This article has been withdrawn by the authors. The actin immunoblots in Fig. 2, A and B, are the same. Lanes 4 and 6 of the CYP1A1 and CYP1B1 panels in Fig. 3B are the same. Also in Fig. 3B, lanes 2 and 4 of the 18S rRNA panel are the same. The Hsp90 lanes are all the same in Fig. 6F. The control lanes in Fig. 6H were all reused in the control siRNA lanes. Additionally, the TSA lane for Hsp90 in Fig. 6H was reused in the HDAC6 siRNA lane. The first lane of the IP: Hsp90, IB: HDAC6 lane in Fig. 6I was reused in the TSA lane for Hsp90. The first lane in the input panel in Fig. 8F was reused in Fig. 8G. Additionally, lanes 2 and 3 of the input panel in Fig. 8G are duplicated. Lane 2 of the AhR panel in Fig. 8E was reused in the actin panel in Fig. 9D and in Subbaramaiah, K., et al. (2008) J. Biol Chem. 283, 33955-33968. In Fig. 9B, lanes 2 and 3 of the input are the same. Part of the input panel in Fig. 9C was reused in Subbaramaiah, K., et al. (2008) J. Biol. Chem. 283, 33955-33968. Parts of the actin panel in Fig. 9D were reused in Subbaramaiah, K., et al. (2008) J. Biol. Chem. 283, 33955-33968. The lanes in the p23 panel of Figs. S1A are the same. In Fig. S1B, lanes 3 and 4 of the Hsp90 panel are the same as well as lanes 1 and 2 of the AhR panel, lanes 1 and 2 of the actin panel, and lanes 3 and 4 of the actin panel. In Fig. S1C, the lanes in the XAP-2 panel are the same. In Fig. S1E, the first lane of the Hsp90 panel were reused in lanes 1 and 2 of the HDAC6 panel. Also in Fig. S1E, lane 3 of the Hsp90 panel was reused in lane 3 of the XAP-2 panel. In Fig. S2, the first lane of the Hsp90 panel was reused in lanes 4 and 6 of the same panel as well as in lane 4 of the IP: Hsp90, IB: Ack panel. Also in Fig. S2, the third lane of the Hsp90 panel was reused in lane 5 of the same panel as well as lane 6 of the IP: Hsp90, IB: Ack panel. Additionally, some portions of the IP: Hsp90, IB: Ack panel were reused in Subbaramaiah, K. et al. (2008) J. Biol. Chem. 283, 33955-33968.
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ability to both bind ligand and activate transcription (27, 29). An effort has been made to identify a functionally important Hsp90 acetylation site. Acetylation/deacetylation of Hsp90 Lys\textsuperscript{294} was found to play an important role in regulating the Hsp90 chaperone cycle (30).

In the current study, our primary objective was to determine whether modulating Hsp90 acetylation would affect ligand-mediated activation of AhR signaling. We demonstrate for the first time that HDAC6 activity is important for ligand-mediated activation of AhR signaling. Silencing or inhibition of HDAC6 resulted in an immature form of AhR with a reduced capacity to bind PAH, translocate to the nucleus, and activate the transcription of CYP1A1 and CYP1B1. Acetylation of the Lys\textsuperscript{294} residue of Hsp90 was important for regulating the activation of AhR signaling. Taken together, these findings suggest that inhibitors of HDAC6 will suppress the activation of AhR-dependent genes, which could in turn impact on both chemical carcinogenesis and drug metabolism.

EXPERIMENTAL PROCEDURES

Reagents—TSA and SAHA were from Biomol (Plymouth Meeting, PA). Antibodies to Hsp90, AhR, XAP-2, CYP1A1, and FLAG were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). B[a]P, protein quantitation assay kits, horseradish peroxidase-conjugated secondary antibody, and antibodies to actin were from Sigma. Antibodies to acetylated lysine and HDAC6 were from Abcam (Cambridge, MA). XRE-luciferase constructs were from Promega (Madison, WI). CYP1B1 and CYP1A1 cDNAs were from Origene Technologies, Inc. (Rockville, MD). Flag-Hsp90 lactosidase were measured in cellular extracts. FLAG-Hsp90 constructs were transfected into A549 cells using Amaxa (Lafayette, CO). Western blotting assay were from Amersham Biosciences. Reagents for the luciferase assay were from Analytical Luminescence (San Diego, CA). Tubacin was a kind gift of Dr. S. L. Schreiber (Lake Placid, NY). Immunoprecipitation kits were from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Northern Blotting—Total RNA was prepared from cell monolayers using an RNA isolation kit from Qiagen. Ten micrograms of total RNA were electrophoresed in a formaldehyde-containing 1% agarose gel and transferred to nylon-supported membranes. CYP1A1, CYP1B1, and 18 S rRNA probes were prepared by random priming. The blots were then probed as previously described (16). All of the Northern blot data are shown. Experiments were repeated three times, and representative blots were then probed as previously described (16).

Cell Lines—KYSE450 (esophageal squamous cell carcinoma) (31), HCA7 (colon adenocarcinoma) (32), 1483 (head and neck squamous cell carcinoma) (33), A549 (lung adenocarcinoma) (34), and MSK-Leuk1 (oral leukoplasia) cells (35) were grown as previously described. A549 cells stably expressing S-layer control plasmid or HDAC6 siRNA (knockdown (KD)) were a kind gift from Dr. Tso-Pang Yao of Duke University (Durham, NC) (27). In all of the experiments, the cells were incubated in serum-free medium for 24 h before treatment. The treatments were carried out in serum-free medium.

Tobacco Smoke Preparation—Cigarettes (2R4F; Kentucky Tobacco Research Institute) were smoked in a Borgwaldt piston-controlled apparatus (model RG-1) using the Federal Trade Commission standard protocol. Cigarettes were smoked one at a time in the apparatus, and the smoke was drawn under sterile conditions into premeasured amounts of sterile phosphate-buffered saline, pH 7.4, representing whole, trapped mainstream smoke (TS). Quantitation of smoke content is expressed in puffs/ml of phosphate-buffered saline with 1 cigarette yielding about 8 puffs drawn into a 5 ml volume. The final concentration of TS in the cell culture medium is expressed as puffs/ml medium. All of the treatments were carried out with 0.03 puffs/ml because this concentration has been used to activate AhR signaling in previous studies (16, 36, 37).

Immunoprecipitation—This was performed with a kit from Upstate Biotechnology, Inc. (Lake Placid, NY) according to the manufacturer’s instructions. Two hundred fifty μg cell lystate protein were used for immunoprecipitation at room temperature. The immunoprecipitases were then analyzed by SDS-PAGE.

Western Blotting—The lysates were prepared from treating cells with lysis buffer (150 mM NaCl, 100 mM Tris, pH 8.0, 1% Tween 20, 50 mM diethyldithiocarbamate, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml trypsin inhibitor, and 10 μg/ml leupeptin). The lysates were sonicated for 20 s on ice and centrifuged at 10,000 × g for 10 min to sediment the particulate material. The protein concentration of the supernatant was measured by the method of Lowry et al. (38). SDS-PAGE was performed under reducing conditions as described by Laemmli (39). The resolved proteins were transferred onto nitrocellulose sheets as detailed by Towbin et al. (40), and the membrane was incubated with primary antisera.
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Secondary antibody to IgG conjugated to horseradish peroxidase was used. The blots were probed with the ECL Western blot detection system.

Ligand Binding Assay—The cells were lysed in 1.5 volumes of buffer (10 mM HEPES, pH 7.3, 1 mM EDTA, and 20 mM sodium molybdate) and centrifuged at 100,000 × g. Aliquots of cytosol were incubated overnight at 4 °C with 100 nm [3H] B[a]P ± a 1000-fold excess of nonradioactive B[a]P. Free B[a]P was removed with dextran-coated charcoal. B[a]P binding is expressed as cpm of [3H] B[a]P/100 μl of cell cytosol.

Chromatin Immunoprecipitation (ChIP) Assay—ChIP assay was performed with a kit (Upstate Biotechnology) according to the manufacturer’s instructions. The cells (2 × 106) were cross-linked in a 1% formaldehyde solution for 10 min at 37 °C. The cells were then lysed in 200 μl of SDS buffer and sonicated to generate 200–1000-bp DNA fragments. After centrifugation, the cleared supernatant was diluted 10-fold with ChIP buffer and incubated with 1.5 μg of the indicated antibody at 4 °C. Immune complexes were precipitated, washed, and eluted as recommended. DNA-protein cross-links were reversed by heating at 65 °C for 4 h, and the DNA fragments were purified and dissolved in 50 μl of water. Ten microliters of each sample were used as a template for PCR amplification. The forward and reverse primers used for amplifying the CYP1A1 promoter are: 5'-ACCCGCCACCCTTGGACAGTTCT-3' and 5'-TGC-CCAGGCGTTGCTGGAGAAG-3' (41). Forward and reverse primers used to amplify the CYP1B1 promoter are: 5'-GTT-CCCTTATAAAGGGGAG-3' and 5'-CTCGCTGAGGAAAGCGGTTG-3' (42). PCR was performed at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s for 30 cycles. The PCR products generated from the indicated antibodies were sequenced, and the identity of the ChIP products was confirmed.

Statistical comparisons between groups were made by Student’s t test. Differences of p < 0.05 were considered significant.

FIGURE 1. HDAC inhibitors suppress TS-mediated induction of CYP1A1 and CYP1B1 in human aerodigestive epithelial cells. MSK-Leuk1, KYSE450, 1483, A549, and HCA7 cells were pretreated with indicated concentrations of TSA, SAHA or vehicle for 2 h. Subsequently, the cells were treated with vehicle or TS for 5 h. Cellular lysate protein was then isolated and loaded (100 μg/lane) on a 10% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose as described under “Experimental Procedures.” The immunoblot was probed with antibodies specific for CYP1A1 (A), CYP1B1 (B), or β-actin.

FIGURE 2. HDAC inhibitors suppress B[a]P-mediated induction of CYP1A1 and CYP1B1 in human aerodigestive epithelial cells. KYSE450 and MSK-Leuk1 cells were pretreated with indicated concentrations of TSA, SAHA, or vehicle for 2 h. Subsequently, the cells were exposed to vehicle or 1 μμ B[a]P for 5 h. Cellular lysate protein was then isolated and loaded (100 μg) on a 10% SDS-polyacrylamide gel, electrophoresed, and subsequently transferred onto nitrocellulose as described under “Experimental Procedures.” The immunoblot was probed with antibodies specific for CYP1A1 (A), CYP1B1 (B), or β-actin.
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the HDAC that regulates ligand-mediated activation of AhR signaling. Previously, Kovacs et al. (27) reported that Hsp90 is a substrate of HDAC6 and that its chaperone activity is regulated by acetylation. Because the AhR is a client protein of Hsp90, we hypothesized that HDAC6 could be important for ligand-mediated induction of CYP1A1 and CYP1B1. To test this possibility, a series of experiments were carried out in which HDAC6 was silenced. Initially, A549 cells in which HDAC6 was stably knocked down were used (Fig. 4A). Treatment with TS (Fig. 4B), TCDD (Fig. 4C), or B[a]P (data not shown) induced CYP1B1 in vector-expressing cells but not in A549 HDAC6 knockdown (KD) cells. Silencing of HDAC6 also suppressed TS-mediated induction of XRE-luciferase activity in these cells (Fig. 4D). Consistent with the findings in A549 cells, silencing of HDAC6 (Fig. 4F) also suppressed the induction of CYP1A1 and CYP1B1 by TS (Fig. 4F) and B[a]P (data not shown) in MSK-Leuk1 cells. To complement this genetic strategy, a pharmacological approach was utilized to confirm the role of HDAC6 in ligand-mediated activation of AhR signaling. HDAC6 possesses tubulin deacetylase activity. Tubacin, a targeted inhibitor of HDAC6 (43), caused dose-dependent increases in tubulin acetylation consistent with its ability to inhibit HDAC6 (Fig. 5A). Tubacin also suppressed TS- and TCDD-mediated induction of CYP1A1 and CYP1B1 and blocked TS-mediated stimulation of XRE-luciferase activity in MSK-Leuk1 cells (B–D). Additionally, tubacin caused dose-dependent suppression of both TS- and TCDD-mediated induction of CYP1B1 in A549 cells (Fig. 5, E and F). Collectively, these data imply
that HDAC6 is important for regulating the ability of AhR ligands to activate the expression of target genes.

We next investigated the mechanism by which HDAC inhibitors suppress ligand-mediated activation of AhR signaling. Previously, HDAC6 was found to interact with Hsp90 and regulate its acetylation (27). Immunoprecipitation of HDAC6 coprecipitated Hsp90 in both MSK-Leuk1 and A549 cells (Fig. 6A). Silencing HDAC6 or treatment with TSA, SAHA, or tubacin prevented the coprecipitation of HDAC6 with Hsp90 (Fig. 6A). Levels of Hsp90, AhR, p23, and XAP-2 were unaffected by these treatments (supplemental Fig. S1, A–D). Because HDAC6 is a Hsp90 deacetylase (27), we next determined whether HDAC inhibition caused an increase in Hsp90 acetylation. Treatment with a broad spectrum HDAC inhibitor (TSA or SAHA), tubacin, or silencing of HDAC6 stimulated the acetylation of Hsp90 (Fig. 6, E–G, and supplemental Fig. S2). In cells in which HDAC6 was silenced, further treatment with TSA or SAHA (data not shown) did not lead to an additional enhancement of Hsp90 acetylation (Fig. 6, F and G). These results suggest that HDAC6 is the dominant TSA/SAHA-sensitive Hsp90 deacetylase.

Next, we investigated whether hyperacetylation of Hsp90 would alter its interaction with AhR, p23, and XAP-2. p23 and XAP-2 are normally found in a cytoplasmic complex with Hsp90 and AhR. As shown in Fig. 6, (H, MSK-Leuk1 cells, and I, A549 cells), immunoprecipitation of Hsp90 pulled down p23,
FIGURE 6. HDAC6 associates with Hsp90 and regulates its acetylation and chaperone complex formation with p23 and XAP-2. A, cell lysates (250 μg) from MSK-Leuk1 and A549 cells were subjected to immunoprecipitation (IP) with antibody to HDAC6 or IgG. The immunoprecipitates were then subjected to immunoblotting (IB) and probed with antibodies to Hsp90 or HDAC6. B, MSK-Leuk1 cells were treated with vehicle (lanes C), TSA (500 nM), SAHA (20 μM), or tubacin (20 μM) for 1 h. The cell lysates were prepared and immunoprecipitated with an antibody to HDAC6. Immunoprecipitates were then subjected to immunoblotting and probed with antibodies to Hsp90 or HDAC6. C, cell lysates were prepared from A549 cells stably expressing pSUPER (Control) or HDAC6 siRNA (HDAC6 KD). D, cell lysates were prepared from MSK-Leuk1 cells transiently transfected with nonspecific (control) siRNA or HDAC6 siRNA. In C and D, cell lysates were subjected to immunoprecipitation with antibody to HDAC6. The immunoprecipitates were then subjected to immunoblotting and probed for Hsp90 and HDAC6. Additionally, cell lysates were directly subjected to immunoblotting for Hsp90. In E–G, MSK-Leuk1 and A549 cells were treated as indicated with vehicle (lanes C), TSA (500 nM), SAHA (20 μM), or tubacin (20 μM) for 1 h. The cell lysates were then subjected to immunoprecipitation with antibody to acetyl lysine (AcK). The immunoprecipitates were then subjected to immunoblotting and probed for Hsp90 and HDAC6. Additionally, cell lysates were directly subjected to immunoblotting for Hsp90. In H, cell lysates were prepared from MSK-Leuk1 cells that received vehicle (control) or TSA (500 nM) for 1 h or were transfected with nonspecific siRNA (control siRNA) or HDAC6 siRNA. I, cell lysates were prepared from A549 cells that received vehicle or TSA (500 nM) for 1 h. In H and I, cell lysates were subjected to immunoprecipitation with antibody to Hsp90. The immunoprecipitates were then subjected to immunoblotting and probed for p23, AhR, HDAC6, and XAP-2. Additionally, cell lysates were directly subjected to immunoblotting for Hsp90.
AhR, HDAC6, and XAP-2. This interaction was not observed in cells treated with an HDAC inhibitor or in which HDAC6 was silenced. Levels of Hsp90, AhR, p23, and XAP-2 were unaffected by treatment with TSA or silencing of HDAC6 (supplemental Fig. S1E). Hence, HDAC6 activity is necessary for Hsp90 complex formation, which in turn plays a significant role in regulating the induction of CYP1A1 and CYP1B1.

**HDAC6 Is Important for AhR Ligand Binding, Translocation, and Transcriptional Activity**—We next evaluated whether HDAC6-regulated Hsp90 acetylation was important for the chaperone function of Hsp90. Efficient binding of ligand and the subsequent translocation of activated AhR from cytosol to nucleus are known chaperone functions of Hsp90. Several experiments were conducted to determine whether HDAC6 was a determinant of ligand binding to the AhR, translocation of AhR from cytosol to nucleus, and activation of the CYP1A1 and CYP1B1 transcription. Silencing HDAC6 did not alter the levels of AhR or Hsp90 in A549 cells (Fig. 7A). The effect of silencing HDAC6 on the binding of ligand to the AhR was investigated. Cytosols prepared from A549 control and A549 HDAC6 KD cells were incubated with radiolabeled B[a]P, and ligand binding to the AhR was determined. The binding of [3H]B[a]P to AhR was reduced in cytosols prepared from A549 HDAC6 KD cells compared with A549 control cells (Fig. 7A). Consistent with this finding, a dramatic decrease in the binding of [3H]B[a]P to the AhR was also found in MSK-Leuk1 cells treated with TSA or SAHA (Fig. 7B). Subsequently, the same experiments were repeated in MSK-Leuk1 cells. Once again, silencing of HDAC6 did not lead to reduced amounts of AhR but did cause a significant decrease in the binding of [3H]B[a]P to the AhR (Fig. 7C). Furthermore, reduced binding of [3H]B[a]P to the AhR was also found in MSK-Leuk1 cells treated with TSA or SAHA (Fig. 7D).

Although comparable amounts of AhR are present in the cytosols of cells in which HDAC6 was silenced, the binding of B[a]P was markedly reduced relative to control cells. These results suggest that the AhR produced in cells in which HDAC6 is silenced is defective in ligand binding. The chaperone function of Hsp90 is deficient in association with hyperacetylation of Hsp90.

Ligand-induced nuclear accumulation of AhR was also evaluated. In untreated MSK-Leuk1 cells, the AhR is located predominantly in the cytosol. Treatment with SAHA (Fig. 8, A and B) or silencing HDAC6 (data not shown) blocked TS- and B[a]P-mediated translocation of the AhR from cytosol to nucleus. ChIP assays were conducted to determine whether recruitment of AhR to CYP1A1 and CYP1B1 promoters was altered by inhibition of HDAC6. Treatment with SAHA (Fig. 8, C–E) or silencing HDAC6 (data not shown) blocked TS-, TCDD-, and B[a]P-mediated recruitment of the AhR to the CYP1A1 promoter. Similar effects were observed for the CYP1B1 promoter (data not shown). In A549 cells, TS-mediated recruitment of AhR to the CYP1B1 promoter was blocked by SAHA (Fig. 8F) or silencing of HDAC6 (Fig. 8G). Thus, Hsp90-dependent binding of ligand to the AhR, nuclear translocation, and stimulation of gene transcription are all suppressed when HDAC6 is inhibited.

**Acetylation of Lys294 Weakens the Interaction with AhR**—The above findings indicate that Hsp90 acetylation inhibits its activity. Recently, the acetylation state of Hsp90 Lys294 was found to be
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In this study, we showed that HDAC6 activity is important for ligand-mediated activation of AhR signaling. Several lines of evidence support this point. First, TSA and SAHA, two broad spectrum HDAC inhibitors, suppressed both TS- and B[a]P-mediated induction of CYP1A1 and CYP1B1 in cell lines derived from the aerodigestive tract (Figs. 1–3). Moreover, silencing of HDAC6 suppressed the induction of CYP1A1 and CYP1B1 by ligands of the AhR (Fig. 4). Consistent with this finding, tubacin, a HDAC6 inhibitor, also inhibited AhR ligand-mediated induction of CYP1A1 and CYP1B1 (Fig. 5).

Previously, HDAC6 was found to function as an Hsp90 deacetylase (27, 28). The known chaperone functions of Hsp90 include maintaining the AhR in a high affinity ligand binding conformation (12, 44). Hence, we next investigated whether inhibiting HDAC6 caused hyperacetylation of Hsp90, leading in turn to reduced chaperone function. In agreement with a previous report (27), we found that HDAC6 physically interacted with Hsp90. Inhibiting or silencing HDAC6 caused hyperacetylation of Hsp90, resulting in the loss of interaction between Hsp90 and HDAC6 (Fig. 6). Interestingly, neither treatment with TSA nor SAHA caused a significant increase in Hsp90 acetylation in HDAC6 knockdown cells. This finding is consistent with another report (27) and suggests that HDAC6 may be the principal deacetylase involved in the regulation of Hsp90 acetylation. In untreated cells, Hsp90 exists in a cytosolic complex that includes the AhR, p23, and XAP-2 (7–11). The interaction between Hsp90 and AhR, p23 and XAP-2 was lost when HDAC6 was silenced or cells were treated with an HDAC inhibitor (Fig. 6). Thus, the accumulation of hyperacetylated Hsp90 in HDAC6-deficient cells prevents stable complexes from forming with both the cochaperone p23 and the AhR. As

DISCUSSION

In this study, we showed that HDAC6 activity is important for ligand-mediated activation of AhR signaling. Several lines of evidence support this point. First, TSA and SAHA, two broad spectrum HDAC inhibitors, suppressed both TS- and B[a]P-mediated induction of CYP1A1 and CYP1B1 in cell lines derived from
mentioned above, the AhR must be in a complex with Hsp90 and p23 to exhibit high affinity ligand binding activity. Hence, we also investigated whether hyperacetylation of Hsp90 altered the ligand binding capacity of the AhR. Silencing HDAC6 or treatment with HDAC inhibitors markedly suppressed the binding of B[a]P to AhR without altering levels of the receptor (Fig. 7). Consistent with this finding, the ability of ligand to induce the nuclear translocation of AhR and activate the transcription of CYP1A1 and CYP1B1 was suppressed when HDAC6 was silenced or cells were treated with HDAC inhibitors (Fig. 8). Taken together, these results indicate that HDAC6 activity regulates the acetylation of Hsp90, the chaperone function of Hsp90, and the ability of ligand to activate AhR-dependent gene expression. Although our study is the first to show the importance of the HDAC6-Hsp90 axis for regulating the activation of AhR signaling, similar results have been reported for the GR (27). More specifically, in HDAC6-deficient cells, Hsp90-dependent maturation of the GR was defective, resulting in reduced ligand binding, nuclear translocation, and gene activation. If HDAC6 regulates the Hsp90-dependent maturation of client proteins including the AhR and GR, it is reasonable to speculate that other nuclear hormone receptors, which are client proteins, e.g. progesterone receptor, will also be affected. Additional studies will be needed to evaluate this possibility.

The above findings clearly show that hyperacetylation of Hsp90 inhibits ligand-mediated activation of AhR signaling. Recently, Scroggins et al. (30) found that acetylation of a specific lysine residue (Lys294) in the beginning of the middle domain of Hsp90 was critical for both co-chaperone and client protein binding. In our study, conservative mutation of Lys294 to amino acids that mimic the unacetylated (arginine) or acetylated (alanine or glutamine) state indicated that the acetylation status of Lys294 affected the interaction between Hsp90, the co-chaperone p23, and AhR (Fig. 9). Consistent with the behavior of hyperacetylated Hsp90, point mutations of Lys294 affected the interactions with p23 and AhR. More specifically, when acetylation of this residue was mimicked, a decrease in both p23 and AhR binding was observed. Importantly, mutation of Lys294 to an unacetylated state of Hsp90 also blocked both TS- and B[a]P-mediated induction of CYP1B1. Therefore, reversible hyperacetylation of Hsp90 and possibly other sites in Hsp90 appear to provide an important level of posttranslational control that regulates AhR signaling.

Inhibitors of Hsp90 including geldanamycin and its derivatives bind within the ATP-binding pocket of the NH2-terminal domain of Hsp90. These agents cause a rapid decrease in ligand binding, reduced ligand-induced nuclear translocation, and reduced ligand-mediated activation of highly carcinogenic metabolites, creating a link to atoxic metabolite, anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydroxy-benzo[a]pyrene. In the present study, we found that HDAC6 inhibition caused hyperacetylation of Hsp90 that in turn suppressed PAH-mediated induction of CYP1A1 and CYP1B1. In contrast to the findings with Hsp90 inhibitors, levels of AhR were stable in HDAC6 knockdown cells, but the ability of AhR to bind ligand and thereby activate gene expression was compromised. This result is consistent with previous findings for the GR (27). Thus, Hsp90 inhibitors and HDAC inhibitors, two classes of molecularly targeted agents, both inhibit Hsp90 function and AhR-dependent gene expression but by different mechanisms. In contrast to the findings for AhR and GR, depletion of HDAC6 has been reported to enhance the proteasomal degradation of other Hsp90 client proteins (28). It seems likely, therefore, that HDAC6 inhibition modulates the function of Hsp90 client proteins by different mechanisms including both inhibition of receptor maturation and increased proteasomal degradation.

Recently, HDAC6 null mice were found to be resistant to PAH-induced skin tumors (47). The results of the current study may help to explain this finding. The induction of CYP1A1 and CYP1B1 by ligand-activated AhR can increase the production of highly carcinogenic metabolites, creating a link to the AhR and chemical carcinogenesis. For example, B[a]P, a potent ligand of the AhR, induces its own metabolism to a toxic metabolite, anti-7,8-dihydroxy-9,10-epoxy-7,8,9,10-tetrahydroxy-benzo[a]pyrene, which covalently binds to DNA, forming bulky adducts that induce mutations (20, 49). We showed that inhibiting or silencing HDAC6 suppressed PAH-mediated activation of AhR signaling and thereby blocked the induction of CYP1A1 and CYP1B1. It is reasonable...
to speculate, therefore, that HDAC6 deficiency will suppress carcinogen activation, which would help to explain why HDAC6 null mice are resistant to PAH induced skin tumors. Elevated levels of CYP1A1 and CYP1B1 are found in the upper aerodigestive tracts of human smokers (16, 37, 50). Our results raise the possibility that both broad spectrum HDAC inhibitors and HDAC6 inhibitors will alter the metabolism and clearance of therapeutic drugs, which could affect both their efficacy and toxicity. The metabolism and clearance of xenobiotics and endogenous substrates could also be affected. In future studies of HDAC6 inhibitors, our findings suggest that it will be important to monitor blood levels of coadministered drugs that are metabolized by enzymes encoded by AHR-dependent genes.

Acknowledgments—We are grateful to Dr. Ralph Mazitschek and Dr. Stuart L. Schreiber (Broad Institute of Harvard University and MIT Chemical Biology Program), Initiative for Chemical Genetics-NCI, for providing tabacin.

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