supplementary **Table 2**). Surprisingly, the number of genes identified as hyperacetylated (the predicted direct effect of Zn-HDAC inhibition) was comparable to the total deacetylated (which is necessarily an indirect effect of such inhibition) by SAHA in regenerating liver (**Table 3**). As with the analysis of regenerating and sham-operated liver, the acetylated

Table 2. Cell proliferation & cell death-associated genes differentially acetylated 12 hr after PH vs. Sham Surgery

Increased Acetylation by PH vs. Sham Surgery			Decreased Acetylation by PH vs. Sham Surgery		
Gene category terms[1]	Cell Proliferation[2]	Cell Death[2]	Gene category terms[1]	Cell Proliferation[2]	Cell Death[2]
Cell Proliferation	Adipor2	Dap	**Cell Proliferation**	Acvr2b	*Bcl6*
-Bladder cancer	Atf5	Lgals9	-Cell cycle control	Arid5b	Bik
-Oncogenesis	Bcr	*Myc*	-Cell proliferation and differentiation	Bcar1	Birc5
-Endometrial cancer	Calml3	Mycl1	-Cell cycle arrest	*Bcl6*	Bmf
-Glioma	**Ccnd1**	Nlrp12	-Developmental growth	**Cebpa**	Bok
-Melanoma	Cda	Tnfrsf1a	-Growth	Coro1c	Cadm1
-Prostate cancer	Cdkn1a	Tnfsf14	-Negative regulation of cell proliferation	Csk	Clec2d
-Prostate gland growth	Cish	*Tns1*	Cell death	Ddah1	Dapk1
-Regulation of cell cycle	Cxadr		-Apoptosis	Dusp6	Dpm1
-Regulation of growth	Cyr61			*Erbb3*	*Erbb3*
-Regulation of mitotic cell cycle	**Egfr**		**Cell Death**		
	Elf3		-Death	*Ern1*	*Ern1*
Cell Death	Epgn		-Induction of apoptosis	Fgfrl1	***Foxo1***
-Induction of apoptosis	Ets2		-Induction of programmed cell death	Foxa3	***Foxo3***
	Gadd45g		-Negative regulation of apoptosis	***Foxo1***	*Gas1*
	Gpam		-Negative regulation of cell death	***Foxo3***	Gclc
	Hpgd		-Negative regulation of neuron apoptosis	Fyn	Gclm
	Igf1		-Negative regulation of programmed cell death	*Gas1*	H2-bl
	Kdm2b		-Positive regulation of apoptosis	Gtpbp4	H2-K1
	Lcn2		-Positive regulation of cell death	Hdac5	H2-q6
	Myc		-Positive regulation of cell killing	Il6ra	Itm2b
	Npm2		-Positive regulation of programmed cell death	*Jmy*	*Jmy*
	Pik3r1		-Programmed cell death	Lats2	Khdc1a
	Prlr		-Regulation of apoptosis	Lst1	Lst1
	Prox1		-Regulation of cell death	Mafb	Map3k5
	Psap		-Regulation of cell killing	Mkl2	Msh2
	Rai1		-Regulation of neuron apoptosis	Mreg	*Notch2*
	Rarg		-Regulation of programmed cell death	Msh2	**Nr3c1**
	Sertad2			Ndfip1	Pim1
	Sik1			Neo1	Sgpl1
	Smad7			*Notch2*	Tmbim6
	Smc1a			Prl2c5	Traf4
	Tcf7l2			Sesn2	
	Tns1			Smarca2	
	Vav2			Sned1	
	Zfp655			Tbce	
				Tspan3	
				Ulk1	

[1]Identified using DAVID bioinformatics database as described in text (see references 11–12).
[2]Listed by "Official gene symbol" (genes in bold are over-connected in the interactome analysis in Figures 2A-B; 2 hyper- and 8 deacetylated genes shown in italics were sorted by DAVID to both cell proliferation- and cell death-related gene category terms).

sequences identified in this experiment also clustered around TSS. In this case, 65.8% of acetylated sequences recovered from SAHA-treated mouse liver and 74.4% of such sequences from vehicle-treated controls were within ±2000 bp of those sites (Supplementary **Fig. 1B**). This experiment also demonstrated selective enrichment of genes hyper- (but not de-) acetylated by SAHA after PH in classification terms associated with cell cycle regulation and cell death (**Table 3**). Functional annotation analyses identified 227 (of 1229) genes hyperacetylated by SAHA and 38 (of 1162) deacetylated genes as linked to cell cycle regulation terms, and 99 hyperacetylated and 36 deacetylated genes as associated with cell death terms (**Table 3** and Supplementary **Table 3**). Thus, in contrast to the histone acetylomic analysis comparing early regenerating to sham-operated liver, more pro-apoptotic genes were hyperacetylated than deacetylated by SAHA in early regenerating liver.

Next, gene-specific differences in liver histone acetylation during early regeneration in SAHA- and vehicle-treated mice (Supplementary **Table 2**) were compared to those observed between regenerating and sham-operated liver (Supplementary **Table 1**). Using $q < 0.1$ to identify differentially acetylated genes in each experiment, this analysis showed that SAHA reversed PH-induced deacetylation of 57 genes and prevented regenerative hyperacetylation of 80 genes (Supplementary **Table 4**). To further evaluate the impact of Zn-HDAC inhibition on early regenerative changes in liver histone acetylation, a similar evaluation was conducted using a threshold of $q < 0.2$ for identification of differentially acetylated genes in each experiment. In this case, the results showed SAHA-dependent reversal of regenerative deacetylation of 157 genes and disruption of hyperacetylation of 116 genes (Supplementary **Table 4**). Further examination revealed that among the

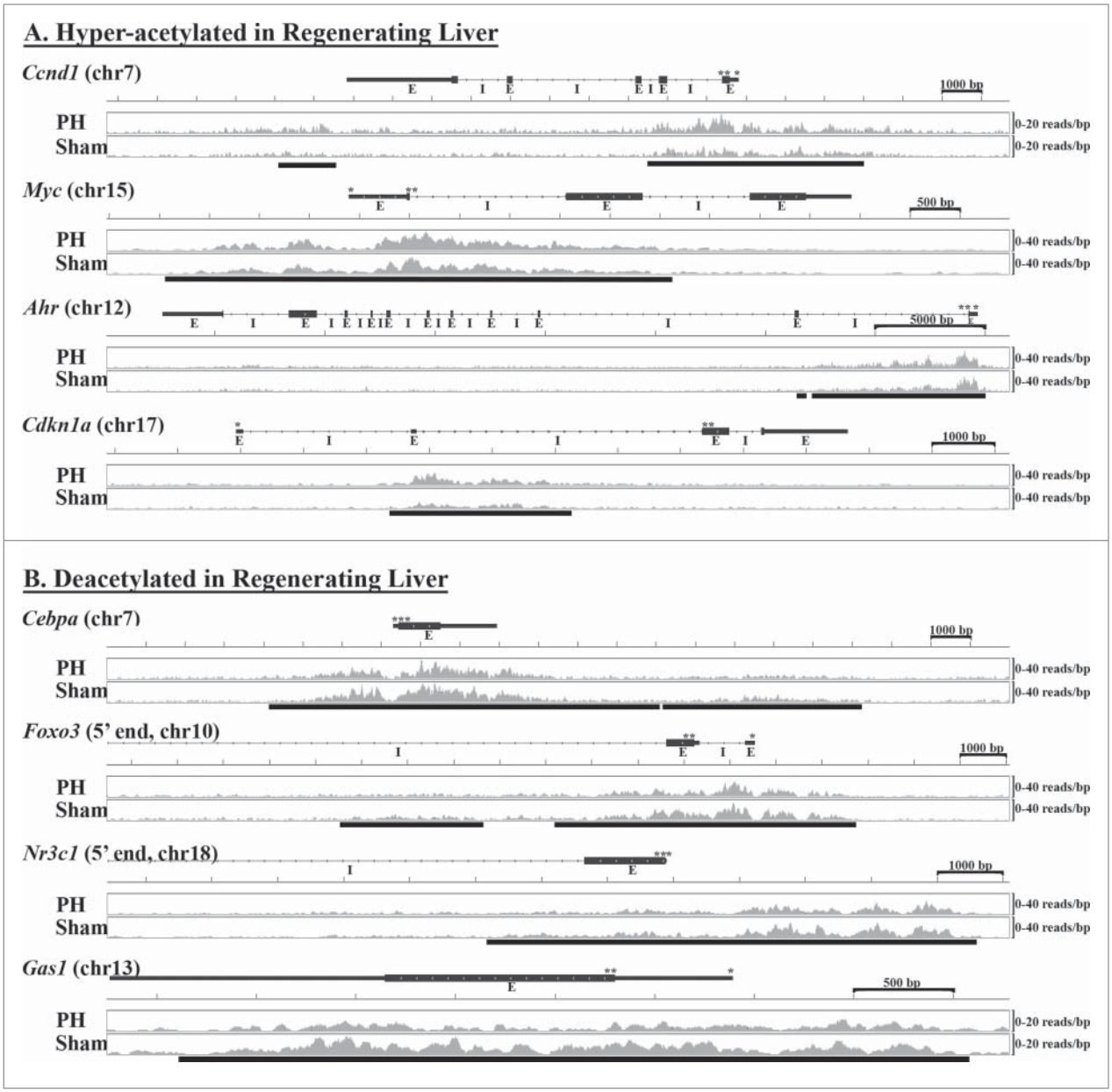

Figure 1. Patterns of Histone H3K9 Acetylation in Early Regenerating Liver. Examples of gene-specific patterns of histone acetylation in regenerating vs. sham-operated liver are shown for several specific genes identified as (**A**) hyperacetylated (*Ccnd1*, *Myc*, *Ahr*, *Cdkn1a*) or (**B**) deacetylated (*Cebpa*, *Foxo3*, *Nr3c1*, *Gas1*) 12 hours after PH. UCSC gene maps (transcription (*) and translation (**) start sites, exons (E), and introns (I) as designated) are aligned with abundance of immunoprecipitated sequence (sequence reads per bp with scale indicated to the right) integrated from livers of 3 replicates each after PH or sham surgery. The sequence abundance images were generated using the Integrative Genomics Viewer (IGV) genome browser.[40,41] The bars below the sequence abundance data indicate specific sequence(s) identified as differentially acetylated.

cell proliferation- and cell death-associated genes differentially acetylated during normal early regeneration (**Table 2**), SAHA reversed PH-induced changes in liver histone acetylation of 17 that were normally deacetylated and 12 that were hyperacetylated (**Table 4**). Similarly, evaluation of the impact of SAHA on regenerative histone hyper- and de-acetylation of the over-connected genes identified by the interactome analyses (**Figs. 2A, B**) showed that Zn-HDAC inhibition disrupts PH-induced effects on acetylation of many of the most over-connected hyperacetylated (e.g., *Ahr*, *Egfr*, *Myc*, *Pik3r1*, and *Tcf7l2*) and deacetylated (e.g., *ChREBP/Mxlpil*, *Dbp*, *Foxo1*, *Foxo3*, *Nr3c1*, *Srebf1*)) genes (Supplementary **Table 4**). Taken together, these data show that SAHA-mediated Zn-HDAC inhibition reverses regenerative changes in both liver histone de- and hyperacetylation, and they identify several specific targets of such regulation. Examples of gene-specific, SAHA-dependent disruption of PH-induced changes in liver histone acetylation are illustrated in **Figure 3**.

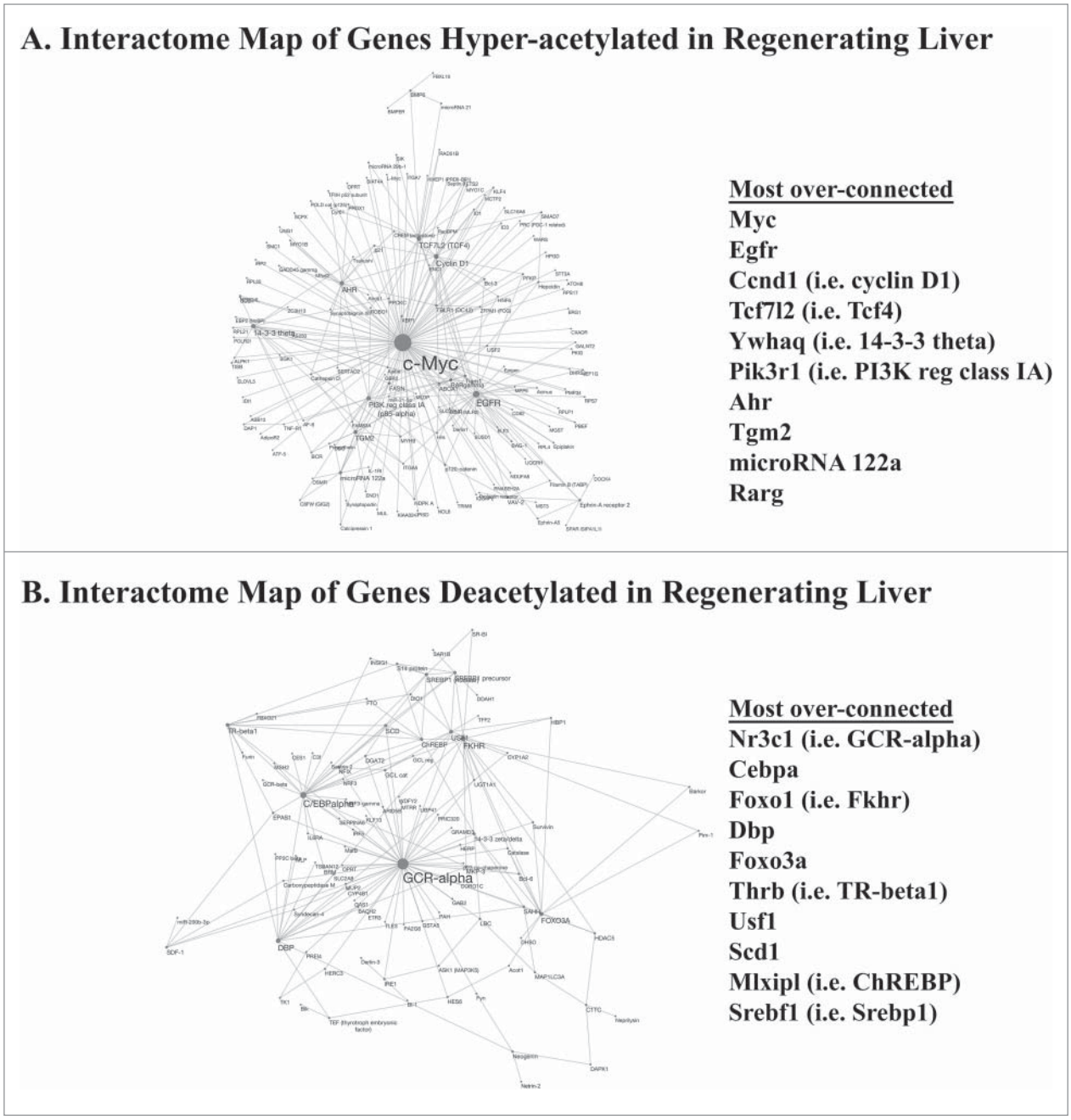

Figure 2. Interactome Plots of Differentially Acetylated Genes in Early Regenerating Liver. Patterns of biological interaction between (**A**) hyper- or (**B**) de-acetylated genes in regenerating vs. sham-operated liver are illustrated (with interactions identified using MetaCore™ from GeneGo and images generated using Cytoscape version 3.1.1 (http://cytoscape.org)). Functional interactions are indicated by lines between designated genes, with increased connectivity represented by increased node size. The most over-connected genes in each set are listed.

Examination of the concordance between histone acetylation and mRNA expression in SAHA- vs. vehicle-treated regenerating liver

Finally, the correlation between (a) SAHA-mediated reversal of regenerative changes in liver histone acetylation and (b) corresponding changes in mRNA expression of the cell cycle and cell death-associated genes listed in **Table 4** was assessed. This analysis defined the subset of these genes that demonstrate concordant changes in histone acetylation (i.e., hyper- or de-acetylation) and mRNA expression (i.e., increased or decreased expression, respectively). For example, the anti-proliferative/pro-apoptotic genes *Foxo3*, *Bik*, and *Bmf*, whose liver histone acetylation (**Fig. 1**) and corresponding mRNA expression (**Fig. 4A** and [13]) are normally suppressed during early regeneration, were each hyperacetylated and induced by SAHA 12 hours after PH (**Figs. 3**, **4**, **Table 4**). Hepatic expression of several of the other cell cycle- and cell

Table 3. Summary of genes differentially acetylated 12 h after PH by SAHA vs. Vehicle (FDR<0.1)

Effect of SAHA	Total (unique) genes[1]	Cell proliferation- and cell death-associated GO terms enriched for[2] differentially acetylated genes (FDR<0.1)	Number of genes linked to[3]: Cell proliferation[4]	Cell Death[4]
Decreased Acetylation	1198 (1162)	none	38	36
Increased Acetylation	1257 (1229)	pathways in cancer, programmed cell death, regulation of apoptosis, apoptosis, regulation of programmed cell death, regulation of cell death, cell cycle control, colorectal cancer, cell death, non-small cell lung cancer, oncogenesis, death, positive regulation of apoptosis, apoptosis, positive regulation of programmed cell death, positive regulation of cell death, cell cycle, cell proliferation and differentiation	227	99

[1]In SAHA- vs. vehicle-treated replicates.
[2]Identified using DAVID bioinformatics database (see references 11–12) from GOTERM_BP_ALL, PANTHER_BP_ALL, KEGG_PATHWAY, PANTHER_PATHWAY on significantly de- or hyper-acetylated genes, respectively.
[3]Based on examination of all terms into which DAVID sorted differentially acetylated genes.
[4]See Supplementary Table 3 for specific genes.

death-associated genes deacetylated during normal regeneration, including *Bcar1, Ern1, Jmy,* and *Traf4,* were also induced but that of others, including *Csk*, *Ddah1*, *Dpm1*, *Foxo1*, *Gclc*, *Ndfip1*, *Nr3c1*, *Sesn2, and Tmbim6,* were not significantly affected by SAHA at this time point (**Table 4** and Supplementary **Fig. 3**). Conversely, *Myc*, which is normally hyperacetylated (**Fig. 1**) and induced (**Fig. 4A** and [17]) by PH, was deacetylated and suppressed by SAHA (**Figs. 3**, **4**, **Table 4**), but expression of other cell cycle-associated genes normally hyperacetylated in regenerating liver, including *Adipor2*, *Dap*, *Egfr*, *Gadd45g*, *Igf1*, *Lcn2*, *Nlrp12*, *Pik3r1*, *Prox1*, *Sik1*, and *Tcf7l2,* were not significantly altered by SAHA (**Table 4** and Supplementary **Fig. 3**). Thus, SAHA causes coincidental, concordant effects on the expression of some but not all genes whose PH-induced regulation of histone acetylation is disrupted by this anti-regenerative Zn-HDAC inhibitor. These effects include disruption of both the hyperacetylation and induction of mRNA expression of *Myc* and the deacetylation and suppression of expression of *Foxo3, Bik*, and *Bmf* in early regenerating liver (**Fig. 4A**).

Discussion

We recently described the regulation and functional importance of Zn-HDACs during experimental liver regeneration.[2] Based on those findings, we undertook the analyses here to characterize regenerative patterns of change in liver histone acetylation during early regeneration and determine the effect of Zn-HDAC inhibition on such patterns. To our knowledge, this is the first report to ever describe the results of acetyL-histone ChIP-Seq analyses of experimental liver regeneration. Those results define distinct, gene-specific patterns of pro-regenerative hyper- and de-acetylation in liver after PH. Several of the hyperacetylated genes are known to be induced or activated during or to promote liver regeneration (**Table 2**), with others reported to promote cell proliferation in other settings but not yet investigated in regenerating liver. Of note, *Cdkn1a*, which is hyperacetylated 12 hours after PH (**Fig. 1**), encodes a CDKI whose expression is also known to be up regulated during early liver regeneration.[7] Similarly, many of the genes identified as deacetylated are known to be downregulated during liver regeneration and to suppress cell proliferation during regeneration or in other models (**Table 2**). Based on these findings, studies to evaluate the time course of patterns of change in liver histone acetylation throughout experimental regeneration should now be conducted both to define the dynamic nature of such epigenetic regulation and as a prelude to investigating whether disruption of such regulation mediates impaired regeneration in SAHA-treated and other experimental models. Ultimately, those efforts could inform consideration of the importance of epigenetic regulation of liver regeneration in human liver diseases.

The data reported here show that an anti-regenerative treatment regimen of SAHA reverses PH-induced changes in both the deacetylation of anti-proliferative and pro-apoptotic genes and the hyperacetylation of pro-regenerative genes (**Table 4**). While the former result (SAHA-dependent gene-specific hyperacetylation) could directly result from the Zn-HDAC inhibitory activity of SAHA, the latter must occur by other indirect mechanisms. One possibility is that SAHA might directly promote hyperacetylation and thereby prevent transcriptional downregulation of specific Zn-HDAC isoforms that are normally suppressed during early regeneration (and relatively resistant to SAHA-mediated inhibition). With this in mind, it is intriguing that HDAC11 expression is downregulated during normal liver regeneration [2] and that the studies here show that SAHA induces HDAC11 hyperacetylation in regenerating liver (Supplementary **Tables 2, 3**). Zn-HDAC inhibition might also promote hyperacetylation and induce the expression of other SAHA-resistant HDACs, e.g.,

TABLE 4. Cell-Proliferation & Cell-Death Associated Genes[1] with PH-Induced Changes in Histone Acetylation that are Reversed by SAHA

Hyper-acetylated by PH and Deacetylated by SAHA[2]					Deacetylated by PH and Hyper-acetylated by SAHA[2]				
Gene[3]	**FDR[4] PH/Sham**	**FDR[5] SAHA/Veh**	**Effect of PH on Expression[6]**	**Effect of SAHA on Expression[6]**	**Gene[3]**	**FDR[4] PH/Sham**	**FDR[5] SAHA/Veh**	**Effect of PH on Expression[6]**	**Effect of SAHA on Expression[6]**
Adipor2	0.04	0.2	no effect	no effect	Bcar1	0.01	<0.001	no effect	increased
Dap	0.04	<0.001	decreased	no effect	Bik	0.04	<0.001	decreased	increased
Egfr	0.04	0.2	no effect	no effect	Bmf	<0.001	<0.001	decreased	increased
Gadd45g	<0.001	<0.001	increased	no effect	Csk	0.05	<0.001	no effect	no effect
Igf1	0.05	0.009	no effect	no effect	Ddah1	0.09	<0.001	no effect	no effect
Lcn2	<0.001	0.003	increased	no effect	Dpm1	0.09	0.02	no effect	no effect
Myc	0.03	0.02	increased	decreased	Ern1	0.08	0.1	no effect	increased
Nlrp12	<0.001	0.002	increased	no effect	**Foxo1**	0.03	0.2	no effect	no effect
Pik3r1	0.03	0.2	increased	no effect	**Foxo3***	0.06	0.04	decreased	increased
Prox1	<0.001	<0.001	no effect	no effect	Gclc	0.08	<0.001	no effect	no effect
Sik1	<0.001	<0.001	increased	no effect	H2-K1	0.03	<0.001	increased	no effect
Tcf7l2	0.09	0.06	no effect	no effect	Jmy	0.05	0.1	no effect	increased
					Ndfip1	<0.001	0.03	decreased	no effect
					Nr3c1	0.006	<0.001	decreased	no effect*
					Sesn2	0.002	0.003	no effect	no effect
					Tmbim6	0.02	0.006	decreased	no effect
					Traf4	0.09	0.009	no effect	increased

[1]Identified using DAVID bioinformatics database [2]as described in text; [3]Listed by "Official gene symbol" (genes in bold are over-connected in the interactome analysis in Figures 2A-B). See [4]Supplementary Table 1 and [5]Supplementary Table 2. [6]Using $p < 0.05$; see Figure 4A and Supplementary Figure 3.

NAD-dependent sirtuins. Interestingly, a recent report demonstrated the anti-regenerative activity of the sirtuin Sirt1,[18] and the data reported here show *Sirt1* is also hyperacetylated by SAHA during early regeneration (Supplementary **Tables 2, 3**). Based on these considerations, future analyses should examine whether induction of HDAC11 or Sirt1 contribute to SAHA's anti-regenerative activity, and, if so, attempt to define the specific mechanisms that mediate this effect.

To begin to investigate the functional consequences of SAHA-dependent inhibition of PH-induced changes in liver histone acetylation, we compared gene-specific patterns of such change to corresponding effects on hepatic mRNA expression. The results showed concordant effects of PH vs. sham surgery and SAHA vs. vehicle administration on alterations in histone acetylation and mRNA expression of Myc (whose PH-induced hyperacetylation and induction is suppressed by SAHA; **Figs. 1, 3, 4**) and Foxo3 (whose deacetylation and suppression of expression is reversed by SAHA; **Figs. 1, 3, 4**). SAHA also prevented the PH-induced deacetylation and suppression of Bik and Bmf (**Figs. 3, 4**), which, like Foxo3, promote apoptosis. Interestingly, Myc has been reported to antagonize the anti-proliferative/pro-apoptotic activity of Foxo3[19,20] and, similarly, Foxo3 to disrupt Myc function [21] in various models. A recent study reported that Myc might be dispensable for recovery of liver mass after PH; however, that report also showed a reduction in PH-induced hepatocellular proliferation in liver-specific Myc null mice.[22] Nevertheless, the data reported here suggest that a Myc/Foxo3 switch is epigenetically regulated during normal liver regeneration, and that disruption of such regulation might contribute to impaired regeneration in SAHA-treated animals (**Fig. 4B**).

Finally, the findings here have provocative implications with respect to our own and other previous studies identifying alterations in metabolism that occur in response to hepatic insufficiency as the source of essential signals that promote liver regeneration.[23] Although the specific molecular mechanisms that couple metabolism to liver regeneration require further elucidation, several indirect observations suggest epigenetic regulation of histone acetylation as an attractive candidate. For example, supplemental glucose affects patterns of histone acetylation in cell culture,[24-26] PH-induced hypoglycemia occurs during experimental liver regeneration, and glucose supplementation inhibits regeneration.[7,23,27] In addition, HDAC5 undergoes PH-induced nuclear localization in regenerating liver,[2] and this Zn-HDAC also exhibits hypoglycemia-induced nuclear localization and regulates FOXO target gene expression in other models.[28] Finally, recent studies suggest that specific metabolites modulate isoform-specific HDAC activity in vivo.[29] Together, these considerations suggest that investigating patterns of liver histone acetylation in experimental models in which PH-induced alterations in metabolism are disrupted and regeneration is impaired could further elucidate epigenetic mechanisms linking metabolism and established pro-regenerative signaling pathways to liver regeneration. The long-term goal of such effort should be translation of the findings into clinical trials investigating metabolic strategies with which to promote hepatic regeneration and thereby improve patient outcomes in acute and chronic human liver diseases.

Materials and Methods

Animal husbandry and surgery

PH or sham surgery was performed on mice as described in Supplementary Material and previously.[5,7,30-32] Some mice were treated with SAHA (or vehicle control) as previously described.[2]

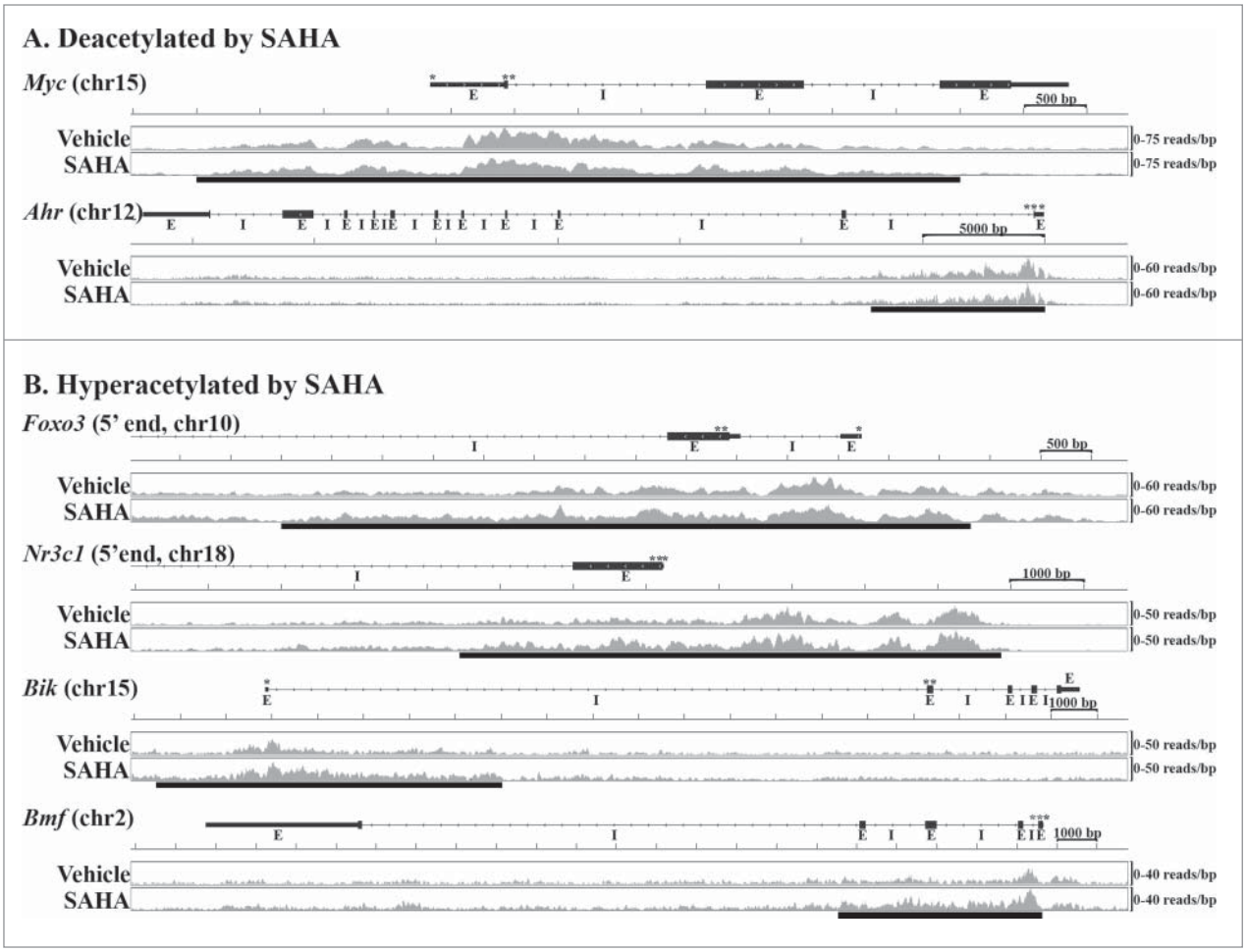

Figure 3. The Influence of SAHA on Histone Acetylation in Early Regenerating Liver. Examples of gene-specific patterns of histone acetylation in regenerating liver from animals treated with SAHA or vehicle control are shown for specific genes identified as (**A**) deacetylated (*Myc*, *Ahr*) or (**B**) hyperacetylated (*Foxo3*, *Nr3c1*, *Bik, Bmf*) 12 hours after PH. UCSC gene maps (transcription (*) and translation (**) start sites, exons (E), and introns (I) as designated) are aligned with abundance of immunoprecipitated sequence (sequence reads per bp with scale indicated to the right) integrated from livers of 4 replicates each treated with SAHA or vehicle. Sequence abundance images were generated as in **Figure 1**, and bars below these data indicate specific sequence(s) identified as differentially acetylated.

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 revised 1985).

Ac-H3K9 ChIP-Seq

Ac-H3K9 ChIP of livers from n = 3-4 replicates in each experimental group was performed as described in Supplementary Material and previously.[2] DNA recovered from the acetyL-histone enriched chromatin and the corresponding input samples were submitted to the Washington University Genome Technology Access Center for blunt ending, adaptor ligation, size selection, and amplification according to established protocols. These libraries were sequenced using the Illumina HiSeq-2500 as single 50 bp reads. Raw data were de-multiplexed and aligned to the most recent mouse reference genome assembly using Novoalign (Novocraft; Selangor, Malaysia). Sequence peaks were identified using MACS software.[33] Determination of significant differences in the abundance of peak sequences between experimental groups was performed with DiffBind, an open source Bioconductor package that utilizes edgeR software for statistical analysis of replicated sequence count data.[34,35] Genes identified as differentially acetylated were analyzed by gene ontology (GO) and other classification schema using the DAVID bioinformatics database (http://david.abcc.ncifcrf.gov/.[36,37]). Patterns of interaction between differentially acetylated genes within experimental groups (i.e., *interactome* analyses) were examined using MetaCore^{TM} from GeneGo (Thomson Reuters).

With respect to the analyses described above, Benjamini and Hochberg false discovery rate (FDR) thresholds (q values) were employed to either (a) identify DNA sequences (and corresponding genes) as significantly differentially-acetylated between replicates of regenerating vs. sham-operated- or vehicle- vs. SAHA-treated regenerating-liver (using the data analysis pipeline

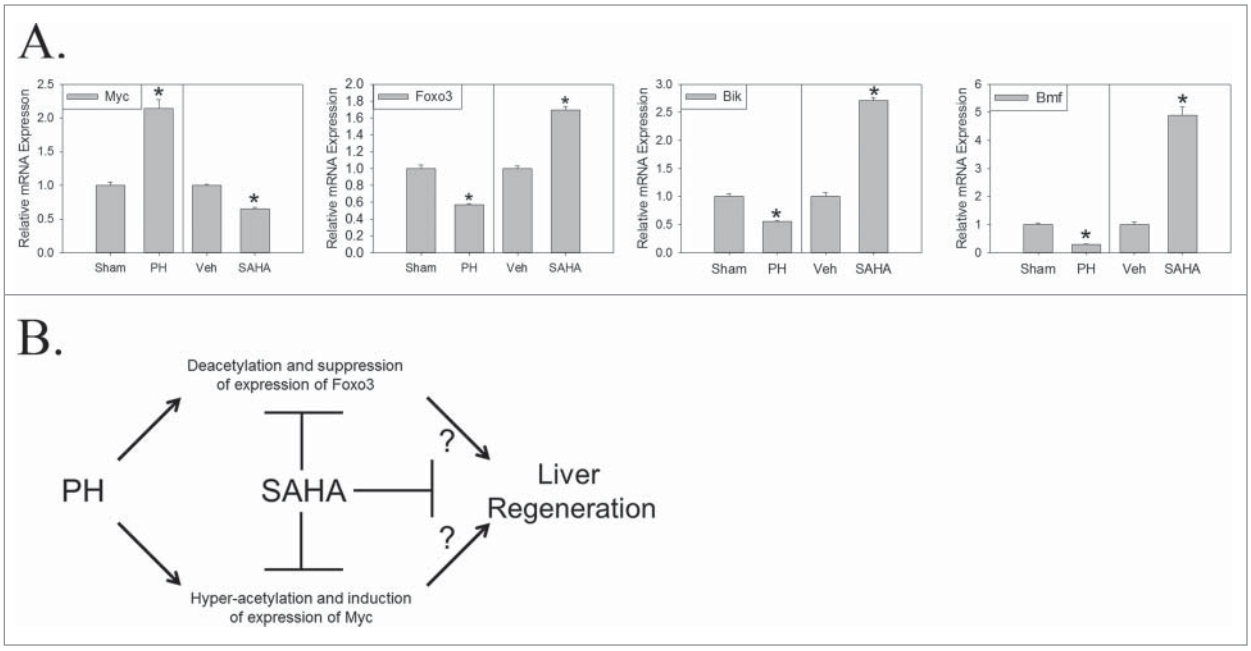

Figure 4. Hepatic mRNA Expression of Differentially Acetylated Genes during and Model of Epigenetic Regulation of Liver Regeneration. (**A**) Relative mRNA expression (± standard error) 12 hours after PH or sham surgery (indexed to sham; *$P < 0.05$ for PH vs. sham; n = 6 replicates per experimental group) or 12 hours after PH in vehicle- (Veh) or SAHA-treated mice (indexed to Veh; *$P < 0.05$ for SAHA vs. vehicle; n = 6 replicates per experimental group). (**B**) Proposed model of epigenetically regulated Myc/Foxo3 switch in regenerating liver (known positive (→) and negative (—|) regulation and hypothesized (?) regulation as indicated; see text for additional discussion).

described above) or (b) identify gene term categories, using the DAVID bioinformatics database and the ontology classification schema enumerated in the Tables, in which genes identified as differentially acetylated in (a) were significantly enriched. Thus, **Tables 1 and 3** list the number of genes (in column 2) identified as significantly hyper- or de-acetylated in regenerating vs. sham-operated (or vehicle- vs. SAHA-treated) liver using an FDR threshold of $q<0.1$, and the ontology terms (column 3) identified by DAVID as significantly enriched for those genes, also using $q<0.1$. Supplementary **Tables 1 and 2** list the specific FDR q values calculated for each sequence (and associated gene) on which the differential acetylation analysis was performed. The FDR q values in **Table 4** (columns 2 and 3) are also listed in Supplementary **Tables 1, 2**, respectively. In Supplementary **Tables 3, 4**, the FDR thresholds refer to the comparison described above in (a) for identification of sequences (and corresponding genes) as significantly hyper- or de-acetylated between replicates of experimental groups (i.e., regenerating vs. sham-operated- and/or vehicle- vs. SAHA-treated) as described.

qRT-PCR: Hepatic mRNA expression in livers from n = 6 animals in each experimental group was determined using semi-quantitative real-time reverse-transcription polymerase-chain-reaction (qRT-PCR) as described in Supplementary Material and previously.[5,7,31,38,39] Gene-specific oligonucleotide primers are listed in Supplementary Material.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Rudnick DA. Liver regeneration: the developmental biologists approach. In: Orlando G, Lerut JP, Soker S, Stratta RJ, eds. Regenerative Medicine Applications in Organ Transplantation. 1st ed. Waltham, MA: ElsevierAcademic Press, 2014, 353-74.
2. Huang J, Barr E, Rudnick DA. Characterization of the regulation and function of zinc-dependent histone deacetylases during rodent liver regeneration. Hepatology 2013 May; 57(5):1742-51; http://dx.doi.org/10.1002/hep.26206
3. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J Roy Stat Soc B 1995; 57:289-300
4. Liao Y, Shikapwashya ON, Shteyer E, Dieckgraefe BK, Hruz PW, Rudnick DA. Delayed hepatocellular mitotic progression and impaired liver regeneration in early growth response-1-deficient mice. J Biol Chem 2004 Oct 8; 279(41):43107-16; PMID:15265859; http://dx.doi.org/10.1074/jbc.M407969200
5. Shteyer E, Liao Y, Muglia LJ, Hruz PW, Rudnick DA. Disruption of hepatic adipogenesis is associated with impaired liver regeneration in mice. Hepatology 2004

Dec; 40(6):1322-32; PMID:15565660; http://dx.doi.org/10.1002/hep.20462
6. Kienzl-Wagner K, Moschen AR, Geiger S, Bichler A, Aigner F, Brandacher G, Pratschke J, Tilg H. The role of lipocalin-2 in liver regeneration. Liver Int 2014 Jul 10; PMID:25040147; http://dx.doi.org/10.1111/liv.12634
7. Weymann A, Hartman E, Gazit V, Wang C, Glauber M, Turmelle Y, Rudnick DA. p21 is required for dextrose-mediated inhibition of mouse liver regeneration. Hepatology 2009 Mar 19; 50:207-15; PMID:19441104; http://dx.doi.org/10.1002/hep.22979
8. Nelsen CJ, Rickheim DG, Tucker MM, Hansen LK, Albrecht JH. Evidence that cyclin D1 mediates both growth and proliferation downstream of TOR in hepatocytes. J Biol Chem 2003 Feb 7; 278(6):3656-63; PMID:12446670; http://dx.doi.org/10.1074/jbc.M209374200
9. Moreno-Carranza B, Goya-Arce M, Vega C, Adan N, Triebel J, Lopez-Barrera F, Quintanar-Stéphano A, Binart N, Martínez de la Escalera G, Clapp C. Prolactin promotes normal liver growth, survival, and regeneration in rodents: effects on hepatic IL-6, suppressor of cytokine signaling-3, and angiogenesis. Am J Physiol Regul Integr Comp Physiol 2013 Oct 1; 305(7):R720-6; PMID:23948778; http://dx.doi.org/10.1152/ajpregu.00282.2013
10. Paranjpe S, Bowen WC, Tseng GC, Luo JH, Orr A, Michalopoulos GK. RNA interference against hepatic epidermal growth factor receptor has suppressive effects on liver regeneration in rats. Am J Pathol 2010 Jun; 176(6):2669-81; PMID:20395437; http://dx.doi.org/10.2353/ajpath.2010.090605
11. Natarajan A, Wagner B, Sibilia M. The EGF receptor is required for efficient liver regeneration. Proc Natl Acad Sci U S A 2007 Oct 23; 104(43):17081-6; PMID:17940036; http://dx.doi.org/10.1073/pnas.0704126104
12. Monga SP, Pediaditakis P, Mule K, Stolz DB, Michalopoulos GK. Changes in WNTbeta-catenin pathway during regulated growth in rat liver regeneration. Hepatology 2001 May; 33(5):1098-109; PMID:11343237; http://dx.doi.org/10.1053/jhep.2001.23786
13. Kurinna S, Stratton SA, Tsai WW, Akdemir KC, Gu W, Singh P, Goode T, Darlington GJ, Barton MC. Direct activation of forkhead box O3 by tumor suppressors p53 and p73 is disrupted during liver regeneration in mice. Hepatology 2010 Sep; 52(3):1023-32; PMID:20564353; http://dx.doi.org/10.1002/hep.23746
14. Kurinna S, Stratton SA, Coban Z, Schumacher JM, Grompe M, Duncan AW, Barton MC. p53 regulates a mitotic transcription program and determines ploidy in normal mouse liver. Hepatology 2013 May; 57(5):2004-13; PMID:23300120; http://dx.doi.org/10.1002/hep.26233
15. Sacilotto N, Espert A, Castillo J, Franco L, Lopez-Rodas G. Epigenetic transcriptional regulation of the growth arrest-specific gene 1 (Gas1) in hepatic cell proliferation at mononucleosomal resolution. PLoS One 2011; 6(8):e23318; PMID:21858068; http://dx.doi.org/10.1371/journal.pone.0023318
16. Jin J, Hong IH, Lewis K, Iakova P, Breaux M, Jiang Y, Sullivan E, Jawanmardi N, Timchenko L, Timchenko NA. Cooperation of CEBP family proteins and chromatin remodeling proteins is essential for termination of liver regeneration. Hepatology 2014 Jul 9; PMID:25043739; http://dx.doi.org/10.1002/hep.27295
17. Fausto N, Webber EM. Control of liver growth. Crit Rev Eukaryot Gene Expr 1993; 3(2):117-35; PMID:8324292
18. Garcia-Rodriguez JL, Barbier-Torres L, Fernandez-Alvarez S, Juan VG, Monte MJ, Halilbasic E, et al. SIRT1 controls liver regeneration by regulating BA metabolism through FXR and mTOR signaling. Hepatology 2013 Dec 12; 59:1972-83; PMID:24338587; http://dx.doi.org/10.1002/hep.26971
19. Chandramohan V, Mineva ND, Burke B, Jeay S, Wu M, Shen J, Yang W, Hann SR, Sonenshein GE. c-Myc represses FOXO3a-mediated transcription of the gene encoding the p27(Kip1) cyclin dependent kinase inhibitor. J Cell Biochem 2008 Aug 15; 104(6):2091-106; PMID:18393360; http://dx.doi.org/10.1002/jcb.21765
20. Chandramohan V, Jeay S, Pianetti S, Sonenshein GE. Reciprocal control of Forkhead box O 3a and c-Myc via the phosphatidylinositol 3-kinase pathway coordinately regulates p27Kip1 levels. J Immunol 2004 May 1; 172(9):5522-7; PMID:15100294; http://dx.doi.org/10.4049/jimmunol.172.9.5522
21. Peck B, Ferber EC, Schulze A. Antagonism between FOXO and MYC regulates cellular powerhouse. Front Oncol 2013; 3:96; PMID:23630664 ; http://dx.doi.org/10.3389/fonc.2013.00096
22. Sanders JA, Schorl C, Patel A, Sedivy JM, Gruppuso PA. Postnatal liver growth and regeneration are independent of c-myc in a mouse model of conditional hepatic c-myc deletion. BMC Physiol 2012; 12:1; PMID:22397685; http://dx.doi.org/10.1186/1472-6793-12-1
23. Huang J, Rudnick DA. Elucidating the metabolic regulation of liver regeneration. Am J Pathol 2014 Feb; 184(2):309-21; PMID:24139945; http://dx.doi.org/10.1016/j.ajpath.2013.04.034
24. Zhao S, Xu W, Jiang W, Yu W, Lin Y, Zhang T, Yao J, Zhou L, Zeng Y, Li H, et al. Regulation of cellular metabolism by protein lysine acetylation. Science 2010 Feb 19; 327(5968):1000-4; PMID:20167786; http://dx.doi.org/10.1126/science.1179689
25. Wellen KE, Hatzivassiliou G, Sachdeva UM, Bui TV, Cross JR, Thompson CB. ATP-citrate lyase links cellular metabolism to histone acetylation. Science 2009 May 22; 324(5930):1076-80; PMID:19461003; http://dx.doi.org/10.1126/science.1164097
26. Rathmell JC, Newgard CB. Biochemistry. A glucose-to-gene link. Science 2009 May 22; 324(5930):1021-2; PMID:19460991; http://dx.doi.org/10.1126/science.1174665
27. Rudnick DA, Davidson NO. Functional relationships between lipid metabolism and liver regeneration. Int J Hepatol 2012 Jan 26; 2012:549241; PMID:22319652; http://dx.doi.org/10.1155/2012/549241
28. Mihaylova MM, Vasquez DS, Ravnskjaer K, Denechaud PD, Yu RT, Alvarez JG, Downes M, Evans RM, Montminy M, Shaw RJ. Class IIa histone deacetylases are hormone-activated regulators of FOXO and mammalian glucose homeostasis. Cell 2011 May 13; 145(4):607-21; PMID:21565617; http://dx.doi.org/10.1016/j.cell.2011.03.043
29. Shimazu T, Hirschey MD, Newman J, He W, Shirakawa K, Le Moan N, Grueter CA, Lim H, Saunders LR, Stevens RD, et al. Suppression of oxidative stress by beta-hydroxybutyrate, an endogenous histone deacetylase inhibitor. Science 2013 Jan 11; 339(6116):211-4; PMID:23223453; http://dx.doi.org/10.1126/science.1227166
30. Huang J, Glauber M, Qiu Z, Gazit V, Dietzen DJ, Rudnick DA. The influence of skeletal muscle on the regulation of liver:body mass and liver regeneration. Am J Pathol 2012 Feb; 180(2):575-82; PMID:22155110; http://dx.doi.org/10.1016/j.ajpath.2011.10.032
31. Gazit V, Weymann A, Hartman E, Finck BN, Hruz PW, Tzekov A, Rudnick DA. Liver regeneration is impaired in lipodystrophic fatty liver dystrophy mice. Hepatology 2010 Dec; 52(6):2109-17; PMID:20967828; http://dx.doi.org/10.1002/hep.23920
32. Gazit V, Huang J, Weymann A, Rudnick DA. Analysis of the role of hepatic PPARgamma expression during mouse liver regeneration. Hepatology 2012 Jun 18; 56:1489-98; PMID:22707117; http://dx.doi.org/10.1002/hep.25880
33. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W, et al. Model-based analysis of ChIP-Seq (MACS). Genome Biol 2008; 9(9):R137; PMID:18798982 ; http://dx.doi.org/10.1186/gb-2008-9-9-r137
34. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 2010 Jan 1; 26(1):139-40; PMID:19910308; http://dx.doi.org/10.1093/bioinformatics/btp616
35. Ross-Innes CS, Stark R, Teschendorff AE, Holmes KA, Ali HR, Dunning MJ, Brown GD, Gojis O, Ellis IO, Green AR, et al. Differential oestrogen receptor binding is associated with clinical outcome in breast cancer. Nature 2012 Jan 19; 481(7381):389-93; PMID:22217937; http://dx.doi.org/10.1038/nature10730
36. Huang dW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 2009; 4(1):44-57; PMID:19131956
37. Huang dW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 2009 Jan; 37(1):1-13.
38. Turmelle YP, Shikapwashya O, Tu S, Hruz PW, Yan Q, Rudnick DA. Rosiglitazone inhibits mouse liver regeneration. FASEB J 2006; 20:2609-11; PMID:17077279; http://dx.doi.org/10.1096/fj.06-6511fje
39. Clark A, Weymann A, Hartman E, Turmelle Y, Carroll M, Thurman JM, Holers VM, Hourcade DE, Rudnick DA. Evidence for non-traditional activation of complement factor C3 during murine liver regeneration. Mol Immunol 2008 Jun; 45(11):3125-32; PMID:18452991; http://dx.doi.org/10.1016/j.molimm.2008.03.008
40. Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Lander ES, Getz G, Mesirov JP. Integrative genomics viewer. Nat Biotechnol 2011 Jan; 29(1):24-6; PMID:21221095; http://dx.doi.org/10.1038/nbt.1754
41. Thorvaldsdottir H, Robinson JT, Mesirov JP. Integrative genomics viewer (IGV): high-performance genomics data visualization and exploration. Brief Bioinform 2013 Mar; 14(2):178-92.