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Requirement of a Novel Splicing Variant of Human Histone Deacetylase 6 for TGF- β 1-mediated Gene Activation

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Abstract

Histone deacetylase 6 (HDAC6) belongs to the family of class IIb HDACs and predominantly deacetylates non-histone proteins in the cytoplasm via the C-terminal deacetylase domain of its two tandem deacetylase domains. HDAC6 modulates fundamental cellular processes via deacetylation of α -tubulin, cortactin, molecular chaperones, and other peptides. Our previous study indicates that HDAC6 mediates TGF- β 1-induced epithelial-mesenchymal transition (EMT) in A549 cells. In the current study, we identify a novel splicing variant of human HDAC6, hHDAC6p114. The hHDAC6p114 mRNA arises from incomplete splicing and encodes a truncated isoform of the hHDAC6p114 protein of 114 kD when compared to the major isoform hHDAC6p131. The hHDAC6p114 protein lacks the first 152 amino acids from N-terminus in the hHDAC6p131 protein, which harbors a nuclear export signal peptide and 76 amino acids of the N-terminal deacetylase domain. hHDAC6p114 is intact in its deacetylase activity against α -tubulin. The expression hHDAC6p114 is elevated in a MCF-7 derivative that exhibits an EMT-like phenotype. Moreover, hHDAC6p114 is required for TGF- β 1-activated gene expression associated with EMT in A549 cells. Taken together, our results implicate that expression and function of hHDAC6p114 is differentially regulated when compared to hHDAC6p131.

Introduction

Substrate and functional diversity of lysine acetylation revealed by a proteomic survey highlights lysine acetylation as a key component of the signaling network that coordinates fundamental cellular processes [1]. Reversible and dynamic lysine acetylation is tightly controlled by HDAC and histone acetyltransferase. HDAC6 belongs to class IIb and predominantly deacetylates non-histone proteins in the cytoplasm [2]. Human HDAC6 harbors a unique SE14-repeat domain which is pivotal for the cytoplasmic retention [3]. Distinct from other HDACs, HDAC6 possesses two tandem HDAC domains and one ubiquitin binding motif (BUZ) at its C-terminus [4;5]. HDAC6 deacetylates α -tubulin and

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overexpression of HDAC6 causes hypoacetylation of α -tubulin as well as increased cell motility [6]. Moreover, HDAC6-mediated deacetylation of heat shock protein 90 facilitates its chaperone function for the glucocorticoid receptor [7]. HDAC6 binds polyubiquitinated proteins via its BUZ domain and regulates the cellular management of polyubiquitinated proteins [8]. The function of the nuclear HDAC6 which accounts for a minor portion of the total HDAC6 in the cell, remains largely unknown, although several reports suggest that the nuclear HDAC6 acts as a transcriptional co-repressor for transcription factors, such as Runx2 and sumoylated p300 [9;10]. Our previous report indicates that HDAC6 tubulin deacetylase activity is up-regulated post-translationally in TGF- β 1-induced epithelial-mesenchymal transition (EMT) in A549 cells [11]. This concept is supported by a previous study showing that the tubulin deacetylase activity of HDAC6 is up-regulated via Aurora A-mediated phosphorylation during cilium disassembly [12].

Splicing variants of human HDAC6 (hHDAC6) are predicted by high throughput and bioinformatic analyses (AceView Genes, <http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly>). The best characterized isoform, hHDAC6aApr07 (Figure 1A), encodes an hHDAC6 protein of a molecular mass around 131 kD (named hHDAC6p131 or p131 hereafter), although its apparent molecular mass on a SDS page gel is around 160 kD [3]. However, validation and characterization of other hHDAC6 isoforms have not been attempted. The current study identified a novel splicing variant of human HDAC6 that is named hHDAC6p114. Our findings suggest a critical role of hHDAC6p114 in the TGF- β 1-activated gene expression associated with EMT.

Materials and Methods

Reagents and Plasmids

Recombinant human TGF- β 1 was purchased from R&D Systems. 3TP-LUX, a firefly luciferase reporter, which contains the TGF- β 1 responsive elements in the PAI-1 promoter was a gift from Dr. J. Massague at Sloan-Kettering Institute. Ectopic expression of the hHDAC6p114 protein was accomplished using two different expression vectors. The cDNA clone that encodes the full-length hHDAC6p114 mRNA in the pAMP vector (GenBank, AL137696.1) was purchased from imaGenes and subcloned into pCDNA3 (Invitrogen). The expression vector is designated here as p114-f. The cDNA clone that encodes only the translated regions of the hHDAC6p114 mRNA (GenBank, BC013737.1) in the pCMV-SPORT6 vector was purchased from OpenBiosystems. The p131 expression vector in the pCDNA3 backbone was kindly provided by Dr. Tso-Pang Yao at Duke School of Medicine. The expression vector is designated here as p114-t. Integrated DNA Technologies provided the scrambled siRNAs and the hHDAC6p114-targeting siRNAs (sense, 5'-GUAGGAUUUGGUGUGGCUACUGC-3'; anti-sense, 5'-CUGCAGUAGCCACACCAAUCCUACUU-3').

Cell Culture and Exposure to TGF- β 1

A549 cells, a human lung adenocarcinoma cell line, were purchased from ATCC. A549 cells were cultured as previously described [11]. In the selected experiments, A549 cells were serum-starved in the medium containing 0.5% FBS for 24 hrs followed by exposure to TGF- β 1 (5 ng/ml) for 48 hrs. MCF-7 cells, (N variant), and its TNF-resistant derivative MCF-7TNR cells were cultured as previously [13]. The MCF-7TNR subpopulation was derived from the parental MCF-7 after chronic exposure to lethal dose of TNF- α and has acquired resistance to TNF- α -induced apoptosis.

Transient Transfection and Luciferase Reporter Assays

A549 cells were transfected with the HDAC6 expression vectors, or the reporter constructs (3TP-LUX and RL-TK), and/or siRNAs using Lipofectamine 2000 (Invitrogen) as previously described [14]. In the selected transfections, A549 cells were exposed to TGF- β 1 as described above. Total cell RNA and protein were extracted to examine expression of HDAC6 and the TGF- β 1-activated genes. For luciferase reporter assays, the treated cells were harvested and assayed for luciferase activity using the Dual-Luciferase Reporter Assay Systems (Promega). Ratios of firefly luciferase activity from 3TP-LUX vs renilla luciferase activity from the co-transfected pRL-TK (Promega) were compared across the treated groups.

RT-PCR

Two pairs of primers were designed to amplify and reveal the unique intron-converted-exon region in p114 as depicted in Figure 1A. Quantitative RT-PCR (qRT-PCR) was performed to determine the mRNA levels of the hHDAC6 isoforms and the TGF- β 1-regulated genes as previously described [14]. The sequences for each primer were listed in Supplementary Materials Table 1. The ratios of each mRNA vs that of the housekeeping gene 36B4 were obtained and compared across the treated groups.

Immunoblots

Total cell protein was extracted and immunoblotted for the proteins of interest as previously described [14]. The following primary antibodies were used for immunoblots: rabbit polyclonal antibodies specific for HDAC6 (H-300, Santa Cruz Biotechnology); and for α -tubulin (CS-2144, Cell Signaling Technology); a mouse monoclonal antibody specific for acetylated α -tubulin (6-11B-1, Sigma-Aldrich). The secondary antibodies (IRDye800 or IRDye700 conjugated anti-rabbit or anti-mouse IgG) were detected using an Odyssey Infrared Imaging System (LiCor Biosciences). The ratios of the proteins of interest over the α -tubulin loading control were determined by densitometry using ImageJ (NIH) and compared across the treated groups.

Statistical Analysis

The results were obtained from at least three independent experiments and presented as mean $\pm$ standard deviation. Statistical evaluation was performed among individual groups using the unpaired two-tailed Student's T-test (GraphPad Prism, Version 5). A P value of less than 0.05 was considered significant.

Results

The AceView Genes predicts 21 splicing variants of human HDAC6 (<http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?db=human&l=HDAC6>). hHDAC6bApr07, a splicing variant that is predicted to produce a protein of 114 kD is the focus of the current study and designated as hHDAC6p114 or p114 hereafter. As predicted by the AceView Genes, the p114 mRNA is transcribed from the hHDAC6 gene via incomplete splicing which results in a unique exon-6 that consists of exon-6, exon-7, and intron-6 located between exon-6 and -7 in p131 (Figure 1A). To confirm cellular expression of the p114 mRNA, we designed a pair of primers that amplify the mRNA region spanning from exon-2 to exon-7 in p131 and from exon-2 to exon-6 in p114. As illustrated in Figure 1A, PCR using this pair of primers is predicted to yield a product of 644 bp in p131 and a product of 1340 bp in p114. For the HDAC6 gene or the HDAC6 pre-mRNA, a product of 3,872 bp is predicted because of inclusion of the introns 2-5. The PCR products corresponding to p131 and p114, but not to the HDAC6 gene or the HDAC6 pre-mRNA,

were amplified in both A549 and MCF-7 cells (Figure 1B). The p114-specific amplicon appeared to be much less abundant than the p131-specific PCR amplicon. PCR of reverse transcriptase minus control failed to yield either PCR product, which confirmed the PCR products were indeed derived from the mRNA template rather than from trace genomic DNA in the RNA extraction (Figure 1B). To further confirm expression of the p114 mRNA, we used a second pair of primers to specifically amplify the unique intron-converted-exon region. We recovered the 1344 bp product and used the purified amplicon as a template to amplify the p114 unique intron-converted-exon region as depicted in Figure 1A. Indeed, the p114-specific 198 bp PCR product was amplified in the 1344 bp PCR amplicon from A549 and MCF-7 cells (Figure 1C). The mRNA levels of hHDAC6p114 and hHDAC6p131 were further compared between MCF-7 and its derivative MCF-7TNR, which has acquired resistance to TNF- α -induced apoptosis and an EMT-like phenotype [13] (M.E. Burow, personal communication). When RT-PCR was carried out using a pair of non-selective primers for both HDAC6 isoforms, the mRNA levels of p114 and p131 combined (p114+p131) were significantly higher in MCF-7TNR than that in MCF-7 (0.0045 ± 0.0011 vs. 0.002 ± 0.0003 , arbitrary unit). The mRNA levels of p114 were also higher in MCF-7TNR than that in MCF-7 ($1.33535E-05 \pm 1.85012E-06$ vs. $2.07135E-06 \pm 7.66833E-07$, arbitrary unit). More importantly, p114 was elevated to a greater extent than p131 in MCF-7TNR as evidenced by the ratios of p114/(p114+p131) standing at 0.0031 ± 0.0009 in MCF-7TNR vs that at 0.001 ± 0.0002 in MCF-7 (Figure 1D). These findings demonstrated expression of the p114 mRNA in human cells.

As predicted by the AceView Genes, the p114 splicing variant is translated into a smaller protein than the p131 mRNA, despite harboring an additional intron-converted-exon region. The paradox is speculated to be caused by introduction of three in-frame stop codons that possibly favor alternative translation initiation starting at an internal AUG rather than the most 5' AUG utilized by translation of the p131 mRNA (Supplementary Materials Table 2). Thus, the p114 conceptual protein is expected to be a truncated isoform that lacks the N-terminal 152 amino acids when compared to the p131 protein (Figure 2A). The truncation results in the loss of a validated nuclear export signal (NES), and the N-terminal 76 amino acids of the first HDAC domain when compared to the p131 protein [3] (Figure 2A). To confirm expression of the p114 protein, we searched for additional protein bands that were recognized by an HDAC6-specific antibody in the protein lysates extracted from A549 cells in which HDAC6 expression was knocked down by RNAi [11]. The HDAC6 C-terminal region specific antibody recognized two major protein bands that were substantially reduced in the A549 cells when HDAC6 was knocked down by RNAi (Figure 2B). The larger protein band was the p131 protein although it displayed an apparent molecular mass of around 160 kD [3]. The identity of the larger protein band was further confirmed by our own experiments demonstrating that ectopic expression the p131 mRNA resulted in greater intensity of the larger protein band (Figure 2D). Because the RNAi utilized, by design, non-selectively targets both the p114 and the p131 mRNAs, we speculated that the smaller protein band might represent the p114 protein. To test this postulate, we transfected A549 cells with the siRNA that is specific for the unique intron-converted-exon in p114. The p114-specific siRNA substantially reduced intensity of the protein band right below the p131 protein (reduced from 9.52 ± 2.42 to 4.74 ± 2.31 , arbitrary unit), whereas the p131 protein exhibited only a modest decrease (reduced from 17 ± 2.1 to 13.5 ± 2.29) (Figure 2C). The modest decrease in the hHDAC6p131 protein may be attributed to the ability of p114-targeting siRNAs to hybridize with HDAC6 pre-mRNA that is a precursor to both p131 and p114. A similar disparate response to p114-targeting siRNA was observed at the mRNA levels of p114 and p131 (Figure 3B). Efficiency and selectivity of p114-targeting RNAi was further confirmed in MCF-7 cells (Figure 2C). Notably, selective knockdown of p114 was insufficient to cause hyperacetylation of α -tubulin (Figure 2C) whereas non-selective knockdown of both p114 and p131 resulted in a remarkable increase in acetylated α -tubulin

(Figure 2B). The differential response of acetylated α -tubulin to the two sets of siRNAs appeared to be proportional to relative abundance of the p131 and p114. We attempted a second approach to determine the identity of the smaller protein band by ectopic expression of the p114 mRNA. A549 cells were introduced with two p114 cDNAs, one harboring only the translated region (p114-t) and the other one harboring the full length (p114-f) as described in Materials and Methods. Transfection of either p114-t or p114-f yielded a robust increase in the protein band that was reduced by the p114-targeting RNAi (Figure 2, C & D). In contrast, transfection of the p131 expression vector yielded a robust increase in the larger protein band that corresponds to the endogenous p131 protein, although the increase was accompanied by a modest increase in the protein band that corresponds to p114 (Figure 2, B & D). Moreover, elevated expression of either p114 or p131 correlated with a substantial decrease in acetylated α -tubulin (Figure 2D). These findings confirmed the expression of the p114 protein in the cell although p114 is much less abundant relative to p131. The results further suggested active tubulin deacetylase activity in hHDAC6p114 despite truncation of the N-terminal deacetylase domain.

We have demonstrated the requirement of HDAC6 for TGF- β 1-induced expression of EMT markers [11]. However, the experimental approaches employed to inactivate HDAC6 in our previous work non-selectively targeted both p131 and p114. We set to use p114-targeting RNAi to determine the role for p114 in the expression of EMT markers induced by TGF- β 1. A549 cells were transiently transfected with either p114-targeting or scrambled siRNA followed by exposure to TGF- β 1. RNAi-mediated selective knockdown of p114 was confirmed by qRT-PCR and immunoblots for p114 and p131 (Figure 3). Consistent with the modest effect of p114-targeting siRNA on p131, selective knockdown of p114 was insufficient to abrogate TGF- β 1-induced deacetylation of α -tubulin (Figure 3B), whereas non-selective knockdown of p131 and p114 restored acetylation of α -tubulin in TGF- β 1-stimulated A549 cells [11]. The mRNA levels of a panel of TGF- β 1-regulated genes were examined using qRT-PCR. When compared to the groups transfected with the scrambled siRNA, p114-targeting RNAi inhibited TGF- β 1-mediated activation of PAI-1 (Figure 4A, reduced from 144 to 57), PDGF-B (Figure 4B, reduced from 53 to 26), and type I collagen (Figure 4C, reduced from 19.5 to 10.7). However, p114-targeting RNAi did not restore the expression of E-cadherin, an epithelial marker that is repressed by TGF- β 1 during EMT (Figure 4D). In accord with its impact on TGF- β 1-activated expression of the endogenous genes, p114-targeting RNAi nearly abrogated TGF- β 1-mediated activation of 3TP-LUX, a luciferase reporter that is regulated by the PAI-1 promoter (Figure 4E). These findings indicated a critical role for p114 in TGF- β 1-activated gene expression.

Discussion

The current study identifies and characterizes a novel splicing variant of human HDAC6, hHDAC6p114. Our results suggest that the hHDAC6p114 mRNA results from incomplete splicing and gives rise to the hHDAC6p114 protein via alternative translation initiation. Moreover, hHDAC6p114 is required for gene activation associated with TGF- β 1-induced EMT.

Alternative splicing is a common theme for the cell to confer a single gene multiple protein products. The novel HDAC6 splicing variant p114 results from incomplete splicing at the junction of exon-6 and intron-6 in the p131 mRNA and thereby intron-6 is converted into a part of exon-6 in the p114 mRNA, an exon corresponding to the sum of exon-6, intron-6, and exon-7 in p131. A paradoxical feature of the longer splicing variant p114 is that it is translated into a smaller protein when compared to p131. The intron-converted-exon appears to favor translation from an internal in-frame AUG site other than the most 5' AUG site and thus leads to the truncated p114 protein relative to the p131 protein. A sequence analysis of

the additional intron-converted-exon reveals introduction of several in-frame stop codons (Supplementary Materials Table 2). It is likely that the translation from the most 5' AUG utilized by p131 is prematurely terminated by the introduced stop codons and thus favors translation from the internal AUG site. A second possibility involves a potential internal ribosomal entry site (IRES) introduced by the intron-converted-exon. IRES relies heavily on RNA folding and prediction for IRES purely based on sequences or secondary structure has been challenging. An experimental approach will be to fuse the 5' end of the p114 cDNA to a Flag or GFP tag and examine whether two proteins can be separately produced from the tag-p114 fused transcript. It is notable that ectopic expression of the p131 expression vector yields a modest increase in a protein band that corresponds to p114 (Figure 2). One plausible speculation can be obtained from scrutiny of the two AUG sites utilized in translation of the p114 and p131 mRNAs. The likelihood for an efficient translation initiation involves recognition of the Kozak consensus sequence. Neither the most 5' AUG for p131 nor the internal AUG site for p114 presents perfect match of the Kozak consensus sequence. Therefore, it is likely that forced expression of the p131 mRNA results in less frequent translation initiation from the internal AUG site and consequently the p114 protein. Similarly, the endogenous p114 protein can potentially be translated from the p131 mRNA as well as the p114 splicing variant.

p114 is expressed at a much lower level than p131, which indicates the alternative splicing to produce p114 is a less frequent event than that for p131 (Figure 2 & 3). Alternative splicing to produce p114 appears to be regulated in the cell. MCF-7TNR exhibits higher ratios of p114 vs. p131 than MCF-7N (Figure 1). Moreover, elevated expression of p114, and to a lesser degree of p131 in MCF-7TNR, correlates with resistance to TNF- α -induced apoptosis and EMT-like phenotype (Burow ME, personal communication). These preliminary findings suggest that expression of the two hHDAC6 isoforms is differentially regulated in physiological and/or pathological conditions. It is also noteworthy that the p114 splicing variant may be a consequence of evolution because the genomic sequences encoding the intron-converted-exon is conserved only in the primate genomes (Supplementary Materials Table 3). Similarly, the coding sequences for the C-terminal SE14-repeat that is critical for cytoplasmic retention of human HDAC6 is highly conserved only in primates, but absent in other available genomic sequences, such as rodents [3].

At the protein level, our results indicate that p114 appears to be the minor form relative to p131, which is consistent with the abundance of their mRNAs (Figures 1 & 2). With respect to structure, p114 lacks the first 76 amino acids of the N-terminal HDAC domain. Our results indicate that p114 appears to be active in terms of tubulin deacetylase activity because overexpression of p114 results in reduced acetylated α -tubulin (Figure 2). This piece of data supports a previous report that the C-terminal HDAC domain in HDAC6 mediates tubulin deacetylation [15]. Likely due its low abundance relative to p131, the endogenous level of p114 does not account for maintenance of acetylated α -tubulin because selective knockdown of p114 does not cause an increase in acetylated α -tubulin (Figure 2). Neither does knockdown of p114 restore acetylated α -tubulin that is reduced during TGF- β 1-induced EMT (Figure 3). In contrast, non-selective knockdown of both p131 and p114 leads to hyperacetylation of α -tubulin and restores the protein levels of acetylated α -tubulin that are reduced during TGF- β 1-induced EMT, which likely results from reduced expression of p131 [11]. These findings suggest disparate function of the two isoforms, which is caused by difference in their abundance and structure. Indeed, our investigation of the role of p114 in the EMT response of A549 cells to TGF- β 1 supports this postulate. Selective knockdown of p114 imposes little effect on repression of E-cadherin by TGF- β 1 but significantly diminishes TGF- β 1-activated expression of PAI-1, type I collagen, and PDGF-B (Figure 4). We extrapolate that p114 participates in signaling that leads to gene activation, but not gene repression associated with TGF- β 1-induced EMT. In contrast, p131 may act in a broader

spectrum of TGF- β 1 signaling because it appears to be required for deacetylation of α -tubulin, the consequent remodeling of microtubules and cytoskeleton, and expression of EMT markers that includes both gene activation and repression [11]. It is conceivable that subcellular localization of p114 might be differentially regulated in comparison to p131 during EMT because of absence of the N-terminal NES in p114 (Figure 2). Although a previous report indicates dominance of the C-terminal SE14-repeat in cytoplasmic retention of hHDAC6 [3], p114 may demonstrate more nuclear accumulation than p131 when the function of the SE14-repeat is compromised by undefined mechanisms. It is plausible that p114 rather than p131 accounts for the function of the nuclear HDAC6 that remains largely unclear [9; 10].

In summary, this work identifies a novel splicing variant of human HDAC6, hHDAC6p114, that is structurally distinct from hHDAC6p131. Our results further implicate that expression and function of hHDAC6p114 are differentially regulated when compared to hHDAC6p131. Our initial characterization of this novel isoform warrants further investigation of expression and function of hHDAC6p114 in diverse physiological and pathological settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Kim SC, Sprung R, Chen Y, Xu Y, Ball H, Pei J, Cheng T, Kho Y, Xiao H, Xiao L, Grishin NV, White M, Yang XJ, Zhao Y. Substrate and functional diversity of lysine acetylation revealed by a proteomics survey. *Mol Cell* 2006;23:607–18. [PubMed: 16916647]
2. Boyault C, Sadoul K, Pabion M, Khochbin S. HDAC6, at the crossroads between cytoskeleton and cell signaling by acetylation and ubiquitination. *Oncogene* 2007;26:5468–76. [PubMed: 17694087]
3. Bertos NR, Gilquin B, Chan GK, Yen TJ, Khochbin S, Yang XJ. Role of the tetradecapeptide repeat domain of human histone deacetylase 6 in cytoplasmic retention. *J Biol Chem* 2004;279:48246–54. [PubMed: 15347674]
4. Pandey UB, Batlevi Y, Baehrecke EH, Taylor JP. HDAC6 at the intersection of autophagy, the ubiquitin-proteasome system and neurodegeneration. *Autophagy* 2007;3:643–5. [PubMed: 17912024]
5. Boyault C, Zhang Y, Fritah S, Caron C, Gilquin B, Kwon SH, Garrido C, Yao TP, Vourc'h C, Matthias P, Khochbin S. HDAC6 controls major cell response pathways to cytotoxic accumulation of protein aggregates. *Genes Dev* 2007;21:2172–81. [PubMed: 17785525]
6. Hubbert C, Guardiola A, Shao R, Kawaguchi Y, Ito A, Nixon A, Yoshida M, Wang XF, Yao TP. HDAC6 is a microtubule-associated deacetylase. *Nature* 2002;417:455–8. [PubMed: 12024216]
7. Kovacs JJ, Murphy PJ, Gaillard S, Zhao X, Wu JT, Nicchitta CV, Yoshida M, Toft DO, Pratt WB, Yao TP. HDAC6 regulates Hsp90 acetylation and chaperone-dependent activation of glucocorticoid receptor. *Mol Cell* 2005;18:601–7. [PubMed: 15916966]
8. Kawaguchi Y, Kovacs JJ, McLaurin A, Vance JM, Ito A, Yao TP. The deacetylase HDAC6 regulates aggregate formation and cell viability in response to misfolded protein stress. *Cell* 2003;115:727–38. [PubMed: 14675537]
9. Westendorf JJ, Zaidi SK, Cascino JE, Kahler R, van Wijnen AJ, Lian JB, Yoshida M, Stein GS, Li X. Runx2 (Cbfa1, AML-3) interacts with histone deacetylase 6 and represses the p21(CIP1/WAF1) promoter. *Mol Cell Biol* 2002;22:7982–92. [PubMed: 12391164]

10. Girdwood D, Bumpass D, Vaughan OA, Thain A, Anderson LA, Snowden AW, Garcia-Wilson E, Perkins ND, Hay RT. P300 transcriptional repression is mediated by SUMO modification. *Mol Cell* 2003;11:1043–54. [PubMed: 12718889]
11. Shan B, Yao TP, Nguyen HT, Zhuo Y, Levy DR, Klingsberg RC, Tao H, Palmer ML, Holder KN, Lasky JA. Requirement of HDAC6 for transforming growth factor-beta1-induced epithelial-mesenchymal transition. *J Biol Chem* 2008;283:21065–73. [PubMed: 18499657]
12. Pugacheva EN, Jablonski SA, Hartman TR, Henske EP, Golemis EA. HEF1-dependent Aurora A activation induces disassembly of the primary cilium. *Cell* 2007;129:1351–63. [PubMed: 17604723]
13. Weldon CB, Parker AP, Patten D, Elliott S, Tang Y, Frigo DE, Dugan CM, Coakley EL, Butler NN, Clayton JL, Alam J, Curiel TJ, Beckman BS, Jaffe BM, Burow ME. Sensitization of apoptotically-resistant breast carcinoma cells to TNF and TRAIL by inhibition of p38 mitogen-activated protein kinase signaling. *Int J Oncol* 2004;24:1473–80. [PubMed: 15138590]
14. Shan B, Zhuo Y, Chin D, Morris CA, Morris GF, Lasky JA. Cyclin-dependent kinase 9 is required for tumor necrosis factor-alpha-stimulated matrix metalloproteinase-9 expression in human lung adenocarcinoma cells. *J Biol Chem* 2005;280:1103–11. [PubMed: 15528190]
15. Haggarty SJ, Koeller KM, Wong JC, Grozinger CM, Schreiber SL. Domain-selective small-molecule inhibitor of histone deacetylase 6 (HDAC6)-mediated tubulin deacetylation. *Proc Natl Acad Sci U S A* 2003;100:4389–94. [PubMed: 12677000]

Figure 1.

Manifestation of the p114 mRNA. A) The schemes to amplify p131 and p114. The shaded boxes indicate exons and the connecting lines indicate introns. B) Total cell RNA was extracted from A549 (A) and MCF-7 (M) cells. The p131 and p114 mRNAs were detected by RT-PCR using a pair of non-selective primers. C) Similar to B except that cDNA was amplified using a pair of the p114 mRNA-specific primers. D) The mRNA levels of p114 (p114), and p114 and p131 combined (p114+p131) were determined using quantitative RT-PCR. Ratios of p114/(p114+p131) were obtained after normalization to the Ct values of the housekeeping gene 36B4. Mean and standard deviations were obtained from 3 independent experiments. *, P value < 0.05.

Figure 2.

Manifestation of the p114 protein. A) Comparison of the structure of the conceptual proteins of p131 and p114. B) Expression of potential isoforms of the hHDAC6 proteins and acetylated α -tubulin was examined in A549 cells stably expressing either scrambled (sc) or hHDAC6-targeting siRNA (HD6) using immunoblots. C) A549 and MCF-7 cells were transfected with either scrambled (–) or p114-targeting siRNA (+). The protein levels of p131, p114, and acetylated α -tubulin were examined using immunoblots. D) A549 cells were transfected with either the backbone or p114/p131 expression vectors. The protein levels of p131, p114, and acetylated α -tubulin were evaluated using immunoblots. The results are representative of at least 2 independent experiments.

Figure 3.

Impact of p114-targeting RNAi on TGF-β1-induced deacetylation of α-tubulin. A) Total cell RNA was isolated from A549 cells transfected with either the scrambled (scramble) or p114-targeting (p114si) siRNA followed by exposure to TGF-β1 (5 ng/ml) for 48 hrs. The mRNA levels of p114 (p114) and the combined p114 and p131 were determined using quantitative RT-PCR. Ratios of the genes of interest over the housekeeping gene 36B4 were compared across the groups. B) The culture conditions were similar to part A except that the protein levels of p131, p114, and acetylated α-tubulin were determined using immunoblots. Mean and standard deviations were obtained from 4 independent transfections.

Figure 4.

Requirement of p114 for TGF-β1-activated gene expression. A-D) Total cell RNA was isolated from A549 cells transfected with either the scrambled (scramble) or hHDAC6p114-targeting (p114si) siRNA followed by exposure to TGF-β1 (5 ng/ml) for 48 hrs. The mRNA levels of PAI-1 (A), PDGF-B (B), Collagen I (C), and E-cadherin (D) were determined using quantitative RT-PCR. Ratios of the genes of interest over the housekeeping gene 36B4 were compared across the groups. E) A549 cells were co-transfected with luciferase reporter constructs and siRNAs followed by exposure to TGF-β1 for 24 hrs. The ratios of TGF-β1 responsive 3TP-LUX activity over the co-transfected RL-TK activity were compared across the groups. Mean and standard deviations were obtained from 4 independent transfections. *, P value < 0.05.