**HIF1α Protein Stability Is Increased by Acetylation at Lysine 709**

Hao Geng, Qiong Liu, Changhui Xue, Larry L. David, Tomasz M. Beer, George V. Thomas, Mu-Shui Dai, and David Z. Qian

From the Oregon Health & Science University Knight Cancer Institute, Oregon Health & Science University, Portland, Oregon 97239

**Background:** HIF1α and p300 are key components of HIF-1 transcription complex.

**Results:** Lysine 709 of HIF1α is acetylated by p300, which increases protein stability and HIF-1 activity.

**Conclusion:** p300 has a novel function in stabilizing HIF1α by Lys-709 acetylation.

**Significance:** New insights in how HIF1α is post-translationally regulated by its cofactor to ensure HIF-1 activity.

Lysine acetylation regulates protein stability and function. p300 is a component of the HIF-1 transcriptional complex and positively regulates the transcriptional activity of HIF-1. Here, we show a novel molecular mechanism by which p300 facilitates HIF-1 activity. p300 increases HIF-1α (HIF1α) protein acetylation and stability. The regulation can be opposed by HDAC1, but not by HDAC3, and is abrogated by disrupting HIF1α-p300 interaction. Mechanistically, p300 specifically acetylates HIF1α at Lys-709, which increases the protein stability and decreases polyubiquitination in both normoxia and hypoxia. Compared with the wild-type protein, a HIF1α K709A mutant protein is more stable, less polyubiquitinated, and less dependent on p300. Overexpression of the HIF1α wild-type or K709A mutant in cancer cells lacking the endogenous HIF1α shows that the K709A mutant is transcriptionally more active toward the HIF-1 reporter and some endogenous target genes. Cancer cells containing the K709A mutant are less sensitive to hypoxia-induced growth arrest than the cells containing the HIF1α wild-type. Taken together, these data demonstrate a novel biological consequence upon HIF1α-p300 interaction, in which HIF1α can be stabilized by p300 via Lys-709 acetylation.

Hypoxia-inducible factor 1 α (HIF1α) is a critical component of the HIF-1 transcriptional complex that regulates cellular response to hypoxia. In non-hypoxic conditions, HIF1α protein is constantly synthesized, but rapidly degraded by oxygen. The primary mechanism consists of the post-translational hydroxylation of HIF1α at proline residues 402 and 564 by oxygen-dependent prolyl hydroxylases, which is followed by von Hippel-Lindau (VHL)-mediated polyubiquitination and 26 S proteasome-dependent protein degradation. In hypoxic conditions, oxygen is not available for the hydroxylation of HIF1α protein, which is stabilized and dimerized with the oxygen-dependent HIF1α protein (11, 14, 18). However, the biological consequence due to these different acetylation sites can be very different. Currently, both the lysine acetylase and deacetylase families have been discovered within the HIF1α protein stability and HIF-1 transcriptional activity are associated with the protein lysine acetylation-deacetylation system. Multiple members within the lysine acetylase and deacetylase families have been reported to interact with HIF1α (10–14). The biological consequence is a change of HIF-1 activity due to a change in HIF1α protein stability in many cases (9, 15, 16). Lysine acetylation changes protein stability and function (17). Multiple acetylation sites have been discovered within the HIF1α protein (11, 14, 18). However, the biological consequence due to these different acetylation sites can be very different. Currently, both the lysine acetylase and deacetylase are potential therapeutic targets against cancer and other human diseases, in which HIF1α plays a pathological role. It is important to delineate the functional consequence of HIF1α acetylation at specific lysine locations.

p300 is a critical component in the HIF-1 transcriptional complex. In the non-hypoxic condition, p300 and HIF1α are not physically associated due to the rapid HIF1α protein degradation and the hydroxylation at asparagine 803 within the HIF1α (20). Recently, the acetylation at lysine 674 of HIF1α by PCAF has also been reported to play a role in determining HIF1α-p300 interaction (14). Upon hypoxia, the C-terminal of HIF1α interacts with the CH1 (cysteine/histidine-rich) domain of p300, which leads to an increase of HIF-1 transcriptional activity. A small molecule inhibitor, chetomin, has...
been reported to disrupt the interaction between HIF1α and p300 and inhibit the HIF-1 target gene expression (22). p300 is a multiple domain protein with acetylase activity (23). Despite being closely associated with HIF1α in hypoxia, it is unclear whether p300 has a direct effect on HIF1α. In the current study, we identified a novel function of p300 in stabilizing HIF1α proteins by lysine acetylation at Lys-709 within HIF1α.

**EXPERIMENTAL PROCEDURES**

**Cell Culture**—Human embryonic kidney 293T (Hek293T), 293 (Hek293), osteosarcoma U2OS, Hep3Bc1, and renal cell carcinoma RCC10 cells were cultured in DMEM with 10% FBS and 1% penicillin/streptomycin. Cells were maintained in a humidified incubator at 37 °C with 5% CO2. Hypoxia was identified using LI-COR odyssey Infrared software (version 2.0) as described previously (18).

**Cycloheximide HIF1α Half-life**—HIF1α and GFP proteins were expressed in Hek293T cells. Following exposure to normal or hypoxic condition for 5 h, cells were treated with cycloheximide and harvested at different time points. Western blots were used to detect HIF1α and GFP proteins, which were quantified using LI-COR odyssey Infrared software (version 2.0) as described previously (18).

**Real-time PCR and Cell Proliferation**—The ΔΔCt method was used to calculate mRNA fold change. The trypan blue exclusion method was used to calculate viable cells in the proliferation experiments as described (18, 24).

**Statistical Analysis**—Differences between the means of unpaired samples were evaluated by the Student’s t test.

**RESULTS**

**p300 Up-regulates the HIF1α Protein Level**—Although p300 is known to increase HIF-1 transcriptional activity, its effect on HIF1α protein is less clear. In the cancer cell line U2OS, we used siRNA to knockdown p300 and cultured the cells in either normal ambient oxygen (21% O2) or hypoxic (1% O2) conditions for 6 h to induce HIF1α protein accumulation. We found that p300 siRNA knockdown (si-p300) significantly decreased the HIF1α in hypoxia (Fig. 1A, U2OS, and supplemental Fig. S1). This decrease was on the protein level because the mRNA of HIF1α was not changed by p300 knockdown (supplemental Fig. S1). We also tested the effect of p300 siRNA on HIF1α proteins in a renal cell carcinoma cell line RCC10 (25), in which a loss of function in VHL renders HIF1α protein stable in normal oxygen condition (Fig. 1A, RCC10). The siRNA knockdown of p300 significantly decreased HIF1α proteins in both normal and hypoxic conditions (Fig. 1A, RCC10). Next, we measured the effect of p300 overexpression on HIF1α protein levels. In VHL wild-type U2OS cells, p300 overexpression increased HIF1α proteins in the hypoxic condition (Fig. 1B, U2OS). In VHL-negative RCC10 cells, the HIF1α protein was significantly increased by p300 transfection in both normal and hypoxic conditions (Fig. 1B, RCC10). The effect of p300 on HIF1α appears to be specific because HIF2α and HIF1β protein levels were not changed by p300 (supplemental Fig. S2). Next, we co-overexpressed a FLAG-tagged HIF1α wild-type plasmid with either empty vector (Ev) or p300 in Hek293T cells. To ensure the regulation of FLAG-HIF1α by p300 is specific, we also included a plasmid encoding the green florescence protein (GFP) that is regulated by the same promoter of FLAG-HIF1α as a control. In Ev cells, hypoxia increased the FLAG-HIF1α protein level, and the overexpressed HIF1α protein was present in cells cultured in normal oxygen condition (Fig. 1C); presumably, this was because the vector-based FLAG-HIF1α overexpression was very robust and overcame the endogenous oxygen/VHL-mediated HIF1α degradation. Importantly, p300
overexpression further increased the FLAG-HIF1α in both normal and hypoxic conditions (Fig. 1C). As a control, the co-overexpressed GFP was not changed by either hypoxia or p300 overexpression. To further confirm the HIF1α regulation by p300, we transfected the FLAG-HIF1α into Hek293T cells and treated cells with chetomin, a chemical inhibitor that disrupts the HIF1α-p300 interaction (22). We found that chetomin treatment decreased the FLAG-HIF1α protein expression in both normal and hypoxic conditions (Fig. 1D). These data suggest that p300 can enhance the HIF1α stability in hypoxia or in conditions that the oxygen/VHL-mediated HIF1α degradation is either inactive (RCC10 cell line) or overwhelmed (vector-based HIF1α overexpression).

p300 Acetylates HIF1α—One of the primary functions of p300 is protein lysine acetylation (23). To understand the molecular mechanism underlying the HIF1α up-regulation by p300, we transfected the FLAG-HIF1α and p300 in Hek293T cells, immunoprecipitated the FLAG-HIF1α protein from whole cell lysates (WCL), and measured the HIF1α lysine acetylation and its interaction with p300. In agreement with the reported role of p300, in Ev cells, hypoxia increased the interaction between FLAG-HIF1α and the endogenous p300 (Fig. 2A). The overexpressed p300 can also interact with FLAG-HIF1α proteins in normoxia, and the interaction was further increased by hypoxia (Fig. 2A). Importantly, the level of FLAG-HIF1α lysine acetylation was increased by hypoxia in the Ev cells and was significantly increased by p300 overexpression in both normal and hypoxic conditions (Fig. 2A). Because both hypoxia and p300 overexpression increased the FLAG-HIF1α protein level (Figs. 1C and 2A), we wanted to know whether the increase of HIF1α acetylation was due to the increase of total HIF1α proteins or the increase of de novo HIF1α acetylation. We performed an in vitro HIF1α acetylation assay by mixing equal amount of IP-purified FLAG-HIF1α proteins with IP-purified p300 proteins in the acetylation assay buffer. After 30 min of incubation at 30 °C, the acetylation level of FLAG-HIF1α was measured by Western blot. As shown in Fig. 2B, the de novo HIF1α acetylation was significantly increased in vitro by incubating with the p300 immunocomplex compared with IgG complex, whereas the total FLAG-HIF1α input remained the same. Next, we repeated the HIF1αα and p300 co-overexpression experiment as in Fig. 2A using a plasmid coding for a FLAG-HIF1α proline double mutant (HIF1αα-dm) that is resistant to the oxygen/VHL-mediated degradation. In Ev cells, the HIF1αα-dm protein level in WCL was not significantly changed between normal and hypoxic conditions, and hypoxia increased the interaction between HIF1αα-dm and the endogenous p300 (Fig. 2C) and increased the HIF1αα-dm protein acetylation in the IP samples (Fig. 2C). Co-overexpression of p300 slightly increased the HIF1αα-dm total protein levels in the WCL, increased the interaction between HIF1α and p300, and significantly increased the HIF1αα-dm protein lysine acetylation (Fig. 2C). These results indicated de novo acetylation of HIF1αα by p300. To test whether the HIF1αα-p300 interaction is required for the increase of HIF1αα protein acetylation, we transfected FLAG-HIF1αα into Hek293T cells and treated cells with chetomin. In hypoxia, chetomin treatment significantly reduced the HIF1αα-p300 interaction, which coincided with the reduction of HIF1αα acetylation and total HIF1αα protein level (Fig. 2D). Because HIF1αα can also be associated with lysine deacetylases (26, 27), we further tested whether the p300-induced acetylation can be countered by deacetylation. We co-overexpressed FLAG-HIF1αα with p300 or in combination with either HDAC1 or HDAC3. We found that HDAC1, but not HDAC3, significantly reduced the p300-induced HIF1αα acetylation, which also coincided with a reduction of total FLAG-HIF1αα protein level (Fig. 2E). Recently, HIF1αα was reported to be acetylated by a p300-interacting protein, PCAF (14). We compared the ability of p300 and PCAF in terms of HIF1αα acetylation. When an HA-tagged HIF1αα was co-overexpressed with either p300 or FLAG-PCAF, we observed that the HIF1αα-PCAF interaction was not changed by hypoxia, and the interaction was less robust than HIF1αα-p300 (Fig. 2F). Furthermore, p300 also induced a more robust HIF1αα acetylation than FLAG-PCAF (Fig. 2F). Taken together, these data suggest that HIF1αα-p300 interaction has two novel biological consequences: the increases of HIF1αα lysine acetylation and protein stability, which are
dependent on HIF1α-p300 interaction that can be achieved by hypoxia or p300 overexpression.

Lysine 709 of HIF1α Is the Acetylation Target of p300—To identify the p300 acetylated lysine residue(s) within HIF1α, we co-transfected the full-length wild-type FLAG-HIF1α with either p300 or Ev in Hek293T cells. Next, we immunoprecipitated and gel-purified FLAG-HIF1α proteins, digested them with trypsin, and analyzed the samples by liquid chromatography-tandem mass spectrometry (LC-MS/MS). We observed that lysine 674 within the FLAG-HIF1α was acetylated in both Ev and p300-overexpressed samples (data not shown). Significantly, lysine 709 was only acetylated in p300-overexpressed cells (Fig. 3A and supplemental Fig. S3). Lys-674 has recently been reported as an acetylation target for PCAF (14), and Lys-709 is novel. Based on the FLAG-HIF1α plasmid, we used site-directed mutagenesis to convert lysine to alanine and generated FLAG-HIF1α mutants containing a single point mutation of either K674A or K709A. Then, we overexpressed FLAG-
HIF1α Lys-709 Acetylation Increases Protein Stability

HIF1α-WT, FLAG-HIF1α-K674A, or FLAG-HIF1α-K709A in Hek293T cells with either Ev control or p300. Forty-eight hours after transfection, the FLAG-HIF1α wild-type and mutant proteins were immunopurified from the whole cell lysates. Western blots showed that all HIF1α proteins interact with p300 similarly (Fig. 3B). In both FLAG-HIF1α-WT and FLAG-HIF1α-K674A mutant, the acetylation levels were robustly increased by p300 (Fig. 3B). In contrast, the acetylation level of FLAG-HIF1α-K709A mutant was not significantly increased by p300 (Fig. 3B). To further confirm the specific acetylation at Lys-709 within the HIF1α protein, we co-overexpressed HIF1α-WT or Lys-709 mutant with either the wild-type p300 or a p300 mutant that is defective in the acetyltransferase activity (28). Similar to the results in Fig. 3B, p300 wild-type was able to increase the acetylation in HIF1α-WT, but not in HIF1α-K709A mutant proteins (Fig. 3C). However, the p300 acetylase mutant was unable to increase the acetylation in HIF1α-WT and K709A mutant (Fig. 3C). These data suggest that p300 acetylates HIF1α primarily at Lys-709.

p300 Regulates HIF1α Protein Stability via Lys-709 Acetylation—To investigate the biological significance of Lys-709 acetylation, we overexpressed equal amounts of FLAG-HIF1α-WT or FLAG-HIF1α-K709A mutant in Hek293T cells with GFP plasmids as transfection controls. Then, we measured the normal and hypoxic FLAG-HIF1α protein levels. We observed that the FLAG-HIF1α K709A mutant protein expressed at a higher level than FLAG-HIF1α-WT in both normal and hypoxic conditions by Western blots coupled with fluorescence densitometry (Fig. 4A). Next, we used cycloheximide to inhibit protein synthesis and measured the HIF1α WT and K709A mutant protein stability in the absence (Ev) or presence of p300 overexpression. In normal oxygen condition, the K709A mutant protein was significantly more stable than HIF1α-WT protein in the Ev cells (#, p < 0.01, Fig. 4B). p300 overexpression significantly increased the HIF1α-WT protein stability (a, p < 0.01, Fig. 4B) but had no effect on HIF1α-K709A mutant. A similar trend was observed in the hypoxic condition, in which the HIF1α-K709A mutant was more stable than the HIF1α-WT in Ev cells (#, p < 0.01, Fig. 4C), and p300 overexpression increased the FLAG-HIF1α-WT protein stability (a#, p < 0.01, Fig. 4C) and had no effect on the K709A mutant (Fig. 4C).

Lys-709 within the HIF1α has been reported to be a site for polyubiquitination (29). We hypothesize that p300 increases HIF1α-WT protein stability by acetylation at Lys-709, which prevents polyubiquitination and protein degradation. To investigate the relationship between polyubiquitination and acetylation at Lys-709, we performed similar co-overexpression experiments as in Fig. 4, B and C, and used proteasomal inhibitor MG132 to prevent FLAG-HIF1α degradation. FLAG-HIF1α

FIGURE 3. HIF1α is acetylated by p300 at lysine 709. A, mass spectrum of HIF1α acetylation (Ace) by p300 at lysine 709. FLAG-HIF1α and p300 were co-overexpressed in Hek293T cells. The FLAG-HIF1α was IP and gel-purified. The gel slice containing FLAG-HIF1α was digested by trypsin and subjected to LC-MS/MS analysis. Mass spectrum of b ions and y ions were shown in red and blue, respectively. Acetylation at the location was marked with an ampersand by additional mass of 42 Da. Image was prepared with Scaffold.

B, FLAG-HIF1α WT, FLAG-HIF1α K674A, and FLAG-HIF1α K709A was co-transfected with Ev (−) or p300 (+) in Hek293T cells. 48 h later, WCLs were harvested, IP purified with anti-FLAG antibodies, and analyzed by Western blots.

C, FLAG-HIF1α WT and HIF1α K709A were co-transfected with Ev (−), p300 WT, or p300 mutant that is defective in acetyltransferase activity. 48 h later, WCL was harvested, IP-purified by anti-FLAG antibodies, and analyzed by Western blots.
WT and mutant proteins were IP-purified, and the level of polyubiquitination and acetylation were measured by Western blot. In normal oxygen condition, the polyubiquitination level of HIF1α-WT was much higher than HIF1α-K709A mutant in Ev cells (Fig. 5A). p300 overexpression reduced polyubiquitination and increased acetylation in HIF1α-WT proteins (Fig. 5A). In contrast, p300 did not change the polyubiquitination and acetylation levels of the HIF1α-K709A mutant (Fig. 5A). In hypoxic condition, although both wild-type and mutant HIF1α proteins were less polyubiquitinated than in normoxia, the HIF1α-WT still had a significantly higher level of polyubiquitination than the HIF1α-K709A mutant (Fig. 5A). p300 only decreased polyubiquitination and increased acetylation levels in the HIF1α-WT, but not in the HIF1α-K709A mutant (Fig. 5A). Next, we overexpressed HIF1α-WT or K709A mutant in the presence of p300 siRNA in U2OS cells. In both normoxia and hypoxia, the loss of p300 significantly reduced the HIF1α-WT protein level, and did not change the HIF1α-K709A mutant (Fig. 5B). These data suggest that p300 regulates HIF1α protein stability by Lys-709 acetylation. Lys-709 within the HIF1α protein can be a target of both polyubiquitination and acetylation. Polyubiquitination at normal and hypoxic conditions cause HIF1α protein degradation. Acetylation by p300 or the point mutation (Lys→Ala) eliminates the polyubiquitination possibility and leads to HIF1α protein stabilization.

HIF1α Lys-709 Acetylation Increases Protein Stability

We hypothesized that the up-regulation of HIF1α protein level due to Lys-709 acetylation can lead to an increase of HIF1α transcriptional activity. To directly compare the function of HIF1α WT and K709A mutant in normal and hypoxic conditions, we transduced Hek293 cells with lentivirus containing shRNA against the 3′-UTR of HIF1α. As a result, the transduced Hek293 cell line contained stable shRNA knockdown against the endogenous, but not the exogenous, HIF1α (supplemental Fig. S4). Then, we performed HIF-1 activity reporter assay using the Dual-Luciferase plasmid system, in which the firefly luciferase reporter gene (p2.1) is under the control of hypoxia and HIF-1 and the Renilla luciferase gene is constitutive (30, 31). The HIF1α shRNA effectively inhibited the HIF-1 activity driven by the endogenous HIF1α (supple-
When p300 was co-overexpressed, it significantly increased the mutant had significantly higher activities than the HIF1. Consistent with the Western blot results in Fig. 4, the K709A reporter activity in normal and hypoxic conditions (Fig. 6) p300 overexpression did not significantly increase the HIF-1 mutant activity was not significantly affected (Fig. 6B). This was consistent with the Western blot data in Fig. 5B, in which the protein expression of HIF1α-WT, not K709A mutant, was reduced by p300 knockdown.

We also compared the HIF-1 target gene expression due to HIF1α-WT or the K709A mutant. The Hek293-shHIF1α cell line was transfected with the wild-type or mutant plasmid, and cultured in normal or hypoxic conditions overnight. Similar to Fig. 4A, the Lys-709 mutant protein was more stable than the wild-type (supplemental Fig. S6). The HIF-1 target gene expressions were significantly up-regulated by HIF1α-WT in hypoxia. For a subset of these genes such as VEGFa, Glut 1, and PDK1, the fold of hypoxia up-regulation was significantly higher by an equal amount of HIF1α-K709A plasmid compared with the wild-type (Fig. 6C and supplemental Fig. S6). In contrast, HIF-1 target genes such as hexokinase 2 (HK2) and JMJD1A were not further up-regulated by the K709A mutant (Fig. 6C and supplemental Fig. S6). Taken together, these results suggest that Lys-709 is critical for p300 to exert its positive effect on HIF1α protein stability and HIF-1 activity. The elimination of this lysine residue provides a gain of function effect to HIF-1 to phenotypically mimic p300. Finally, to investigate the effect of HIF1α-WT and K709A mutant on cancer cell growth, we used shRNA to knockdown the endogenous HIF1α in the liver cancer cell line Hep3B and subsequently overexpressed either HIF1α-WT or K709A mutant. Proliferation studies showed that cells expressing the WT or mutant protein had similar growth patterns in normal oxygen condition (Fig. 6D). Importantly, cells with the K709A mutant grew significantly better than the wild-type in hypoxia (Fig. 6D).

**DISCUSSION**

In hypoxic conditions, the increased HIF1α-p300 interaction can enhance HIF-1 transcriptional activity. In this study, we identified a novel biological consequence of HIF1α-p300 interaction. HIF1α is acetylated at lysine 709 by p300. The consequence of this acetylation is to allow the hypoxic cells to have a more sustainable level of HIF1α protein, which can lead to a more sustainable HIF-1 transactivation and HIF-1 target gene transcription. Our data indicate that p300 can acetylate HIF1α-Lys-709 in both normal and hypoxic conditions. However, the acetylation process requires HIF1α-p300 interaction, which is significantly enhanced by hypoxia (26). Therefore, the physiologically relevant Lys-709 acetylation and the biological effect should primarily occur in hypoxia. As suggested in Fig. 6E, in hypoxia, the dehydroxylation at asparagine 803 leads to the increase of HIF1α-p300 interaction, which shifts Lys-709 to an acetylated state and contributes to the HIF1α protein stability and HIF-1 transactivation. HIF1α is known to interact directly and/or indirectly with lysine deacetylases (class I–III HDACs) (10, 13, 14, 18, 27), including HDAC1, which specifically counteracts the Lys-709 acetylation and down-regulates HIF1α. Furthermore, it has been shown that HDAC1 interacts with HIF1α via VHL (27); therefore, it is likely that in normoxia, one or multiple HDACs keep the HIF1α (including Lys-709 and Lys-674) in a deacetylated state. This contributes to the inhibition of HIF-1 activity via increasing protein degradation and/or decreasing HIF1α-p300 interaction.
We have shown that the Lys-709 acetylation is not the result of hypoxia-induced HIF1α protein stability, but rather, it is one of the causes for HIF1α to be stable and/or more sustainable in hypoxic condition. Although the oxygen/VHL pathway provides a major regulatory mechanism for HIF1α protein stability, additional mechanisms do exist to fine-tune the HIF1α protein stability in both normoxia and hypoxia. For example, despite being significantly stabilized by hypoxia, HIF1α still undergoes polyubiquitination (via HAF and CHIP/HSP70) and 26 S-dependent proteasomal degradation (8, 32). In the current study, the post-translational modification at Lys-709 provides a novel mechanism in regulating HIF1α polyubiquitination and protein stability in hypoxia. Our data suggest that the acetylation at Lys-709 by p300 reduces HIF1α polyubiquitination, which serves as a possible mechanism of enhancing the protein stability in hypoxia. Recently, HIF1α Lys-709 has been identified as a possible site of polyubiquitination in a proteomic study (29). In this study, the polyubiquitination of HIF1α-WT was significantly reduced by p300 in normal and hypoxic conditions. In contrast, the polyubiquitination of HIF1α K709A mutant was not dramatically affected by p300. We speculate that Lys-709 is an oxygen/VHL-independent polyubiquitination site. In normoxia, the VHL-dependent mechanism primarily determines the HIF1α protein stability. In hypoxia or VHL loss-of-function conditions, there is a possible competition between the polyubiquitination (degradation) and acetylation by p300 (stability) in regulating HIF1α protein. A more detailed study is required to elucidate the polyubiquitination machinery at Lys-709, and the cross-talk or competition between polyubiquitination and acetylation.

Although the interaction between HIF1α-p300 is functionally critical for HIF-1 transactivation, it is unknown whether all HIF-1 target gene transactivation depends on the presence of p300 to the same level. Because p300 can also serve as a chro-
HIF1α Lys-709 Acetylation Increases Protein Stability

It is possible that the HIF1α-p300 interaction and acetylation occurs at the chromatin level and is only relevant to HIF-1 target genes that require p300. This is a likely interpretation that only a subset of HIF-1 target gene expressions were further increased by p300 or HIF1α Lys-709 mutant overexpression. Therefore, the functional significance of HIF1α-p300 interaction is both gene- and cell type-dependent.

Multiple sites of the HIF1α protein can be modified by lysine acetylation with different biological consequences. Acetylation within the oxygen-dependent degradation domain is related to VHL-dependent HIF1α degradation (11), whereas acetylation within the N-terminal region is related to VHL-independent degradation (18). The acetylation at Lys-674 is related to HIF1α-p300 interaction and HIF1-1 transactivation (14). In the current study, the acetylation at Lys-709 is related to VHL-independent HIF1α stability. Furthermore, the acetylation/deacetylation enzymes regulating these specific modifications can also be different. Our site-directed mutagenesis study indicates that p300 primarily acetylates Lys-709 within the HIF1α. In contrast, a p300 associated protein, PCAF, has no effect on Lys-709 acetylation and is primarily responsible for Lys-674 acetylation (14).

Therapeutically, HIF1α protein stability and HIF-1 transcriptional activity can be targeted by deacetylase inhibitors targeting class I, II, or III HDACs. Currently, most of the inhibitors are not specific to an isozyme; therefore, the current study suggest less than optimal inhibitory results using these deacetylase inhibitors. For example, the HIF1α protein can be stabilized by deacetylation of N-terminal lysine residues via HDAC4 (18). In the current study, HIF1α protein can be destabilized by deacetylation of Lys-709 via HDAC1. This suggests that a hydroxamic-based HDAC inhibitor such as TSA or SAHA can cause both stabilization and destabilization activities toward HIF1α. The protein level after the treatment is just a net result of these two conflicting mechanisms. Interestingly, this may also explain the observation that inhibiting class I HDACs (HDAC1–3) does not inhibit HIF1α proteins (10). Therefore, the current study provides mechanistic rationale of discovering and developing the HDAC isozyme-specific inhibitors, some of which can potentially be used to either increase or decrease HIF1α and HIF-1 activities.

REFERENCES
1. Semenza, G. L. (2012) Hypoxia-inducible factors in physiology and medicine. Cell 148, 399–408
2. Ivan, M., Kondo, K., Yang, H., Kim, W., Valiando, J., Ohh, M., Salic, A., Asara, J. M., Lane, W. S., and Kaelin, W. G., Jr. (2001) HIFα targeted for VHL-mediated destruction by proline hydroxylation: implications for O2 sensing. Science 292, 464–468
3. Yu, F., White, S. B., Zhao, Q., and Lee, F. S. (2001) HIF-1α binding to VHL is regulated by stimulus-sensitive proline hydroxylation. Proc. Natl. Acad. Sci. U.S.A. 98, 9630–9635
4. Semenza, G. L. (2012) Hypoxia-inducible factors: mediators of cancer progression and targets for cancer therapy. Trends Pharmacol. Sci. 33, 207–214
5. Semenza, G. L. (2004) Intratumoral hypoxia, radiation resistance, and HIF-1. Cancer Cell 5, 405–406
6. Isaacs, J. S., Jung, Y. J., Mimnaugh, E. G., Martinez, A., Cuttitta, F., and Neckers, L. M. (2002) Hsp90 regulates a von Hippel Lindau-independent hypoxia-inducible factor-1 α-degradative pathway. J. Biol. Chem. 277, 29936–29944
7. Liu, Y. V., and Semenza, G. L. (2007) RACK1 versus HSP90: competition for HIF-1 α degradation versus stabilization. Cell Cycle 6, 656–659
8. Luo, W., Zhong, J., Chang, R., Hu, H., Pandey, A., and Semenza, G. L. (2010) Hsp70 and CHIP selectively mediate ubiquitination and degradation of hypoxia-inducible factor (HIF)-1α but Not HIF-2α. J. Biol. Chem. 285, 3651–3663
9. Ellis, L., Hammers, H., and Pili, R. (2009) Targeting tumor angiogenesis with histone deacetylase inhibitors. Cancer Lett. 280, 145–153
10. Qian, D. Z., Zachhap, S. K., Collis, S. J., Verheul, H. M., Carducci, M. A., Atadja, P., and Pili, R. (2006) Class II histone deacetylases are associated with VHL-independent regulation of hypoxia-inducible factor 1 α. Cancer Res. 66, 8814–8821
11. Jeong, J. W., Bae, M. K., Ahn, M. Y., Kim, S. H., Sohn, T. K., Bae, M. H., Yoo, M. A., Song, E. J., Lee, K. J., and Kim, K. W. (2002) Regulation and destabilization of HIF-1α by ARD1-mediated acetylation. Cell 111, 709–720
12. Kato, H., Tanimizu-Kato, S., and Shibasaki, F. (2004) Histone deacetylation 7 associates with hypoxia-inducible factor 1α and increases transcriptional activity. J. Biol. Chem. 279, 41966–41974
13. Finley, L. W., Carracedo, A., Lee, J., Souza, A., Egia, A., Zhang, J., Teruya-Feldstein, J., Moreira, P. L., Cardoso, S. M., Clish, C. B., Pandolfi, P. P., and Haigis, M. C. (2011) SIRT3 opposes reprogramming of cancer cell metabolism through HIF1α destabilization. Cancer Cell 19, 416–428
14. Lim, J. H., Lee, Y. M., Chun, Y. S., Chen, J., Kim, J. E., and Park, J. W. (2010) Sirtuin 1 modulates cellular responses to hypoxia by deacetylating hypoxia-inducible factor 1α. Mol. Cell 38, 864–878
15. Laemmle, A., Lechleiter, A., Roh, V., Schwarz, C., Portmann, S., Furer, C., Keogh, A., Tschän, M. P., Candinas, D., Vovorburger, S. A., and Stroka, D. (2012) Inhibition of SIRT1 impairs the accumulation and transcriptional activity of HIF-1α protein under hypoxic conditions. PLoS One 7, e33433
16. Kim, S. H., Jeong, J. W., Park, J. A., Lee, J. W., Seo, J. H., Jung, B. K., Bae, M. K., and Kim, K. W. (2007) Regulation of the HIF-1α stability by histone deacetylases. Oncol. Rep. 17, 647–651
17. Sadoul, K., Boyault, C., Pabion, M., and Khochbin, S. (2008) Regulation of protein turnover by acetyltransferases and deacetylases. Biochimie 90, 306–312
18. Geng, H., Harvey, C. T., Pittsenbarger, J., Liu, Q., Beer, T. M., Xue, C., and Qian, D. Z. (2011) HDAC4 protein regulates HIF1α protein lysine acetylation and cancer cell response to hypoxia. J. Biol. Chem. 286, 38095–38102
19. Arany, Z., Huang, L. E., Eckner, R., Bhattacharya, S., Jiang, C., Goldberg, M. A., Bunn, H. F., and Livingston, D. M. (1996) An essential role for p300/CBP in the cellular response to hypoxia. Proc. Natl. Acad. Sci. U.S.A. 93, 12969–12973
20. Dann, C. E., 3rd, Bruick, R. K., and Deisenhofer, J. (2002) Structure of factor-inhibiting hypoxia-inducible factor 1: An asparaginyl hydroxylase involved in the hypoxic response pathway. Proc. Natl. Acad. Sci. U.S.A. 99, 15351–15356
21. Kung, A. L., Wang, S., Klee, J. M., Kaelin, W. G., and Livingston, D. M. (2000) Suppression of tumor growth through disruption of hypoxia-inducible transcription. Nat. Med. 6, 1335–1340
22. Kung, A. L., Zabludoff, S. D., France, D. S., Freedman, S. J., Tanner, E. A., Vieira, A., Cornell-Kennon, S., Lee, J., Wang, B., Wang, J., Memmert, K., Naegeli, H. U., Petersen, F., Eck, M. J., Bair, K. W., Wood, A. W., and Livingston, D. M. (2004) Small molecule blockade of transcriptional co-activation of the hypoxia-inducible factor pathway. Cancer Cell 6, 33–43
23. Chan, H. M., and La Thangue, N. B. (2001) p300/CBP proteins: HATs for transcriptional bridges and scaffolds. J. Cell Sci. 114, 2363–2373
24. Geng, H., Rademacher, B. L., Pittsenbarger, J., Huang, C. Y., Harvey, C. T., Lafortune, M. C., Myrthue, A., Garzotto, M., Nelson, P. S., Beer, T. M., and Qian, D. Z. (2010) ID1 enhances docetaxel cytotoxicity in prostate cancer cells through inhibition of p21. Cancer Res. 70, 3239–3248
25. Harten, S. K., Esteban, M. A., and Maxwell, P. H. (2009) Identification of novel VHL regulated genes by transcriptomic analysis of RCC10 renal carcinoma cells. Adv. Enzyme Regul. 49, 43–52
26. Semenza, G. L. (2007) Hypoxia-inducible factor 1 (HIF-1) pathway. Sci. STKE 2007, cm8
HIF1α Lys-709 Acetylation Increases Protein Stability