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HDAC-mediated deacetylation of KLF5 associates with its proteasomal degradation

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ABSTRACT

Krüppel-like factor 5 (KLF5) is a basic transcription factor that regulates diverse cellular processes during tumor development. Acetylation of KLF5 at lysine 369 (K369) reverses its function from promoting to suppressing cell proliferation and tumor growth. In this study, we examined the regulation of KLF5 by histone deacetylases in the prostate cancer cell line DU 145. While confirming the functions of HDAC1/2 in KLF5 deacetylation and the promotion of cell proliferation, we found that the knockdown of HDAC1/2 upregulated KLF5 protein but not *KLF5* mRNA, and the increase in KLF5 protein level by silencing HDAC1/2 was at least in part due to decreased proteasomal degradation. Deacetylase activity was required for HDAC1/2-mediated KLF5 degradation, and mutation of KLF5 to an acetylation-mimicking form prevented its degradation, even though the mutation did not affect the binding of KLF5 with HDAC1/2. Mutation of K369 to arginine, which prevents acetylation, did not affect the binding of KLF5 to HDAC1 or the response of KLF5 to HDAC1/2-promoted degradation. These findings provide a novel mechanistic association between the acetylation status of KLF5 and its protein stability. They also suggest that maintaining KLF5 in a deacetylated form may be an important mechanism by which KLF5 and HDACs promote cell proliferation and tumor growth.

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1. Introduction

The transcription factor Krüppel-like factor 5 (KLF5) regulates a variety of biological processes including cell proliferation, apoptosis, angiogenesis, stemness and the epithelial-mesenchymal transition (EMT) in cancer cells [1–6]. In regulating cell proliferation and tumor growth, KLF5 has been shown to play opposing roles depending on TGF-β and likely other signaling molecules [7]. In prostate cancer cells, KLF5 suppresses tumor growth in the presence of TGF-β, which induces the acetylation of KLF5 at lysine 369 (K369) [8–10], but promotes tumor growth when $KLF5^{K369}$ acetylation is interrupted [7]. Mechanistically, $KLF5^{K369}$ acetylation is essential for the transcriptional regulation of KLF5 downstream target genes. For example, acetylation of KLF5 is critical for KLF5 to bind and activate the p15 promoter [10]. Moreover, acetylation of KLF5 is also required to transcriptionally activate PDGF-A [11]. Acetylation of KLF5 is also necessary for KLF5 to bind to the promoter of p21 and form a repressive complex [12].

The coactivator/acetylase p300 interacts with the DNA-binding domain of KLF5 to acetylate KLF5 at K369 [11]. On the other hand, HDAC1 [13] and HDAC2 [12] have been shown to remove the acetyl group from KLF5. In addition, the oncogenic regulator SET, which masks histone lysines from being acetylated, also masks KLF5 from being acetylated [11]. In our previous study, we found that TGF-β treatment resulted in the recruitment of p300 to acetylate KLF5 [8]; and our unpublished data suggests that oncogenic signaling from RAS or the inactivation of PTEN also affects the acetylation of KLF5 at K369. These findings indicate that both acetylases and deacetylases regulate the acetylation of KLF5 to alter its transcriptional activities. Our previous studies have shown that

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KLF5 is an unstable protein with a short half-life [14] and is degraded at least through the ubiquitination-proteasome pathway [14]. WWP1, FBW7, SMURF2 and EFP have been identified as E3 ligases that target KLF5 for ubiquitination and subsequent degradation [15–18]. However, whether the acetylation of KLF5 affects its protein stability is unknown at present.

In this study, we investigated whether and how the deacetylases HDAC1 and HDAC2, which regulate KLF5 acetylation, regulate KLF5 protein stability. We found that acetylated KLF5 is more stable than deacetylated KLF5, and that HDACs not only control the acetylation of KLF5 but also affect KLF5 protein stability.

2. Material and methods

2.1. Cell lines and reagents

The DU 145 prostate cancer cell line and the 293T cell line were purchased from the American Type Culture Collection (ATCC, Manassas, VA) and propagated as previously described [7]. Trichostatin A (TSA), z-Leu-Leu-Leu-al (MG132) and cycloheximide (CHX) were purchased from Sigma (St. Louis, MO).

2.2. Establishment of DU 145 cells expressing KLF5, KLF5^{K369R} and KLF5^{K369Q}

The CRISPR-cas9 system was used to eliminate KLF5 protein according to the protocol from the Zhang laboratory [19]. Single clones without KLF5 expression were identified by Western blotting, and truncation of both *KLF5* genes was confirmed by sequencing. Retroviruses expressing wild-type *KLF5*, the acetylation-deficient mutant KLF5^{K369R} and the acetylation-mimicking mutant KLF5^{K369Q} were packaged and applied to infect a KLF5-null clone of DU 145 cells according to the protocol described in our previous study [7].

2.3. shRNAs and lentiviruses

PLKO.1 lentiviral vectors expressing shRNAs targeting HDAC1 (TRCN0000004814 and TRCN0000004818) and HDAC2 (TRCN0000004819 and TRCN0000004821) mRNA were purchased from Sigma, and were used following the lentiviral protocols described on the Addgene website (http://www.addgene.org/lentiviral/protocols-resources/). PLKO.1 empty vector (Sigma, SHC001) was used as the control.

2.4. Immunoprecipitation

Plasmids of Flag-HDAC1 or Flag-KLF5 were co-transfected into 293T cells using the JetPrime polyplus transfection reagent (Radnor, PA) according to the manufacturer's protocol. Empty vector pcDNA3.1 was used as a negative control. Immunoprecipitation was performed following a standard protocol using the KLF5 antibody from R&D Systems with protein G sepharose (Sigma).

2.5. Statistical analysis

Results from all experiments were expressed as means ± standard errors. The statistical significance of differences between two groups was determined by unpaired Student *t*-test. Two-way ANOVA tests were used for analyses of protein degradation curves.

3. Results

3.1. Silencing HDAC expression enhances KLF5 acetylation

Previously we found that acetylation of KLF5 determines its functions in cell proliferation and transcriptional regulation in normal epithelial cells. TGF-β treatment induces KLF5 acetylation at K369, which reverses the role of KLF5 in cell proliferation from promotion to suppression [8–10]. To investigate whether TGF-β also regulates the acetylation of KLF5 in cancer cells, we treated DU 145 prostate cancer cells with TGF-β for 24 h, and found that TGF-β treatment clearly induced the acetylation of KLF5 (Fig. 1A and B). Acetylation of KLF5 is also regulated by HDACs [11,12], so we further tested whether HDACs also regulate KLF5 acetylation in DU 145 cells. As expected, knockdown of HDAC1 or HDAC2 by RNA interference (RNAi) increased KLF5 acetylation; and the knockdown further enhanced the acetylation of KLF5 induced by TGF-β treatment (Fig. 1A and B). Interestingly, silencing HDAC1 or HDAC2 also increased the protein level of total KLF5 without TGF-β

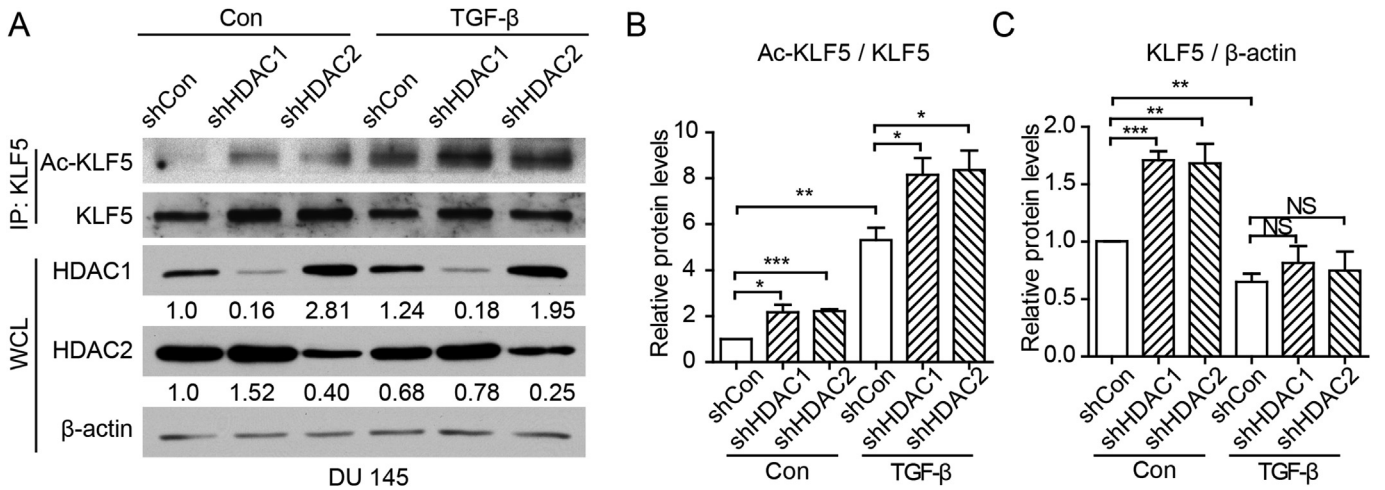

Fig. 1. Silencing HDAC1 and HDAC2 enhances TGF-β-promoted KLF5 acetylation. (A) Detection of indicated proteins by Western blotting in whole cell lysates (WCL) and endogenous KLF5 proteins immunoprecipitated (IP) from DU 145 prostate cancer cells with or without TGF-β treatment. shCon, control shRNA; shHDAC1, shRNA mixture for HDAC1; shHDAC2, shRNA mixture for HDAC2. Numbers below HDAC panels indicate ratios of HDAC protein levels to β-actin. (B) Relative protein level of acetylated KLF5 (Ac-KLF5) in reference to total input KLF5 level, as calculated based on band intensities from panel A. Ac-KLF5 was detected by acetylated lysine antibody after IP with KLF5 antibody. (C) Relative protein level of KLF5 in reference to β-actin level for each sample, as calculated according to band intensities from panel A.

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